Mechanism of antifreeze proteins action, based on Hierarchic theory of water and new ”clusterphilic” interaction
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A basically new Hierarchic theory, general for solids and liquids (Kaivarainen, 2001, 2000, 1995, 1992), has been briefly described and illustrated by computer simulations on examples of water and ice. Full description of theory and its numerous applications are presented in series of articles at the arXiv of Los-Alamos (see http://arXiv.org/abs/physics/01022086).

New clusterphilic interactions, intermediate between hydrophilic and hydrophobic, are introduced. They can be subdivided into: intramolecular - when water cluster is localized in the ”open” states of big interdomain or intersubunit cavities and intermolecular clusterphilic interactions. Intermolecular clusterphilic interactions can be induced by very different macromolecules. The latter displays themselves in bordering of water cluster by macromolecules and forming so-called ”clustrons”.

Clusterphilic interactions can play an important role in self-organization of biosystems, especially multiglobular allosteric enzymes, microtubules and the actin filaments. Cooperative properties of the cytoplasm, formation of thixotropic structures, signal transmission in biopolymers, membranes and distant interactions between different macromolecules may be mediated by both types of clusterphilic interactions.

The selected review of literature, devoted to antifreeze proteins (AFP) and ice-nucleation proteins (INP) interaction with water is presented. Corresponding experimental results were analyzed on the base of Hierarchic theory. The possible mechanism of cryoproteins influence on water, changing its freezing point has been proposed. The consequences of new model of AFP action and ways of its experimental verification are described also.

I. INTRODUCTION

A quantum and quantitative theory of liquid state, as well as a general theory of condensed matter, was absent till now. This fundamental problem is crucial for different branches of science and technology. The existing solid states theories did not allow to extrapolate them successfully to liquids.

Widely used molecular dynamics method is based on classical approach and corresponding computer simulations. It cannot be considered as a general one.
The understanding of hierarchic organization of matter and developing of general theory include a mesoscopic bridge between microscopic and macroscopic properties of condensed matter. The biggest part of molecules of solids and liquids did not follow classical Maxwell-Boltzmann distribution. This means that only quantum approach is valid for elaboration of general theory of condensed matter.

Our theoretical study of water and aqueous systems was initiated in 1986. It was stimulated by necessity to explain the nontrivial phenomena, obtained by different physical methods in our investigations of water-protein solutions. For example, the temperature anomalies in water physical properties, correlating with changes in large scale protein dynamics were found in our group by specially elaborated experimental approaches (Kaivarainen, 1985). It becomes evident, that the water clusters and water hierarchical cooperative properties are dominating factors in self-organization, function and evolution of biosystems. The living organisms are strongly dependent on water properties, representing about 70% of the body mass.

Due to its numerous anomalies, water is an ideal system for testing a new theory of condensed matter. If the theory works well with respect to water and ice, it is very probable, that it is valid for other liquids, glasses or crystals as well. For this reason we have made the quantitative verification of our hierarchic concept (Kaivarainen, 1989, 1992, 1995, 1996, 2000, 2001) on examples of water and ice.

Our theory considers two main types of molecular heat motion: translational (tr) and librational (lb) anharmonic oscillations, which are characterized by certain distributions in three-dimensional (3D) impulse space. The most probable impulse or momentum (p) determine the most probable de Broglie wave (wave B) length ($\lambda_B = h/p = v_{ph}/\nu_B$) and phase velocity ($v_{ph}$). Conformational intramolecular dynamics is taken into account indirectly, as far it has an influence on the intermolecular dynamics and parameters of waves B in condensed matter. Solids and liquids are considered as a hierarchical system of collective excitations - metastable quasiparticles of the four new type: effectons, transitons, convertons and deformons, strongly interrelated with each other.

When the length of standing waves B of molecules exceed the distances between them, then the coherent molecular clusters may appear as a result of high temperature molecular Bose-condensation (BC). The possibility of BC in liquids and solids at the ambient temperatures is one of the most important results of our model, confirmed by computer simulations. Such BC is mesoscopic one, in contrast to macroscopic BC, responsible for superfluidity and superconductivity.

The interaction between atoms and molecules in condensed matter is much stronger and thermal mobility/impulse much lesser, than in gas phase. It means that the temperature of Bose condensation can be much higher in solids and liquids than in the gas phase. The lesser is interaction between molecules or atoms the lower temperature is necessary for initiation of Bose condensation.

This is confirmed in 1995 by Ketterle’s group in MIT and later by few other groups, showing the Bose-Einstein condensation in gas of neutral atoms,
like sodium (MIT), rubidium (JILA) and lithium (Rice University) at very low temperatures, less than 10 K. However, at this temperatures the number of atoms in the primary effectons (Bose condensate) was about 20,000 and the dimensions were almost macroscopic: about 15 micrometers.

For comparison, the number of water molecules in primary librational effecton (coherent cluster), resulting from mesoscopic BC at freezing point 273 0 K is only 280 and the edge length about 20 A (see Fig. 7).

Our Hierarchic theory of matter unites and extends strongly two earlier existing most general models of solid state (Ashcroft and Mermin, 1976):

a) the Einstein model of condensed matter as a system of independent quantum oscillators;
b) the Debye model, taking into account only collective phenomena - phonons in a solid body as in continuous medium.

Among earlier models of liquid state the model of flickering clusters by Frank and Wen (1957) is closest of all to our model. In our days the quantum field theoretical approach to description of biosystems with some ideas, close to our ones has been developed intensively by Umesawa’s group (Umezawa, et. al., 1982; Umezawa, 1993) and Italian group (Del Giudice, et al., 1983; 1988, 1989).

Arani et al. introduced in 1998 the notion of Coherence Domains (CD), where molecules are orchestrated by the internal electromagnetic waves (IR photons) of matter. This idea is close to our notion of collective excitations of condensed matter in the volumes of 3D translational and librational IR photons, named primary electromagnetic deformons (see next section).

The new physical ideas require a new terminology. It is a reason why one can feel certain discomfort at the beginning of this work reading. To facilitate this process, we present below a description of a new quasiparticles, notions and terminology, introduced in our Hierarchic Theory of matter (see Table 1). Most of notions and excitation properties, presented below are not postulated, but are the result of our computer simulations.

II. THE NEW NOTIONS AND DEFINITIONS, INTRODUCED IN HIERARCHIC THEORY OF MATTER

The most probable de Broglie wave (wave B).

In composition of condensed matter the dynamics of particles could be characterized by the thermal anharmonic oscillations of two types: translational (tr) and librational (lb).

The length of the most probable wave B of thermally activated molecule, atom or atoms group in condensed matter can be estimated by two following ways:

\[
\lambda_{1,2,3} = \frac{\hbar}{m v_{gr}^{1,2,3}} = \frac{v_{ph}^{1,2,3}}{\nu_B^{1,2,3}} \]  

(2.1)
where the most probable impulse $p^{1,2,3} = mv^{1,2,3}_{gr}$ is equal to product of the particle mass ($m$) and most probable group velocity ($v^{1,2,3}_{gr}$). The wave B length also could be evaluated as the ratio of its most probable phase velocity ($v^{1,2,3}_{ph}$) to most probable frequency ($v^{1,2,3}_{B}$).

The indices (1,2,3) correspond to selected directions of motion in 3D space, related to the main axes of the molecules symmetry and their tensor of polarizability. In the case when molecular motion is anisotropic one, we have inequalities:

$$\lambda_{B}^{1} \neq \lambda_{B}^{2} \neq \lambda_{B}^{3}$$  \hspace{1cm} (2.2)

Due to anharmonicity of oscillations: $(mv^2/2) < (kT/2)$ - the most probable kinetic energy of molecules $(T_{kin})_{tr,lb}$ is lesser than potential one $(V)_{tr,lb}$. Consequently, the most probable wave B length may be bigger than space, occupied by one molecule in condensed matter:

$$(V_0/N_0)^{1/3} < \lambda_{B}^{1,2,3} > h/(mk_BT)^{1/2}$$ \hspace{1cm} (2.2a)

where $V_0$ and $N_0$ are molar volume and Avogadro number correspondingly. The left part of this inequality represents condition of mesoscopic Bose condensation.

**The most probable (primary) effectons (tr and lb).** Such a new type of quasiparticles (excitations) is represented by 3D superposition of three most probable standing waves B of molecules. The shape of primary effectons in a general case can be approximated by a parallelepiped, with the length of its 3 edges determined by 3 most probable waves B length. The volume of primary effectons is equal to:

$$V_{ef} = (9/4\pi)\lambda_{1}\lambda_{2}\lambda_{3}.$$  \hspace{1cm} (2.3)

The number of molecules or atoms forming effectons is:

$$n_{m} = (V_{ef})/(V_{0}/N_{0}).$$ \hspace{1cm} (2.4)

where $V_0$ and $N_0$ are molar volume and Avogadro number, correspondingly. The $n_{m}$ increases with temperature decreasing and may reach hundreds or even thousands of molecules.

In liquids, primary effectons may be checked as a clusters and in solids as domains or microcrystalline.

The thermal oscillations in the volume of corresponding effectons are synchronized. It means the coherence of the most probable wave B of molecules and their wave functions. We consider the **primary effectons as a result of partial Bose condensation** of molecules of condensed matter. Primary effectons correspond to the main state of Bose-condensate with the packing number $n_{p} = 0$, i.e. with the resulting impulse equal to zero.
Primary effectons, as a coherent clusters, represent self-organization of condensed matter on mesoscopic level, like I. Prigogin dissipative structures. However, it is a quantum phenomenon.

The volume of primary translational effectons in liquids is less than the volume, occupied by one molecule \((V_0/N_0)\), this points to the classical behavior of molecules.

"Acoustic" (a) and "optical" (b) states of primary effectons.

The "acoustic" a-state of the effectons is such a dynamic state when molecules or other particles composing the effectons, oscillate in the same phase (i.e. without changing the distance between them).

The "optic" b-state of the effectons is such dynamic state when particles oscillate in the counterphase manner (i.e. with periodical change of the distance between particles). This state of primary effectons has a common features with Frölich’s mode (Frölich, 1988).

The kinetic energies of "acoustic" (a) and "optical" (b) states are equal \([T_{\text{kin}}^a = T_{\text{kin}}^b]\) in contrast to potential energies \([V^a < V^b]\). It means that the most probable impulses in (a) and (b) states and, consequently, the wave length and spatial dimensions of the effectons in the both states are equal. The energy of intermolecular interaction (Van der Waals, Coulomb, hydrogen bonds etc.) in a-state are bigger than that in b-state. Consequently, the molecular polarizability in a-state also could be bigger than in b-state. It means that dielectric properties of matter may change as a result of shift of the \((a \leftrightarrow b)_{\text{tr,lb}}\) equilibrium of the effectons.

Primary transitons (tr and lb).

Primary transitons represent intermediate transition states between (a) and (b) modes of primary effectons - translational and librational. Primary transitons (tr and lb) - are radiating or absorbing photons corresponding to translational and librational bands in oscillatory spectra. The volumes of primary transitons and primary effectons coincides (see Table 1).

Primary electromagnetic and acoustic deformons (tr, lb).

Electromagnetic primary deformons are a new type of quasiparticles (excitations) representing a 3D superposition of three standing electromagnetic waves. The IR photons originate and annihilate as a result of \((a \leftrightarrow b)_{\text{tr,lb}}\) transitions of primary effectons, i.e. corresponding primary transitons. Such quantum transitions are not accompanied by the fluctuation of density but with the change of polarizability and dipole moment of molecules only. Electromagnetic deformons appear as a result of interception - superposition of 3 pairs of IR photons, penetrating in matter in different selected directions (1,2,3). We assume, that each of these 3 pairs of photons form a standing wave in the volume of condensed matter.

The linear dimension of each of three edges of primary deformon is determined by the wave length of 3 intercepting standing photons:

\[
\lambda_{1,2,3} = \left[\frac{n\tilde{\nu}}{1}\right]_{\text{tr,lb}}^{1,2,3} (2.5)
\]
where: \( n \) is the refraction index and \( (\tilde{\nu})_{tr,lb} \) - the wave number of translational or librational band. These quasiparticles as the biggest ones, are responsible for the long-range space-time correlation in liquids and solids.

In the case when \((b \rightarrow a)_{tr,lb}\) transitions of primary effectons are accompanied by fluctuation of density they may be followed by emission of phonons instead of photons. It happened when primary effectons are involved in the volume of macro- and supereffectons (see below). Primary acoustic deformons may originate or annihilate in such a way. But for primary effectons the probability of collective spontaneous emission of photons during \((b \rightarrow a)_{tr,lb}\) transition is much higher than that of phonons.

The coherent electromagnetic radiation as a result of self-correlation of many dipole moments in composition of coherent cluster, like primary effectons, containing \( N \gg 1 \) molecules is already known as superradiance (Dicke, 1954).

Secondary effectons (tr and lb).

In contrast to primary effectons, this type of quasiparticles is conventional. They are the result of averaging of the frequencies and energies of the "acoustic" (\( \overline{a} \)) and "optical" (\( \overline{b} \)) states of effectons with packing numbers \( n_P > 0 \), having the resulting impulse more than zero.

For averaging the energies of such states the Bose-Einstein distribution was used under the condition when \( T < T_0 \) \( (T_0 \) is temperature of degeneration and, simultaneously, temperature of first order phase transition). Under this condition the chemical potential: \( \mu = 0 \) and distribution has a form of Plank equation.

Secondary transitons (tr and lb).

Secondary transitons, like primary ones are intermediate transition state between \( (\overline{a}) \) and \( (\overline{b}) \) states of secondary effecton - translational and librational. Secondary transitons are responsible for radiation and absorption of phonons. As well as secondary effectons, these quasiparticles are conventional, i.e. a result of averaging. The effective volumes of secondary transitons and secondary effectons - coincides.

Secondary "acoustic" deformons (tr and lb).

This type of quasiparticles is also conventional as a result of 3D superposition of averaged thermal phonons. These conventional phonons originate and annihilate in a process of \((\overline{a} \leftrightarrow \overline{b})_{1,2,3}\) thermoactivated transitions of secondary conventional effectons (translational and librational transitons). In the case of secondary transitons \((\overline{a} \leftrightarrow \overline{b})_{tr,lb}\) transitions are accompanied by the fluctuation of density.

Convertons (tr \( \leftrightarrow \) lb).

These important excitations are introduced in our model as interconversions between translational and librational primary effectons. The \((acon)\) convertons correspond to transitions between the \((a)\) states of these effectons and \((bcon)\) convertons - to that between their \((b)\)-states. As far the dimensions of translational primary effectons are much less than that of librational ones, the convertons could be considered as the dissociation and association of the primary librational effectons (coherent clusters). Both of convertons: \((acon)\) and \((bcon)\)
are accompanied by density fluctuation, inducing phonons with corresponding frequency in the surrounding medium.

**The ca- and cb- deformons, induced by convertons.**

Three-dimensional (3D) superposition of phonons, irradiated by two types of convertons: \( a_{con} \) and \( b_{con} \), represent in our model the acoustic ca- and cb-deformons. They have the properties, similar to that of secondary deformons, discussed above.

**The c-Macrotransitons (Macroconvertons) and c-Macrodeformons.**

Simultaneous excitation of the \( a_{con} \) and \( b_{con} \) types of convertons in the volume of primary librational effectons lead to origination of big fluctuation, like cavitational one, termed c-Macrotransitons or Macroconvertons. In turn, such fluctuations induce in surrounding medium high frequency thermal phonons. The 3D-superposition these phonons forms c-Macrodeformons.

**Macroeffectons (tr and lb).**

Macroeffectons \((A \text{ and } B)\) are collective simultaneous excitations of the primary and secondary effectons in the \([A \sim (a, \bar{a})]_{tr,lb}\) and \([B \sim (b, \bar{b})]_{tr,lb}\) states in the volume of primary electromagnetic translational and librational deformons, respectively. This correlation of similar primary and secondary states results in significant deviations from thermal equilibrium. The A and B states of macroeffectons (tr and lb) may be considered as the most probable volume-orchestrated thermal fluctuations of condensed matter.

**Macrodeformons or macrotransitons (tr and lb).**

This type of conventional quasiparticles is considered in our model as an intermediate transition state of macroeffectons. The \((A \rightarrow B)_{tr,lb}\) and \((B \rightarrow A)_{tr,lb}\) transitions are represented by the coherent transitions of primary and secondary effectons in the volume of primary electromagnetic deformons - translational and librational. The \((A \rightarrow B)_{tr,lb}\) transition of macroeffecton is accompanied by simultaneous absorption of 3 pairs of photons and that of phonons in the form of electromagnetic deformons. If \((B \rightarrow A)_{tr,lb}\) transition occur without emission of photons, then all the energy of the excited B-state is transmitted into the energy of fluctuation of density and entropy of Macroeffecton as an isolated mesosystem. It is a dissipative process: transition from the more ordered structure of matter to the less one, termed Macrodeformons. The big fluctuations of density during \((A \leftrightarrow B)_{tr,lb}\) transitions of macroeffectons, i.e. macrodeformons are responsible for the Raleigh central component in Brillouin spectra of light scattering ([http://arXiv.org/abs/physics/0003070](http://arXiv.org/abs/physics/0003070)). Translational and librational macrodeformons are also related to the corresponding types of viscosity ([http://arXiv.org/abs/physics/0003074](http://arXiv.org/abs/physics/0003074)). The volumes of macrotransitons/macrodeformons (tr or lb) and macroeffectons coincide with that of tr or lb primary electromagnetic deformons correspondingly.

**Supereffectons.**

This mixed type of conventional quasiparticles is composed of translational and librational macroeffectons correlated in space and time in the volumes of superimposed electromagnetic primary deformons (translational and librational - simultaneously). Like macroeffectons, supereffectons may exist in the ground
\((A_S^* \leftrightarrow B_S^*)\) and excited \((B_S^* \leftrightarrow B_S^*)\) states representing strong deviations from thermal equilibrium state.

**Superdeformons or supertransitons.**

This collective excitations have the lowest probability as compared to other quasiparticles of our model. Like macrodeformons, superdeformons represent the intermediate \((A_S^* \leftrightarrow B_S^*)\) transition state of supereffectons. In the course of these transitions the translational and librational macroeffectons undergo simultaneous

\[
[(A \leftrightarrow B)_{tr} \text{ and } (A \leftrightarrow B)_{lb}] \text{ transitions.}
\]

The \((A_S^* \rightarrow B_S^*)\) transition of supereffecton may be accompanied by the absorption of two electromagnetic deformons - translational and librational simultaneously. The reverse \((B_S^* \rightarrow A_S^*)\) relaxation may occur without photon radiation. In this case the big cavitation fluctuation originates.

Such a process plays an important role in the processes of sublimation, evaporation and boiling.

The equilibrium dissociation constant of the reaction:

\[
H_2O \rightleftharpoons H^+ + HO^- \quad (2.6)
\]

should be related with equilibrium constant of supertransitons: \(K_{B_S^* \rightarrow A_S^*}\). The \(A_S^* \rightarrow B_S^*\) cavitation fluctuation of supereffectons can be accompanied by the activation of reversible dissociation of part of water molecules.

**In contrast to primary and secondary transitons and deformons, the notions of [macro- and supertransitons] and [macro- and superdeformons] coincide.** Such types of transitons and deformons represent the dynamic processes in the same volumes of corresponding primary electromagnetic deformons.

Considering the transitions of all types of translational deformons (primary, secondary and macrodeformons), one must keep in mind that the librational type of modes remains the same. And vice versa, in case of librational deformons, translational modes remain unchanged. Only the realization of a convertons and supereffectons are accompanied by the interconversions between the translational and librational modes, between translational and librational effectons.

**Interrelation between quasiparticles forming solids and liquids.**

Our model includes 24 types of quasiparticles (Table. 1):

\[
\begin{bmatrix}
4 \text{ - Effectons} \\
4 \text{ - Transitons} \\
4 \text{ - Deformons}
\end{bmatrix}
\]

translational and librational, including primary and secondary
| Level   | Quasiparticles                                      | Type                          |
|---------|-----------------------------------------------------|-------------------------------|
| (I)     | 1 - Primary effectons                                 | Translational (tr) and librational (lb) |
| (II)    | 2 - Interconversions                                  | The set of interconversions between translational and librational primary effectons |
| (III)   | 2 - Macroeffectons                                   | Translational and librational (spatially separated) |
| (IV)    | 1 - Supereffectons                                   | Translational $\equiv$ librational (superposition of $tr$ and $lb$ effectons and deformons in the same volume) |

Each level in the hierarchy of quasiparticles (I - IV) introduced in our model is based on the principle of correlation of corresponding type of a dynamic process in space and time. All of these quasiparticles are constructed on the same physical principles as 3D-superposition (interception) of different types of standing waves.

Such a system can be handled as an ideal gas of quasiparticles of 24 types.

As far each of the effecton’s types: $tr$ and $lb$, macroeffectons $[tr + lb]$ and supereffectons $[tr/lb]$ has two states (acoustic and optic) the total number of excitations is equal to:

$$N_{ex} = 31$$

Three types of standing waves are included in our model:
- de Broglie waves of particles (waves B);
- acoustic waves (phonons);
- electromagnetic waves (IR photons).

This classification reflects the duality of matter and field and represent their self-organization and interplay on mesoscopic and macroscopic levels.

Our hierarchical system includes a gradual transition from the Order (primary effectons, transitions and deformons) to the Chaos (macro- and superdeformons). It is important, however, that in accordance with the model proposed, this thermal Chaos is "organized" by hierarchical superposition of definite types of the ordered quantum excitations. It means that the final dynamics condensed matter only "looks" as chaotic one. Our approach makes it possible to take into account the Hidden Order of Condensed Matter.

The increasing or decreasing in the concentration of primary deformons is directly related to the shift of $(a \leftrightarrow b)_{tr,lb}$ equilibrium of the primary effectons leftward or rightward, respectively. This shift, in turn, leads also to corresponding changes in the energies and concentrations of secondary effectons, deformons and, consequently, to that of super- and macro-deformons. It means the existing of feedback reaction between subsystems of the effectons and deformons, necessary for long-range self-organization in macroscopic volumes of condensed matter.
Table 1. Schematic representation of the 18 types of quasiparticles of condensed matter as a hierarchical dynamic system, based on the effectons, transitons and deformons. Total number of quasiparticles, introduced in Hierarchic concept is 24. Six collective excitations, related to convertons- interconversions between primary librational and translational effectons and their derivatives are not represented here for the end of simplicity.

The total internal energy of substance is determined by the contributions of all types of quasiparticles with due regard for their own energy, concentration and probability of excitation. Contributions of super- and macroeffectons and corresponding super- and macrodeformons as well as polyeffectons and coherent superclusters to the internal energy of matter normally are small, due to their low probability of excitation, big volume and, consequently, low concentration.

The sizes of primary effectons determine the mesoscopic scale of the condensed matter organization. According to our model, the domains, nods, crystallites, and clusters observed in solid bodies and in liquid crys-
tals, polymers and biopolymers - can be a consequence of primary translational or librational effectons.

III. HIERARCHIC THERMODYNAMICS

3.1. The internal energy of matter as a hierarchical system of collective excitations

The quantum theory of crystal heat capacity leads to the following equation for the density of thermal internal energy (Ashcroft, Mermin 1976):

$$\epsilon = \frac{1}{V} \frac{\sum E_i \exp(-E_i/kT)}{\sum \exp(-E_i/kT)}$$

where $V$ - the crystal volume; $E_i$ - the energy of the i-stationary state.

According to our Hierarchic theory, the internal energy of matter is determined by the concentration ($n_i$) of each type of quasiparticles, probabilities of excitation of each of their states ($P_i$) and the energies of corresponding states ($E_i$). The condensed matter is considered as an "ideal gas" of 3D standing waves of different types (quasiparticles and collective excitations). However, the dynamic equilibrium between types of quasiparticles is very sensitive to the external and internal perturbations.

The total partition function - the sum of the relative probabilities of excitation of all states of quasiparticles (the resulting thermoaccessibility) is equal to:

$$Z = \sum_{tr,lb} \left\{ \left( P_{ef}^a + P_{ef}^b + P_d \right) + \left( P_{ef}^a + P_{ef}^b + \bar{P}_d \right) + \left[ (P_M^A + P_M^B) + P_D^* \right] \right\} + (P_a + P_{bc} + P_{cM}) + (P_S^A + P_S^B + P_D^*)$$

Here we take into account that the probabilities of excitation of primary and secondary transitions and deformons are the same ($P_d = P_1; \; \bar{P}_d = \bar{P}_1$) and related to the same processes:

$$(a \leftrightarrow b)_{tr,lb} \; \text{and} \; (\bar{a} \leftrightarrow \bar{b})_{tr,lb} \; \text{transitions.}$$

The analogous situation is with probabilities of $a$, $b$ and $cM$ convertons and corresponding acoustic deformons excitations: $P_{ac}$, $P_{bc}$ and $P_{cM} = P_{cM}$. So it is a reason for taking them into account in the partition function only ones.

The final formula for the total internal energy of ($U_{tot}$) of one mole of matter leading from mesoscopic model, considering the system of 3D standing waves as an ideal gas is:
The contributions of all types of effectons, transitons, convertons and deformons in total internal energy may be calculated separately as a parts of (3.3). It is shown, that all similar contributions to the total kinetic energy can be also simulated. The corresponding potential energy of quantum excitations is a result of difference between total and kinetic energies.

The small contribution of intramolecular dynamics \( U_{in} \) to \( U_{tot} \), related to energy of fundamental molecular modes \( \nu_i \), may be estimated using Plank distribution.

It has been shown by our computer simulations for the case of water and ice that \( U_{in} \ll U_{tot} \). It should be general condition for any condensed matter.

For the meaning of the variables in formulae (3.2 - 3.4), necessary for the internal energy calculations, see full version of paper, presented at the Archives of Los-Alamos: [http://arXiv.org/abs/physics/0003044](http://arXiv.org/abs/physics/0003044)

**IV. QUANTITATIVE VERIFICATION OF HIERARCHIC THEORY ON EXAMPLES OF ICE AND WATER**

All the calculations, based on Hierarchic mesoscopic concept, were performed on the personal computers. The special software: "Comprehensive analyzer of matter properties" was elaborated (copyright, 1997, Kaivarainen). The program allows to evaluate more than three hundred parameters of any condensed matter if the following data are available in the temperature interval of interest:
1. Positions of translational and librational bands in oscillatory spectra;
2. Sound velocity;
3. Molar volume or density;
4. Refraction index.

The temperature dependences of different parameters for ice and water, computed using the formulas of our mesoscopic theory, are presented in Figs.(1-4). It is only a small part of available information. In principle, it is possible to calculate about 300 different parameters for liquid and solid state of any condensed matter (Kaivarainen, 1995 and [http://arXiv.org/abs/physics/0003044](http://arXiv.org/abs/physics/0003044)).

4.1. Discussion of theoretical temperature dependences and comparison with experiment

It will be shown below that our hierarchic theory makes it possible to calculate a lot of parameters for water and ice. Those of them that could be measured experimentally are in excellent correspondence with results of theory.

On lowering down the temperature the total internal energy of ice (Fig. 1a) and its components decreases nonlinearly with temperature coming closer to absolute zero. The same parameters for water are decreasing almost linearly within the interval (100 − 0)°C (Fig. 1b).

In computer calculations, the values of $C_p(t)$ can be determined by differentiating $U_{tot}$ numerically at any of temperature interval.

**Fig. 1.** (a,b). Temperature dependences of the total internal energy ($U_{tot}$) and different contributions for ice (a) and water (b) (eqs. 4.3 - 4.5). Following contributions to $U_{tot}$ are presented:

$(U_{ef} + \tilde{U}_{ef})$ is the contribution of primary and secondary effectons;
$(U_d + \bar{U}_d)$ is the contribution of primary and secondary deformons;
$(U_{ef} + U_d)$ is the contribution of primary effectons and deformons;
$(\bar{U}_{ef} + \bar{U}_d)$ is the contribution of secondary effectons and deformons.

The contributions of macro- and supereffectons to the total internal energy and corresponding macro- and superdeformons, as well as all types of convertons, are much smaller than those of primary and secondary effectons and deformons.

It follows from Fig. 2a that the mean value of heat capacity for ice in the interval from -75 to 0°C is equal to:

$$C^\text{ice}_p = \frac{\Delta U_{\text{tot}}}{\Delta T} \approx 39 \, \text{J/M} \cdot \text{K} = 9.3 \, \text{cal/M} \cdot \text{K} \quad (4.1)$$

For water within the whole range $\Delta T = 100^\circ C$, the change in the internal energy is: $\Delta U = 17 - 9.7 = 7.3 \, \text{kJ/M}$ (Fig. 2b). This corresponds to mean value of heat capacity of water:

$$C^\text{water}_p = \frac{\Delta U_{\text{tot}}}{\Delta T} = 73 \, \text{J/M} \cdot \text{K} = 17.5 \, \text{cal/M} \cdot \text{K} \quad (4.2)$$

These results of our theory agree well with the experimental mean values $C_p = 18 \, \text{Cal/M} \cdot \text{K}$ for water and $C_p = 9 \, \text{cal/M} \cdot \text{K}$ for ice.

4.2. Temperature dependence of properties of primary librational effectons

The number of $H_2O$ molecules within the primary libration effectons of water, which could be approximated by a cube, decreases from $n_M = 280$ at 0°C to $n_M \approx 3$ at 100°C (Fig. 2a). It should be noted that at physiological temperatures (35 – 40°C) such quasiparticles contain nearly 40 water molecules. This number is close to that of water molecules that can be enclosed in the open interdomain protein cavities judging from X-ray data. The flickering of these clusters, i.e. their (dissociation $\rightleftharpoons$ association) due to $[lb \rightleftharpoons tr]$ conversions in accordance with our model is directly related to the large-scale dynamics of proteins.
Fig. 2. (a) : The temperature dependencies of the number of $H_2O$ molecules in the volume of primary librational effecton ($n^{lib}_{M, ef}$, left axis) and the number of $H_2O$ per length of this effecton edge ($\kappa$, right axis); (b): the temperature dependence of the water primary librational effecton (approximated by cube) edge length [$l^{lib}_{ef} = \kappa(V_0/N_0)^{1/3}$].

It is very important that the linear dimensions of such water clusters (11 Å) at physiological temperature are close to common ones for protein domains (Fig. 2b).

Such spatial correlations indicate that the properties of water exerted a strong influence on the biological evolution of macromolecules, namely, their dimensions and allosteric properties due to cooperativeness of intersubunit water clusters.

We assume here that integer and half-integer values of number of water molecules per effecton’s edge [\(\kappa\)] (Fig. 2a) reflect the conditions of increased and decreased stabilities of water structure correspondingly. It is apparently related to the stability of primary librational effectons as cooperative and coherent water clusters.

Nonmonotonic behavior of water properties with temperature is widely known and well confirmed experimental fact (Drost-Hansen, 1976, 1992; Clegg and Drost-Hansen, 1991; Etzler, 1991; Roberts and Wang, 1993; Roberts and Wang, 1993; Roberts, et al., 1993, 1994; Wang et al., 1994).

We can explain this interesting and important for biological functions phenomenon because of competition between two factors: quantum and structural ones in stability of primary librational effectons. The quantum factor such as wave $B$ length, determining the value of the effecton edge:

$$l_{ef} = \kappa(V_0/N_0)^{1/3} \sim \lambda_B$$

(4.3)
decreases monotonously with temperature increasing. The structural factor is a discrete parameter depending on the water molecules effective length: $l_{H_2O} = \left(\frac{V_0}{N_0}\right)^{1/3}$ and their number $[\kappa]$ in the effecton’s edge.

In accordance with our model, the shape of primary librational effectons in liquids and of primary translational effectons in solids could be approximated by parallelepiped in general case or by cube, when corresponding thermal movements of molecules (lb and/or tr) and are isotropic.

We suggest that when $(l_{ef})$ corresponds to integer number of $H_2O$, i.e.

$$[\kappa = (l_{ef}/l_{H_2O}) = 2, 3, 4, 5, 6...]_{lb}$$

the competition between quantum and structural factors is minimum and primary librational effectons are most stable. On the other hand, when $(l_{ef}/l_{H_2O})_{lb}$ is half-integer, the librational effectons are less stable (the competition is maximum). In the latter case $(a \Leftrightarrow b)_{lb}$ equilibrium of the effectons must be shifted rightward - to less stable state of these coherent water clusters. Consequently, the probability of dissociation of librational effectons to a number of much smaller translational effecton, i.e. probability of [lb/tr] convertons increases and concentration of primary librational effectons decreases. Experimentally the nonmonotonic change of this probability with temperature could be registered by dielectric permittivity, refraction index measurements and by that of average density of water. The refraction index change should lead to corresponding variations of surface tension, vapor pressure, viscosity, self-diffusion in accordance to our hierarchic theory (Kaivarainen, 1995).

4.3. Mechanism of first order phase transition in terms of hierarchic theory

The mechanism of 1st order phase transition - water freezing, leading from our Hierarchic theory, is important for understanding the mechanism of antifreeze molecules action.

The abrupt increase of the total internal energy (U) as a result of ice melting (Fig. 3a), equal to 6.27 kJ/M, calculated from our theory is close to the experimental data (6 kJ/M) (Eisenberg, 1969). The resulting thermoaccessibility or partition function (Z, eq.3.2) during [ice $\rightarrow$ water] transition decreases abruptly, while potential and kinetic energies increase (Fig. 3b).
The total internal energy \((U = T_{\text{kin}} + V_p)\) change during ice-water phase transition and change of the resulting thermoaccessibility \((Z)\) - (a); changes in kinetic \((T_{\text{kin}})\) and potential \((V_p)\) energies (b) as a result of the same transition.

It is important that at the melting point the \(H_2O\) molecules number in a primary translational effecton \((n_M^1)_{\text{ef}}\) decreases from 1 to \(\simeq 0.4\) (Fig. 4a). It means that the volume of this quasiparticle type gets smaller than the volume occupied by \(H_2O\) molecule. According to our model, under such conditions the individual water molecules get the independent translation mobility, increasing the number of \(tr\) degrees of freedom. The number of water molecules forming a primary libration effecton decreases abruptly from about 3000 to 280, as a result of melting. This also increases the number of molecules, participating in translations. The number of \(H_2O\) in the secondary librational effecton decreases correspondingly from \(\sim 1.25\) to 0.5 (Fig. 4b).

Fig. 5 a,b contains more detailed information on changes in primary librational effecton parameters in the course of ice melting.

The theoretical dependences obtained allow us to give a clear interpretation of the first order phase transitions. The condition of melting at \(T = T_{cr}\) is realized in the course of heating when the number of molecules in the volume of primary translational effectons \(n_M\) decreases:

\[
n_M \geq 1(T \leq T_{cr}) \quad T \rightarrow \quad n_M \leq 1(T \geq T_{cr})
\]  

(4.5)
Fig. 4. Changes of the number of $H_2O$ molecules forming primary $(n^{tr}_{M ef})_{ef}$ and secondary $(\bar{n}^{tr}_{M ef})_{ef}$ translational effectons during ice-water phase transition (a). Changes in the number of $H_2O$ molecules forming primary $(n^{lb}_{M ef})_{ef}$ and secondary $(\bar{n}^{lb}_{M ef})_{ef}$ librational effectons (b) as a result of phase transitions.

The process of boiling, i.e. $[\text{liquid} \rightarrow \text{gas}]$ transition, as seen from Fig. 4a, is also determined by condition (4.5), but in this case it is realized for primary librational effectons.

In other words this means that $[\text{gas} \rightarrow \text{liquid}]$ transition is related to origination (condensation) of the primary librational effectons which contain more than one molecule of substance.

In a liquid as compared to gas, the quantity of rotational degrees of freedom is decreased due to librational coherent effectons formation, but the number of translational degrees of freedom remains the same. The latter, in turn also decreases during $[\text{liquid} \rightarrow \text{solid}]$ phase transition, when the wave B length of molecules corresponding to their translations begins to exceed the mean distances between the centers of molecules (Fig. 4a). This process is accompanied by partial Bose-condensation (BC) of translational waves B and by the formation of coherent primary translational effectons, including more than one molecule. The size of librational effectons grows up strongly during this $[\text{water} \rightarrow \text{ice}]$ transition. This enlarged lb-effectons and their associates may fulfill the role of nucleation centers, necessary for $[\text{liquid} \rightarrow \text{solid}]$ transition. Interaction and adsorption of individual water molecules with such big structures damp the translational mobility of water molecules and increase their corresponding kind of de Broglie wave length till critical value, allowing mesoscopic molecular BC.

Consequently, it follows from the theory, that all factors, preventing the enlargement of primary [lb] effectons in the range of molecular numbers: 280-3000 and their possible polymerization will serve as an antifreeze factors.
Fig. 5. Changes of the number of \( H_2O \) molecules forming a primary librational effecton \((n_{hf})_{ef}\), the number of \( H_2O \) molecules \((\kappa)\) in the edge of this effecton \((a)\) and the length of the effecton edge: \( l_{ef} = \kappa(V_0/N_0)^{1/3}\) \((b)\) during the ice-water phase transition.

Our results do not contradict to conventional interpretation of first order phase transitions and give them clear quantum explanation. The number of molecules in volumes of primary effectons: translational and librational can be considered as the ”parameters of order”, characterizing the condition of first order phase transition.

The results of computer simulations, confirms the correctness of new theory of condensed matter, as a hierarchic system of 3D standing waves of different nature and demonstrate its possibilities. It may be applied for understanding the mechanism of antifreeze proteins action.

V. PROPERTIES OF ANTIFREEZE PROTEINS

5.1. Properties of the antifreeze (AFP) and ice-nucleation proteins (INP)

The antifreeze proteins (AFP), discovered in arctic fish by P. Scholander 1957 and studied later profoundly by group, led by Garth Fletcher and Choy Hew are widely spread in the nature. AFP are used for surviving at low temperatures not only by Arctic and Antarctic fish, but also by frogs, insects, plants, crops, microbes, etc.

The interest to AFP is substantial first of all because of undergoing large research programs on the development of genetically manipulated crop plants adapted to cold climate. Quite recently, the importance of AFP has been even intensified by a range of more straightforward technical applications. For example, AFP can be used for:
- preservation of blood substances;
- protection of human organs for transplantation at low temperatures;
- preservation of texture of frozen food after melting.

All these applications exploit AFP’s ability to inhibit formation of big ice crystals, damaging the cytoplasmic supramolecular structure of cells, cell membranes and tissues. However, the mechanism of specific influence of AFP on primary hydrated shell, vicinal water and bulk water is still obscure. On the other hand, this understanding is very important for the full exploitation of AFP.

At the 216th American Chemical Society Meeting in Boston (autumn 1998) possible mechanisms of action of AFP, including the role of hydrogen bonding in the interaction of the protein structure with ice, and contribution of hydrophobic interaction between the protein and water was discussed. The summary by Dr. Fletcher, president of A/F Protein Inc., was not optimistic: “We are less certain of how it works now than we were 10 years ago”. Peter Davies supposed, that specific hydrogen bonds on the surface of protein inhibit crystal growth. The interesting observation is that AFP accumulates at the interface between ice and water. A. Haymet (University of Houston) hypothesized that a hydrophobic reaction between the protein and the neighboring water prevents water from forming ice crystals. To test this idea Harding (University of Sydney) synthesized several mutant flounder proteins and compared them with wild type proteins. Two mutants lacking all threonine residues - the hydroxyl groups necessary for hydrogen bonding - behaved exactly like the wild type proteins without hydroxyls. These results mean that hydrogen [protein-water] bonding does not play the main role in antifreeze activity (Haymet et al., 1998).

In a series of experiments, Davies and colleagues compared antifreeze proteins found in fish with much stronger ones produced in some moths and beetles. The insect proteins inhibited ice growth up to -6 degrees Celsius-about four times the efficacy of known fish proteins. The results point out, in turn, that threonine with its hydroxyl groups plays an important role in AFP activity, since this amino acid is more abundant in insect proteins than in fish proteins.

AFP found in the winter flounder, is structurally long and thin, while the other three types of AFP are more circular. Therefore, it may be misleading to use that protein as a model for how all the other types work.

The fact that all antifreeze proteins studied are rich in hydrogen-bonding amino acid groups cannot be ignored, especially since many other structural and sequential aspects of antifreeze proteins differ drastically from type to type. The understanding of general principles of how the different types of antifreeze mechanisms work could help to build up effective synthetic molecules for different practical purposes.

AFPs lower the non-equilibrium freezing point of water (in the presence of ice) below the melting point, thereby producing a difference between the freezing and melting points that has been termed thermal hysteresis. In general, the magnitude of the thermal hysteresis depends upon the specific activity and concentration of AFP. The study of (Li et al., 1998) describes several low-molecular-mass solutes that enhance the thermal hysteresis activity of an AFP from overwintering larvae of the beetle Dendroides canadensis. The most active of these is citrate, which increases the thermal hysteresis nearly sixfold from 1.2
°C in its absence to 6.8 °C. Solutes which increase activity approximately fourfold are succinate, malate, aspartate, glutamate and ammonium sulfate. Glycerol, sorbitol, alanine and ammonium bicarbonate increase thermal hysteresis approximately threefold. Sodium sulfate at high concentration (0.5 mol/l) eliminated activity of all listed solutes. Solute concentrations between 0.25 and 1 mol/l were generally required to elicit optimal thermal hysteresis activity. Glycerol is the only one of these enhancing solutes that is known to be present at these concentrations in overwintering D. Canadensis. The mechanism of this enhancement of AFP activity by low molecular solutes is unknown. The AFP used in this study was (DAFP-4) type. The mature protein consists of 71 amino acid residues arranged in six 12- or 13-mer repeats with a consensus sequence consisting of Cys-Thr-X3-Ser-X5-X6-Cys-X8-X9-Ala-X11-Thr-X13, where X3 and X11 tend to be charged residues.

For understanding the mechanisms of AFP, it is important to compare them with ice nucleation proteins (INP) with the opposite effect on water structure. INP are responsible for another strategy to survive freezing temperature - to make the animals and plants freeze tolerant. Such organisms secrete INP to the extracellular environment. INP effects that ice-formation outside the cells decreases since it minimizes the extent of supercooling, so that freezing becomes less destructive to cells, making a time for cells to the cold acclimation. Such adaptation should be accompanied by dehydration of cells due to decreasing of water activity in the space out of cells and corresponding passive osmotic diffusion of water from cells.

The tertiary structure of the ice-nucleation protein is likely to be regular, consistent with the expectation of its forming a template for ice-matching. INP stimulates the formation of "ice embryo" - a region with some characteristics of macroscopic ice.

INP catalyze ice formation at temperatures much higher than most organic or inorganic substances (Gurian-Sherman and Lindow, 1993). Models of INPs predict that they form a planar array of hydrogen bonding groups that are closely complementary to the ice crystal. The circular dichroism spectra of the INP indicate the presence of large beta-sheet folds. This may explain the tendency of INPs to aggregate (Schmid, et al., 1997).

Bacterial ice-nucleation proteins are among the most active nucleants. The three-dimensional theoretical model of such proteins, based on molecular modeling, predicts a largely planar extended molecule, with one side serving as a template for ice-like spatial orientation of water molecules and the other side interacting with the membrane (Kajava and Lindow, 1993). This model predicts also that INPs can form big aggregates by interdigitation.

In contrast to INPs, AFPs do not display any tendency to aggregation at physiological concentrations. Moreover, the hydroxyl groups on the surface of AFPs, combined with hydrophobic properties looks to be important for their inhibition of the process of ice formation.

5.2. X-ray and other experimental data on AFP
The X-ray analysis with resolution of 1.25 Å for crystals of globular type III AFP with molecular mass of 7 kD was reported by Jia et al. (1996). It reveals a remarkably flat aphipathic ice-binding site where five hydrogen-bonding atoms match two ranks of oxygens on the [1010] ice prism plane in the <0001> direction, giving high ice-binding affinity and specificity. It looks that type III AFP occupies a niche in the ice surface in which it covers the basal plane while binding to the prism face.

The ice-binding mechanism of the long linear alpha-helical type I AFP (33 kD as been attributed to their regularly spaced polar residues matching into the ice lattice along a pyramidal plane (Sicheri and Yang, 1995).

The AFP of type III was linked through its N-terminals to thiodoxin (12 kD) or maltose-binding protein (42 kD). The resultant 20-kD and 50-kD fusion proteins were larger in diameter than free AFP and thus precluded any extensive [AFP - AFP] contacts on the ice surface. Both fusion proteins had the same activity as free AFP (III) at different concentration tested. This points out that AFP do not require specific intermolecular interaction to bind to ice in a way, inhibiting crystal growth (DeLuca et al., 1998).

AFP are structurally diverse molecules that share an ability to bind to ice crystals and inhibit their growth. The type II fish AFP of Atlantic herring and smelt are unique among known AFPs in their requirement of a cofactor for antifreeze activity. These AFPs are homologous with the carbohydrate-recognition domains of Ca2+-dependent (C-type) lectins and require Ca2+ for their activity. To investigate the role of metal ions in the structure and function of type II AFP, binding of Ca2+ and other divalent cations to herring AFP was investigated (Ewart et al., 1996). The studies showed that AFP has a single Ca2+-binding site.

Proteolytic protection studies and measurement of antifreeze activity revealed a conformational change from a protease-sensitive and inactive apoAFP to a protease-resistant active AFP upon Ca2+ binding. Other divalent metal ions including Mn2+, Ba2+, and Zn2+ bind at the Ca2+-binding site and induce a similar change.

An increase in tryptophan emission intensity at 340 nm also occurred upon Ca2+ addition. Whereas antifreeze activity appeared normal when Ca2+ or Mn2+ were bound, it was much lower in the presence of other metal ions. When Ba2+ was bound to the AFP, ice crystals showed a distinct difference in morphology. These studies demonstrate that herring AFP specifically binds Ca2+ and, consequently, adopts a conformation that is essential for its ice-binding activity.

The ability to control extracellular ice formation during freezing is critical to the survival of freezing-tolerant plants. AFPs, having the ability to retard ice crystal growth, were recently identified as the most abundant apoplastic proteins in cold-acclimated winter rye (Secale cereale L.) leaves.

The thermodynamics of intracellular ice nucleation are important in low-temperature biology for understanding and controlling the process of cell de-
struction by freezing. A new apparatus and technique for studying the physics of intracellular ice nucleation was developed (Tatsutani, Rubinsky, 1998). Employing the principle of directional solidification in conjunction with light microscopy, one can generate information on the temperature at which ice nucleates intracellularly as a function of the thermal history which the cells are encountered. The methods were introduced, and results with primary prostatic cancer cells were described.

AFPs depress the freezing temperature of a solution in a non-colligative manner, by arresting the growth of ice crystals. The kinetics of this effect, as studied using a new technique called temperature gradient thermometry, are consistent with an adsorption-mediated inhibitory mechanism (Chapsky, Rubinsky, 1997). The results provided a new experimental basis for understanding AFP interactions with ice.

A new microsensor that can analyze microliter volume samples was used to measure the viscosity of aqueous solutions of antifreeze glycoproteins as a function of temperature and concentration (Eto, Rubinsky, 1993). The results showed that at physiological concentrations which naturally occur in fish, AFPs significantly increased viscosity of aqueous solution. The probability for ice nucleation is inversely proportional to viscosity. Therefore, the increased viscosity could explain, in part, reports on the beneficial effects of antifreeze glycoproteins during cryopreservation by vitrification. Reducing the probability for ice nucleation could be also beneficial for the survival of cold-water fish in their natural habitat. Millimolar concentrations of AFPs increase viscosity of aqueous solution to values comparable with those of conventional cryoprotectants in molar concentrations.

5.3 Molecular dynamics approach to the mechanism of AFP activity and its biomimetics

Computer assisted approach to design of biomimetic inhibitors controlling crystal growth was used by Wierzbicki and Madura from University of South Alabama, USA. Other efforts alike are the new drug development and the entirely new methods of organ cryopreservation. The focus was on biomimetics that modify crystal growth via stereospecific crystal surface inhibition.

Research efforts since the late 1960s have determined that the antifreeze activity occurs through a unique non-colligative mechanism (DeVries et al., 1984; Feeney et al., 1986). This non-colligative activity is evidenced by a lowering of the freezing point without affecting the osmotic pressure (i.e. water activity) or the melting temperature (Feeney et al., 1973; Tomchaney et al., 1982). Although the antifreeze polypeptides do not function through a colligative mode for dissolved solutes, they must act in some specific way to alter the physical properties and interactions of the ice-water system. Several possible mechanistic explanations for this observed phenomenon of antifreeze activity may involve the solution phase itself, formation of solid solutions, and/or the inhibition of ice-crystal growth at the ice solution interface (Feeney et al., 1986). It is, however,
this latter explanation that has received the main attention. Accommodation of antifreeze molecules onto the ice surface leads to perturbations in both the thermodynamics of the surface interactions and the activation energy of the process. Ice propagation becomes disrupted forcing ice growth to be limited to discrete areas lacking the antifreeze molecules (Raymond and De Vries, 1972; 1977). This effect, known as the Kelvin effect, has been recently accepted as an explanation of the non-colligative freezing point depression mechanism of AFPs (Knight et al., 1991).

Three different types of AFPs, namely of Type I, Type II, and Type III, interacting stereospecifically with appropriate faces of ice crystal, induce the modification of ice crystal shape and cessation of crystal growth within well-defined temperature. For the alpha-helical Type I antifreeze protein, the polypeptide-crystal interaction leads to hexagonal bipyramidal shape of ice crystal.

In a recent reviews by Feeney and Yeh (1993, 1996) several viable practical applications of both native and/or synthetic antifreeze peptides were discussed. These include such areas of application as recrystallization inhibition, protection for non-polar fish, agricultural crops, as well as many foodstuffs that require reduced temperatures or freezing conditions for long term storage.

Since ice crystal growth is responsible for cellular/tissue damages within biological materials, the inhibitors or modifiers of ice crystal growth, analogous to the functional behavior of native antifreeze polypeptides, need to be developed for the reduction of this damage.

Notwithstanding the big efforts, directed to elucidation of AFP mechanisms, they are still quite obscure. Especially poorly understood is the AFPs’ influence on vicinal water: 3-60 Å from biopolymer surface and even more distant from surface water medium. The main difficulty of this problem was the absence of right theoretical approach to analysis of distant water-protein interaction, which is directly interrelated with the absence of general theory of water and ice, including the mechanism of 1st order phase transition.

It is evident from the literature data presented, that the mechanism of action of AFPs cannot be understood by existing methods. The innovative, principally new approaches must be applied for solving this problem.

The advantage of our approach is that one of us (A. K.) during last 12 years has elaborated the new Hierarchic Theory of Condensed Matter, general for liquids and solids, published in a few books. This theory was verified quantitatively on examples of water ice, using theory-based computer program: "Comprehensive Analyzer of Matter Properties (CAMP)" [copyright, 1997, Kaivarainen] as it was partially illustrated at Sections 1-4 of this article. The full description of theory is located at the Archives of Los-Alamos: [http://arXiv.org/abs/physics/01022086](http://arXiv.org/abs/physics/01022086).

It will be illustrated below, how our Hierarchic theory can be applied to explanation of mechanism of AFP action.
VI. HIERARCHIC APPROACH TO THEORY OF SOLUTIONS
AND COLLOID SYSTEMS

The action of the dissolved molecules can lead to the shift of equilibrium to the right or to the left for effectons (tr and lb) of solvent. In the former case the lifetime of unstable state for primary effectons increases, and in the latter case the stabilization of molecular associates (clusters) takes place. The same is true for convertons’ equilibrium: [lb\tr], reflecting [association\dissociation] of water clusters (primary librational effectons). If the wave length of the dissolved molecule or atoms exceeds the dimensions of primary effectons, then it must increase the degree of liquid association due to MESOSCOPIC molecular Bose condensation. In the opposite case the ordering of liquid structure decreases.

In host-guest systems a following situations are possible:

1) guest molecules stabilize (a)-states of host effectons (tr and lb) and increases their dimensions.
   
   As a result, the (a ⇔ b) equilibrium of the effectons and [lb ⇔ tr] equilibrium of convertons (see section II) becomes shifted leftward decreasing potential energy of a system, corresponding to its stabilization effect;

2) guest molecules destabilize (a)-states of host effectons. The (a - b) and [lb - tr] equilibrium of the primary effectons and convertons correspondingly are shifted rightward, inducing general destabilization effect of the system;

3) guest and host molecules form separate individual effectons (mesophase) without separation in two macrophases.

The interaction of a solute (guest) molecule with librational solvent effectons can be subdivided into two cases: when the rotational correlation time of a guest molecule is less (A) and more (B) than the rotational correlation time of librational effectons-coherent water clusters. This time is proportional to the mass and volume of the [lb] effecton. The number of molecules in a librational primary effecton, depending on temperature: in water it decreases from 280 till to 3 in the temperature interval 0-100 degrees of C (Fig.2). This means that the mass of primary [lb] effecton of liquid water near freezing point is about 5000 D.

When the condition (A) is realized, small and neutral guest molecules affect presumably only the translational effectons. In the second case (B) the guest macromolecules can change the properties of both types of effectons: translational and librational and shift the equilibrium [lb\tr] of convertons to the left, stimulating the cluster formation. In accordance with our model, the hydrophilic interaction is related to the shift of the (a - b) equilibrium of translational effectons to the left. As far the potential energy of the (a) state is less than that in the (b) state (Table 1), it means that such solvent-solute (host- guest) interaction will decrease the potential and free energy of the solution. Hydrophilic interaction does not need the realization of condition (II).

Hydrophobic interaction can be explained by the shift of the (a ⇔ b) equilibrium of librational effectons to the right. Such a shift results in the increased
potential energy of the system. The dimensions of coherent water clusters, representing libralional effectons under condition (II) may even increase. However, the decrease in of entropy (S) in this case is more than that in enthalpy (H) and, consequently, free energy will increase:

\[ \Delta G = \Delta H - T \Delta S > 0 \]  

(6.1)

This is a source of hydrophobic interaction, leading to aggregation of hydrophobic particles.

6.1. The new Clusterphilic Interaction of water with macromolecules

A new kind of Clusterphilic Interaction was introduced (Kaivarainen, 1985, 1995; 2000) to describe the cooperative water cluster interaction with nonpolar protein cavities. This idea has got support in the framework of our hierarchic theory.

Clusterphilic interactions are related to:
1) the leftward shift of \((a \rightleftharpoons b)\) equilibrium of primary libralional effectons under condition (B);
2) to the similar shift of the equilibrium of \([lb \rightleftharpoons tr]\) of convertons; and
3) increasing of primary \([lb]\) effectons dimensions due to water molecules immobilization near the surface of macromolecules.

The latter effect is a result of decreasing of the rotational correlation time of libralional effectons and decreasing of the most probable impulses of water molecules, related to librations under the effect of big enough guest particles.

**Clusterphilic interactions can be subdivided into:**

1. Intramolecular - when water cluster is localized in the "open" states of big interdomain or intersubunit cavities;
2. Intermolecular clusterphilic interactions. Intermolecular clusterphilic interactions can be induced by very different sufficiently big macromolecules.

Clusterphilic interactions can play an important role in the self-organization of biosystems, especially multiglobular allosteric enzymes, microtubules and the actin filaments. Cooperative properties of the cytoplasm, formation of thixotropic structures, signal transmission in biopolymers, membranes and distant interactions between different macromolecules can be mediated by both types of clusterphilic interactions.

Clusterphilic interactions and possible self-organization in colloid systems - thixotropic structure formation, are promoted mainly by decreasing of contribution to potential energy, related to librations in the presence of macromolecules. Hydrophilic interactions are the result of decreasing of the contribution of potential energy related to translations of water molecules. Hydrophobic interaction is a consequence of increasing of potential energy of system, related to rightward \([a \rightleftharpoons b]\) equilibrium shift of both kind of the effectons: libralional and translational under the influence of guest molecules, as far \(V_a < V_b\). Macromolecules
or polymers with molecular mass less than 2 kD do not satisfy the condition (B) at the ambient and cannot stimulate the growth of librational effectons.

6.2. The multi-fractional nature and properties of interfacial water

We can present here a classification and description of FOUR interfacial water fraction properties, based on the hierarchic model:

1. First fraction - primary hydration shell with maximum energy of interaction with surface.
   
   The structure and dynamics of this 1st fraction can differ strongly from those of bulk water. Its thickness (3-10 Å) corresponds to 1-3 solvent molecules;

2. Second fraction - vicinal water (VW) is formed by elongated primary effectons with saturated hydrogen bonds. It is a result of lb effecton adsorption on the primary hydration shell from the bulk volume. The thickness of this fraction of interfacial water: (30-75 Å) corresponds to 10-25 molecules and is dependent on the temperature, dimensions of colloid particles and their surface mobility. VW can exist in rigid pores of corresponding dimensions;

3. Third fraction of interfacial water - the surface-stimulated Bose-condensate (SSBC), represented by 3D network of primary librational effectons has a thickness of (50-300 Å). It is a next hierarchical level of interfacial water self-organization on the surface of second fraction (VW). The time of gradual formation of this 3D net of linked to each other coherent clusters (strings of polyeffectons), is much longer than that of VW and it is more sensitive to temperature and other perturbations. The second and third fractions of interfacial water can play an important role activity of biological cells activity;

4. The biggest and most fragile fourth fraction of interfacial water can be a result of slow orchestration of bulk primary effectons in the volume of primary (electromagnetic) lb deformons. The primary deformons appears as a result of three standing IR photons (lb) interception. Corresponding IR photons are superradiated by the enlarged lb effectons of vicinal water. The linear dimension of primary IR deformons is about half of librational IR photons, i.e. 5 microns (5*10^-4 Å). This ”superradiation orchestrated water (SOW)” fraction easily can be destroyed not only by temperature increasing, ultrasound and Brownian movement, but even by mechanical shaking. The time of spontaneous reassembly of this fraction after destruction has an order of hours and is dependent strongly on temperature, viscosity and dimensions of colloid particles. The processes of self-organization of third (SSBC) and forth (SOW) fractions can be interrelated by feedback interaction.

VII. Theoretical model of the antifreeze proteins (AFP) action

Hierarchic model of the formation of ”clustron” structures by AFP and their possible influence on water and ice-formation. We will proceed from our model of 1st order phase transition, described above and experimental data available.
The preliminary model, we are going to elaborate should be able to explain the following results:

a) decreasing of freezing point of water at relatively small concentrations of purified AFP of about (0.5-1) mg/ml at weak buffers at physiological pH (7.0 - 7.5). At these concentrations the water activity and osmotic pressure practically do not change thus explaining the hysteresis between freezing and melting;

b) changing of shape of ice crystals from hexagonal, pertinent for pure water, to elongated bipyramidal one;

c) accumulation of AFPs at the interface between ice and water;

d) "controversy" of AFPs`surface properties, i.e. the presence of regular hydroxyl groups necessary for hydrogen bonding, like threonine residues and their ability to inhibit the ice microcrystal growth;

e) significant increase of viscosity of water in presence of AFPs, directly correlated with their antifreezing activity.

Our mechanism of AFP action, is related to specific influence of these proteins on the properties of primary librational effectons and translational mobility of water molecules.

Our model includes the following stages:

1. Tendency of AFP to surround the primary [lb] effectons with their four-coordinated ideal ice structure, resulted from certain stereo-chemical complementary between distribution of side group on the surface of AFP and ice;

2. Increasing the dimensions of this coherent clusters due to water molecules librations immobilization near the surface and vicinal water, corresponding decreasing of the most probable [lb] impulse (momentum) and increasing of de Broglie wave (wave B) length. Such enlarged water clusters (primary [lb] effecton) surrounded by macromolecules we named "clustron" (Kaivarainen 1995, 1998);

3. Changing the symmetry of librational water molecules, thermal oscillations, from almost isotropic to anisotropic ones. Thus it means redistribution of thermal energy between three librational degrees of freedom again due to special spatial/dynamic properties of AFP surface. The consequence of this effect can be the change of ice-nucleation centers shape from hexagonal to bipyramidal, making the process of freezing less favorable. It should result in corresponding change in ice microcrystal form;

4. Bordering of the enlarged primary [lb] effectons by AFP provides the "insulation effect", preventing the association of these effectons to big enough nucleation centers, which normally accompany the water-ice transition, judging from our computer simulations;

5. Increasing the number of defects in the process of ice lattice formation in presence of AFP, as far the freezing temperature of water inside clustron can be much lower than in free bulk water. It is known experimental fact that the freezing point of water in pores or cavities with dimensions of about nanometers is much lower, than in bulk water (Kaivarainen, 1985). Such the interface effect may explain accumulation of AFP between solid and liquid phase;
6. Shifting the equilibrium between the ground - acoustic (a) state of enlarged primary [lb] effectons and optic (b) state to the latter one. This shift increases the potential energy of the affected [lb] effecton because the potential energy of (b) state is higher that of (a) and the kinetic energy of both states are the same in accordance to our model;

7. Increasing the probability and frequency of excitation of [lb-tr] convertons, i.e. [association - dissociation] of water molecules in the volume of clustron, accompanied by water density fluctuations and corresponding pulsation of the clustron’s volume;

8. Radiation of the ultrasound (US) waves by clustrons, pulsing with frequency of convertons \((10^6-10^7)\) s\(^{-1}\). These US waves destroy the ice-nucleation centers and activate the translational mobility (impulse) of water molecules, decreasing the translational wave B length \((\lambda_{tr} = h/mv_{tr})\). In accordance to condition of the 1st order phase transition (4.5) this effect decreases the freezing point of water. The melting point remains unchanged, explaining the T-hysteresis provided by AFP, because the clustron pulsation in solid phase of system are inhibited;

9. Tendency to association of pulsing clustrons to form thixotropic-like structures in liquid phase, could be a consequence of Bjorkness hydrodynamic attractive forces, existing between pulsing particles, which radiate the acoustic density waves and dipole-dipole interaction between clustrons. The formation of thixotropic structures, resulting from clustron association, explains the increasing of viscosity in AFP solutions.

This preliminary model of AFP action, based on hierarchic theory (Kaivarainen, 2001), includes the mechanism of interfacial water formation and theory of solutions, described above. The goal of our future experimental work is to confirm the main idea of proposed mechanism and evaluate the role of all contributions, listed above.

The self-organization of thixotropic-like structures in aqueous solutions of macromolecules was experimentally shown by Giordano et al., (1981). It seems to be very general phenomena. The results obtained from viscosity, acoustic and light-scattering measurements showed the existence of long-range structures that exhibit a thixotropic behavior. This was shown for solutions of lysozyme, bovine serum albumin (BSA), hemoglobin and DNA. Ordered structure builds up gradually in the course of time to become fully developed after more than 10-15 hours. When a sample is mechanically shaken this type of self-organization is destroyed. The ”preferred distance” between macromolecules in such an ordered system is about 50 Å as revealed by small angle neutron scattering (Giordano et al., 1991). It is important that this distance can be much less than the average statistical distance between proteins at low molar concentrations.

7.1. Consequences and predictions of proposed model of AFP action
1. The radius of clustrons enlarged primary [lb] effectons, surrounded by AFP can be about 30-50 Å, depending on properties of surface (geometry, polarity), temperature, pressure and presence the perturbing solvent structural agents.

2. Water, involved in clustron formation should differ by number of physical parameters from the bulk water. It should be characterized by:
   a) lower density;
   b) bigger heat capacity;
   c) bigger sound velocity
   d) bigger viscosity;
   e) smaller dielectric relaxation frequency, etc.

The formation of thixotropic structure in AFP-water systems should be accompanied by non-monotonic spatial distribution of AFP in the volume due to interaction between clustrons.

The compressibility of primary [lb] effectons should be lower and sound velocity higher than that of bulk water. It is confirmed by results of Teixeira et. al. (1985), obtained by coherent- inelastic-neutron scattering. This was proved in heavy water at 25 C with solid-like collective excitations with bigger sound velocity than in bulk water. These experimental data can be considered as a direct confirmation of primary librational effectons existence.

The largest decrease of water density occurs in pores, containing enlarged primary librational effectons, due to stronger immobilization of water molecules. The existing experimental data point, that the freezing of water inside pores occurs much below 0 °C.

We can conclude, that most of consequences of proposed model of AFP action is in a good accordance with available experimental data and the verification of the rest ones is a matter of future work.

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