Methods of non-destructive testing in studies of self-organization processes in protein films

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Abstract. A non-destructive optical control of dynamics of protein aqueous solution drying under different conditions is considered. The processes of organization of different structures in protein films are discussed.

1. Introduction
Today there are many methods of non-destructive control of a variety of physical, chemical and biological processes [1]. Such a control is especially important for creation and maintenance of essential products, components and structures. To identify various defects, such as corrosion, rusting, and cracking, various methods of non-destructive control are used. There are several types of non-destructive testing: magnetic, electric, radio wave, eddy-current, thermal, optical [2], radiation, acoustic and penetrant. The optical visual observation method is one of the main non-destructive methods used for testing of biological objects, as well as for the study of self-organization processes.

2. Methods of investigation
In the analysis of self-organizing systems the main task is to explain the physics of structure organization. To solve this problem, researchers put forward certain theories and hypotheses. In addition, mathematical modelling and computational approaches provide support for such hypotheses and theories. For example, the "reaction-diffusion" theory provides a well-developed conceptual basis for understanding the patterning and morphogenesis in developing organisms [3]. For the practical study of the processes of self-organization, natural science uses two main methods. These are chemistry and optics, or, to be more exact, the study of the interaction of matter with light in the entire wavelength range: from x-rays to radio waves. Chemistry deciphers the primary structure of biopolymers, the structure of the functional centres of protein globules, etc. However, chemistry cannot establish the spatial structure of a protein or nucleic acid. [4]

To accomplish this task, a large number of physical methods for studying the processes of self-organization are used:

1. X-ray analysis of structure. Radiography gives direct information about the arrangement of atoms in molecules and crystals. With the help of this method, the structure of some vitamins have been revealed, as well as the structure of the first protein, myoglobin. Denaturation of the molecules of this protein was established (globule-coil transition). Predominantly, protein structure formation is studied at the molecular level by this method. It is able to establish a molecular lattice with strong covalent chemical bonds. Owing to this method, a great progress has been made in visualizing protein structures at level A. This method still dominates in the rapidly developing field of biological science called proteomics [5].
2. Some methods of nuclear physics. Synchrotron radiation is a very promising method for studying biological processes associated with conformational and other structural transformations at the molecular and supramolecular levels.

3. Voltammetry. The electric field affects a wide variety of processes, for example, phase transitions of substances. The electric field reduces the heat of water evaporation and increases the rate of heat transfer in liquids. Under the influence of electric field a conformational transformation occurs in the solution of a spiral single-stranded polynucleotide into a glomerulate one. The degree of transition is a linear function of the field strength [6].

4. Non-invasive optical methods. These methods include almost all types of optical and electron microscopy. They allow one to observe biological processes in real time with little or no effect on them [2].

   The most accessible and simple method for studying the processes of self-organization is the method of visual registration of the protein condensation dynamics and its phase transitions under nonequilibrium conditions: in an open protein-water system, far from the thermodynamic equilibrium.

   This method consists in evaporation of water from the colloidal protein-water system (dehydration) with further dynamic visualization of the process of condensation and self-organization of the protein in equilibrium and in nonequilibrium conditions in vitro. The colloidal protein-water system of various natures (albumin, thrombin) is placed on a solid substrate (glass) and dried in an open system at room temperature and atmospheric pressure. The dynamics of the process is visualized with an optical microscope and a sensitive CCD camera.

   Advantages of this method:
   1. This method does not affect the sample properties and the kinetics of the processes proceeding inside it;
   2. The method allows one to evaluate the entire dynamics of the process;
   3. The method allows one to change process conditions during the experiment (temperature, composition, other external factors);
   4. The method does not require special sophisticated equipment.

   Thus, it can be concluded that optical microscopy is the most accessible and quite informative method for studying the self-organization of protein films.

3. Self-organization

Self-organization is seen as a vital concept of intracellular architecture. From simple examples in physics and chemistry, one can note that self-organization can spontaneously appear in systems with a large number of interacting components.

   Analysis of the investigations on the protein film preparation by the dehydration of their aqueous solutions reveals several general principles.

   In most of the studies of drying out of an aqueous solution, the latter was placed on a solid base made of various wettable materials - glass, plastic, etc. Then the process of dehydration, the formation of structures in protein films was observed.

4. Samples and Experimental Results

The interaction of albumin in water solution with a magnetic fluid (magnetite) and kinetics of protein film structure formation were studied by drying the water from the solution and visual control. In our experiments the aqueous solution of albumin and Fe$_3$O$_4$ ferrofluid solution (at a concentration of 1 ml of pure substance to 1 ml of distilled water) were used. The components were mixed in special cuvettes. Experimental data were recorded in the form of photos on the PC through a microscope, with a connected CCD camera. Several series of experiments aimed at studies of the kinetics of protein structure formation of dry films under the influence of a constant magnetic field and pH changes were conducted. Experiments were performed at room temperature (27 °C) and at atmospheric pressure. Fig. 1 shows various pictures of spiral cores in the albumin protein films formed under different initial conditions.
Figure 1. Some results of self-organization investigation of dehydrated protein films, different forms of spiral cores in a film of the solution under different conditions: 1 – aqueous solution of albumin with magnetite without any influence, 2 – aqueous solution of albumin with magnetite in magnetic field $H = 10$ Oe, 3 – aqueous solution of albumin with magnetite in magnetic field $H = 20$ Oe, 4 – aqueous solution of albumin with addition of $0.5 \mu l$ of acetic acid, 5 – aqueous solution of albumin with addition of $1 \mu l$ of acetic acid, 6 – aqueous solution of albumin with the addition of $2 \mu l$ of acetic acid, 7 – aqueous solution of albumin with magnetite with the addition of $2 \mu l$ of acetic acid, 8 – aqueous solution of albumin with magnetite with the addition of $2 \mu l$ of acetic acid in magnetic field $H = 10$ Oe, 9 – aqueous solution of albumin with magnetite with the addition of $2 \mu l$ of acetic acid in magnetic field $H = 20$ Oe, 10, 11, 12 – solution of albumin in NaCl

Experiments have shown that a constant magnetic field affects the structure formation of spiral nuclei: the higher the magnetic field, the smaller the characteristic rings around the central nuclei, and the smaller the size of the "cells".

Experimental data revealed that the increased acidity of the initial solution has a significant effect on the formation of protein structures, violating their “helicity”. As it is seen from the figures, the increased acidity of solutions in an applied external magnetic field does not have a significant effect on the structure formation of spiral nuclei. However, it is clear that the number of turns has increased.

Solution of sodium chloride has an entirely different effect on the formation of protein films, in contrast to distilled water.
To sum up, we revealed that magnetic field is a key factor in formation and appearance of spiral vortices in the film structures.

5. Conclusion
Experimental data show that described optical method of research is the basis for the study of self-organization processes. It allows one to visualize the emergence of a cooperative magnetic phenomenon and turbulent (chaotic) properties of the protein in the transition phase and opens a new and promising approach to the scrutiny of the mechanism of self-organization.

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