Chirality as a physical aspect of structure formation in biological macromolecular systems

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Abstract. A novel regularity of hierarchical structures is found in the formation of chiral biological macromolecular systems. The formation of structures with alternating chirality (helical structures) serves as an instrument of stratification. The ability of a carbon atom to form chiral compounds is an important factor that determined the carbon basis of living systems on the Earth as well as their development through a series of chiral bifurcations. In the course of biological evolution, the helical structures became basic elements of the molecular machines in the cell. The discreteness of structural levels allowed the mechanical degrees of freedom formation in the molecular machines in the cell.

1. Structure formation in chiral systems

One of the efficient methods of theoretical biology is a geometrization of the problem. It is natural to assume that the world of nucleic acids and the world of proteins should operate in the rank-one symmetric space, but with a certain quantitative difference in tangible media. We are talking about symmetry relations in the primary, secondary, etc. structural levels of nucleic acids and proteins. However, both systems must be constructed hierarchically to have executive and regulating subsystems. Chiral duality becomes a natural tool in combinatorics of structural correlations in these subsystems at all levels of structural organization. The purpose of the biological hierarchies is the ability to match the processes of different time and space scales.

Finding the criteria of discreteness and mechanisms of stratification in the hierarchy of primary, secondary, tertiary and quaternary structures of biological macromolecules and, as a result, their functional characteristics is one of the most important physical problems of the molecular biology [1]. The work is aimed at developing a model of the formation of the hierarchical structures in biological macromolecules. We proposed to formulate a new synergetic law that, in our view, is of common physicochemical and biological nature: in the process of self-organization an evolving system, possessing free energy and elements with chiral asymmetry, may change the type of symmetry inside one hierarchical level, thereby increasing its «complexity», but with transition to a higher level the system may also change its scale and chirality sign together with a change in the functionality of an enantiomorph. The change of chirality sign provides for the evolutionary irreversibility of stratification (stratification is understood as the splitting of a hierarchical level of a system into two levels with different chirality signs, i.e. formation of chiral layers in a system). Indeed, to switch its chirality sign a chiral object, which does not possess a symmetry axis, must be disassembled and then reassembled from the same elements. The chirality of biological structures of different levels ensures that the process of L/D stratification is universal and hierarchical levels are deterministic and stable.
An enantiomorph of a higher level, while preserving its mirror equivalence, acquires a broader spectrum of functionality. Earlier we detected the succession of the hierarchical levels with an alternating chirality sign in the structural hierarchy of DNA and proteins: from the "lower" asymmetric carbon atom to the helices, superhelices and supramolecular structures [2]. The regular alternations of the chirality sign D-L-D-L for DNA and L-D-L-D for protein structures (figure 1) were observed during the transition to a higher level of the structural and functional organization [3].

**Figure 1.** The sign-alternating hierarchies from primary to quaternary chiral (helical) structures for DNA (left column) and proteins (right column). L denotes the left enantiomer or the helical configuration; D, the right enantiomer or the helical configuration [3].

In principle, the premises of the proposed paradigm prompt an answer to the question about the basis of precisely this relation between chiral hierarchies of nucleic acids and proteins. Probably the answer is in the complemental principles of chirality and complementarity in the chain DNA-RNA-protein. It appears that the mechanism of complementary interactions that connects DNA and proteins at the level of RNA and ribosomes provides for another level in the chiral hierarchy of informationally determined macromolecular systems (D/L-sequences of DNA and proteins are mirrored). As a matter of fact nucleic acids and proteins were formed in nature as linear polymers for the purpose «to build», in the course of early evolutionary adjustment, a «sensible» chiral pair «DNA - protein».

2. **Structural and functional hierarchy of proteins**

The protein primary structure is a sequence of L-amino acids residues. The polypeptide chain is laid in the helix or in the pleated sheet. Generally, α-helix is D-enantiomer, because the right-handed α-helix is more stable than the left-handed one [4]. Interacting right-handed α-helices tend to form left superhelices. The quaternary structure of proteins is represented by the supramolecular structures, mainly formed with the left-handed superhelices interacting with the right-coiling structures. A phase shift is obvious because the protein hierarchy starts from L-amino acids, and the nucleotide hierarchy starts from D-carbohydrates deoxyriboses.
While the chirality signs are determined for the first two levels of the hierarchy of the protein structures, other levels of more complex construction of tertiary and quaternary structures are not that easy to determine. Soon after the Pauling’s α-helix model, studying complicated structures with several α-helices became interesting topic for researchers. Significant research progress was made by Pauling and Crick independently of each other in the early 1950s. (see [5]). Crick noted that if two α-helices twist around each other at an angle of about 20°, their side-chains would interlock systematically along the core of the structure, repeating the same interactions every 7 residues (or two turns of the α-helix). He called such a bundle of the supercoiled helices the “coiled coil” and such type of interaction – the “knobs into holes” (KIH).

Later Crick’s assumptions were confirmed: a typical sequence of the coiled coils therefore shows a “heptad” repeat of 7 amino acid residues (see [6]). The seven structural positions are labeled a-b-c-d-e-f-g. Usually there are hydrophobic residues in positions a and d (most frequently - Leu, Ile and Val), polar amino acid residues in positions g and e (most frequently - Lys and Glu). Such distribution of amino acid residues makes the helices amphipathic with residues at a and d forming the inter-helical hydrophobic core and residues at e and g forming inter-helical ionic interactions.

The authors of the paper [7] have introduced systematical classification of superhelices by the "periodic table". Protein structures were divided into two groups: classical (structure with one coiled coil and consequently one hydrophobic core) and complex (protein structures that contain two or more classical coiled coils that are connected in some way). Classical coiled coils with two, three, four or five helices form the first row of the table and the heads of the columns, whereas complex coiled-coil structures are separated into classes according to the highest-order classical coiled coil. Each protein containing superhelix can be studied separately using the source [8].

To prove the dominance of the left-handed superhelices in all types of configurations, we analyzed all available protein structures from the "periodic table" [7, 9]. In almost all the cases where intermolecular overlap of α-helices was visually detected there was a tendency for the secondary left superhelix formation. The structure analysis gives quantitative evaluations of the presence of left-handed superhelical motif in each class selected from the table [7]. In the simplest case - for superhelix consisted of two α-helices, - left twisting tendency was observed almost in all structures in this class. Increasing a number of α-helices, number of structures with a similar trend is reduced, but in particular classes, which are presented by more complex superhelices, containing 5-9 α-helices, the percentage of structures with a tendency to left twisting increases sharply. We pay special attention to the fact that the right-handed twisting was observed only in two cases among the variety of structures [7]: in tetrabrahione of Staphylothermus marinus (1FE6 in [8]) and in artificially synthesized tetramer (1RH4 in [8]). In some cases it was difficult to classify the twisting due to accumulations of α-helices and β-sheets near the coiled coil. Sometimes it was difficult to observe the tendency because of too short fragments of α-helices in a superhelix. In addition in some cases (most often in superhelices based on 4 α-helices) there was almost a parallel layer of α-helices with a light left-handed twisting tendency, and we did not rank these structures as having left-handed motif (as an "incomplete criterion").

Thus, we consider as proven our earlier statement regarding the characteristic chirality sign change, which is a general physical principle during transition from the secondary to the tertiary structure. It is worthy to note that the trend of the chirality sign change can be observed in protein quaternary structure (in fibrous proteins: actin protofibril, myosin, α-keratin, tropomyosin, paramyosin and the light chain of meromyosin) [2].

In our paper [3] there is a description of possible solution of Levinthal paradox: complementary sign-changing chiral sequences D–L–D–L and L–D–L–D for DNA and proteins form an Ariadne’s thread that directs macromolecular folding along the necessary trajectory toward Levinthal’s trap. It is known that a homochiral molecular substance (e.g., an amino acid or carbohydrate solution) undergoes racemization to equalize the concentration of enantiomers, raising the system’s entropy to its maximum and lowering its free energy. Meanwhile, a linear homochiral polymer is able to reduce the free energy not only through monomer racemization (“horizontal racemization”) but through “vertical racemization” as well (i.e., by creating higher level structures with the different chirality sign). In this
case the "right-handed" and the "left-handed" structural units of different structural levels are the particles of the system. The system thus distributes its homochirality, lowering its free energy and leading to the development, among some (or all) macromolecules, of a more stable (longer lived, harder) shell, as opposed to the initial state.

3. Conclusions
A model for the chiral periodicity with an alternating chiral sign in hierarchies of protein and nucleic acid structures is proposed and substantiated. The model also describes chiral conformations in interactions of macromolecules of the same or different types. Regular alternation of the chirality sign is revealed in transitions from the lowest to the higher levels of structural-functional organization both in DNA molecules where it is D-L-D-L (the structure of the deoxyribose polymeric chain is taken to be the primary structure), and in proteins where it is shifted in chiral phase to L-D-L-D. Such cross-chiral (antiphase) correspondence between proteins and DNAs forms the basic structural unit of the molecular biology as an achiral invariant allowing to realize chiral motifs in the interactions of molecules of the same or different classes through interactions of specific chemical groups. There are reasons to believe that the helical structures are required not only as rigid constructions in order to provide specific mechanical translational degrees of freedom for the molecular machines, but also as gating devices ensuring unidirectional cyclical motion of the molecular machines that create rotational degrees of freedom. Prospects of practical application of the approach developed here is related to the modeling of the interaction of chiral drugs with macromolecular targets as well as with the selection of chiral cryoprotectants in the methods associated with the cryopreservation of biomaterials.

To summarize, any chiral system, possessing free energy, tends to spontaneous formation of a new, higher structural level with the same type of symmetry but with an opposite sign of chirality and on an enlarged scale. The resulting hierarchy of conjugated sign-alternating chiral structures kinetically stabilizes them by inhibiting spontaneous racemization; forms a conjugated system with selected degrees of freedom, which makes the work of biological machines possible; in living systems macromolecular and autowave chiral structures are conjugated as a consequence of their channeling through the structures of chiral biological machines; defines the vector of the general development of a system in the direction of an upper, “open-ended” hierarchical level [10].

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