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ROLE OF BOUND WATER AND PRECIPITANTS IN THE SELF-ORGANIZATION OF BIOCRYSTALS

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It is shown that the unit cell parameters of biocrystals can be expressed as the sum of the pseudoperiods of a 30/11 helix composed of water molecules, which is the main element in the structure of the hydration shells of biocrystals. It is suggested that bound water molecules in the structure of a biocrystal form a lattice with edges that are essentially 30/11 helices spaced at a distance of the length of the corresponding pseudoperiod. An account is given of the role of precipitant in stabilizing this lattice. The conclusions are used to explain the polymorphism of biocrystals and the possible existence of large cavities and wide through channels in the structures of biocrystals in the case of polyethylene glycol and similar molecules of the corresponding length being used as a precipitant.

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INTRODUCTION

In recent years there has been an apparent crisis in natural sciences, which has the following roots: (1) reductionism and weak interdisciplinary interactions between the various sciences; (2) lack of direct experimental and theoretical methods for the study of aperiodic structures and those of nonequilibrium substances, which are more complex in structure than crystalline ones; and (3) lack of knowledge on the cooperative structural patterns and interactions underlying the various processes (especially such processes as self-organization and morphogenesis) and mutual transformations in biological systems.

The positivist views on the creation of new knowledge, which currently prevail in science, assign the primary role to the accumulation of new experimental knowledge and its further generalization. However, overcoming this crisis requires a transition from an instrumental revolution to a conceptual one. In this respect, an increasing importance is given to design methods, which are currently the only way to create models of potentially possible structures. This is why J.-M. Lehn named design as the main idea of future technology [1].

The development of nanotechnology is one of the signs of the coming scientific revolution, which will result in the creation of a systemic structural interdisciplinary megascience capable of designing potentially possible structures of the subjective reality. The best examples of the implementation of these structures and the cooperative transitions that are possible in these structures are systemic heterogeneous biological structures, which have come a long way of deterministic evolutionary development on the basis of bound-water structures. Therefore, modeling these possible structures of biosystems begins with the construction of bound-water structures that are commensurate with the other components of the biosystems,

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are suitable to them in shape, and form hydrogen bonds both with these components and with other bound-water structures being designed.

This requires the development of a generalized crystallography, some approaches to which were created by J. Bernal and A. Mackay [2, 3]. Judging by the scope of this new field, these approaches are interdisciplinary in nature [4]. Today, it is the first time that science has addressed the world of potentially possible structures and the design method, the results of which should correspond to experimental data. This issue was brought in focus also by A. A. Lyubishchev, who saw the solution to the problem of deterministic biochemical self-organization in creating a new non-Euclidean crystallography [5]. The idea of creating a generalized crystallography of tetrahedral bound-water structures as a common systemically important structural component determining the form of heterogeneous biological systems opens new possibilities for creating a theoretical systems biology, which supports the idea voiced by M. Heidegger [6] that almost all what the modern thought does is about chasing the missing whole, but as long as we see the whole only at the end of our constructions, we will never see them end.

Speaking about crystal structures, their structural motifs depicted in coordination polyhedra [7] provide enough evidence to uniquely identify the minimal repeating fragments in their structures, i.e., *crystalline modules* or *crystalline molecules* according to E. S. Fedorov [8], which fully determine the stoichiometry, symmetry, long-range order, and even morphology of the crystal (the structure and orientation of its major faces and edges). All the atoms in a module are located only on its vertices, edges, and faces; none of them are contained inside the module. Therefore, modular decomposition of a crystalline space is essentially its decomposition into all chemical bonds, including directional and weak hydrogen bonds, and corresponds to complete connectivity in a crystal structure [9].

A cooperative transformation of Euclidean crystalline modules into non-Euclidean structures of curved spaces of constant curvature is possible by the introduction of the corresponding disclinations and dispiratations into their closed bound shell [10]. For example, an ice crystalline module Ih can be transformed into a bound non-Euclidean module by introducing dispirations with an angular component of +60° and an axis parallel to the third-order axis. The resulting module retains the point symmetry subgroup of the original crystalline module D_3. The non-Euclidean modules can be joined into a one-dimensional 30/11 helix structure; these structures can, in turn, be used to build all kinds of system- and shape-forming discontinual bound-water structures, which can be embedded into the three-dimensional Euclidean space of biosystems [11].

The modular concept of self-organization of all stable bound structures embraces all continual crystal structures (equilibrium and nonequilibrium ones) as well as discontinual nonequilibrium strained structures. Tetrahedral non-Euclidean modules can be used to build systemically important ordered modular bound-water structures, which can serve as a basis for the heterogeneous structures of biosystems whose other structural components correspond, in their shape and hydrogen bond positions, to bound-water structures [11, 12]. These modules can be used to build systemically important hierarchical fractal structures forming the basis for hierarchical biosystems [11, 12].

Discontinual bound-water structures can serve as a basis for the formation of crystals of biomolecules, particularly proteins, and their concentrated colloids. The aim of this work is to: (1) verify this assumption by modeling bound-water lattices for different biocrystals; (2) explain the polymorphism of biocrystals; and (3) clarify the role of smaller ions and precipitant molecules in the formation and stabilization of biocrystal structures.

SPECIFIC FEATURES OF THE STRUCTURE AND COMPOSITION OF BIOCRYSTALS

Compared with crystals of other organic or inorganic substances, biomolecule crystals have a number of distinctive features, which are associated primarily with their large molecular weight and complex chemical structure [13].

Firstly, they have larger unit cell dimensions (50-250 Å), which makes it difficult to decode them.

Secondly, they always contain the solvent in whose solution they were grown. The proportion of the solvent in a biocrystal ranges from 30 vol. % to 75-80 vol. %. The presence of the solvent in the crystal is necessitated by the inevitable emergence of large cavities during the packing of large molecules. Furthermore, the packing of biomolecules in a crystal may also be loose, leading to a decrease in the relative volume occupied by biomolecules and an increase in the volume fraction of

the solvent. The role of the solvent is most often performed by an aqueous solution of salts of low molecular weight and polyethylene glycol (PEG).

The role of the aqueous solvent in the self-organization of protein crystals is not only to fill the space between large protein molecules. First of all, already at the stage of solution, the solvent facilitates the formation of the secondary and tertiary (globular) protein structures because the molecule portions with hydrophobic nonpolar residues tend to be shielded from the aqueous solution while those with hydrophilic polar residues tend to contact with it. In so doing, a part of the solvent water molecules find themselves in a bound state, forming hydrogen bonds with the hydrophilic groups of the protein molecule and thus creating its hydration shell.

Since the content of the aqueous solvent in biocrystals remains high, it is believed that the conformation of biomolecules does not change much during crystallization. In a biocrystal, bound water either is present in the form of the hydration shells of biomolecules or is structured under the influence of low molecular weight solutes. The proportion of bound water is up to 30 % of the total water. The rest of the water is present in the crystal in the unbound, disordered state. The bound-water structures formed in the interaction of water molecules with other molecules of the solution affect the packing pattern of biomolecules in the crystal.

These features of biocrystal structures lead to increased brittleness and propensity to fracture when the crystals are dried. Another implication is polymorphism: depending on the composition of the mother liquor and other conditions, molecules of the same protein are packed in the crystal in different ways and with different volume fractions of the solvent. As mentioned above, we believe that the polymorphism is associated with the diversity of the possible bound-water structures in biocrystals.

DISCONTINUAL LATTICE OF 30/11 HELICES AS A BASIS FOR THE SELF-ORGANIZATION OF BIOCRYSTALS

In the self-organization of biosystems, bound-water structures play a determinative role as systemically important components commensurate with the other components of the biosystem. They determine the conformation of the biosystem's organic components, especially at the level of formation of intermediates. A system simulation consists in the selection of a bound-water structure most suitable to the biopolymer in size and shape in order to form native and stable hydrated biosystems. The crucial point in such a simulation is the atomic structure of the biopolymer, which is identified by single crystal XRD analysis. The biosystem structure provides evidence that may be used in a further study of its self-organization and cooperative interactions of the protein with other substances.

It was shown previously [11] that 30/11 helices of bound water are an important element of the hydration shells of biomolecules in an aqueous medium (in particular, mother liquor). The repeating unit (Fig. 1*a*) consists of two water molecules; when thirty such units are attached to the helix, it expands by eleven turns, which corresponds to a length (period) of $a_{30} = 44.12$ Å along the helical axis given that the hydrogen bond length is 2.75 Å. The length of the projection of one repeating unit onto the axis is 44.12/30 = 1.47 Å, and the corresponding rotation angle is 360°× 11/30 = 132°. The 30/11 helix has the following pseudoperiods a_N with the minimal twist angle α_N with respect to the frame origin: (a) $N = 4$, $a_4 = 1.47$ Å × 4 = 5.88 Å and $\alpha_4 = 132° \times 4 = 528° = 180° \times 3 - 12 = -12°$ modulo 180°; (b) $N = 11$, $a_{11} = 1.47$ Å × 11 = 16.18 Å and $\alpha_{11} = 132° \times 11 = 1452° = 1440° + 12° = 360° \times 4 + 12° = +12°$ modulo 360°; and (c) $N = 15$, $a_{15} = 1.47$ Å × 15 = 22.06 Å and $\alpha_{15} = 132° \times 15 = 1980° = 180° \times 11 = 0°$ modulo 180°. Based on these pseudoperiods, we find that $a_{15} = a_4 + a_{11} = 22.06$ Å, $\alpha_{15} = 0°$ modulo 180°; $a_{19} = a_4 + a_{15} = 27.94$ Å, $\alpha_{19} = -12°$ modulo 180°; and $a_{26} = a_{11} + a_{15} = 38.24$ Å, $\alpha_{26} = +12°$ modulo 180°.

It was shown [1] that twelve 30/11 helices can intersect in a cluster of 27 water molecules, which is called a *T*-node; the central molecule of the cluster belongs to each of the helices. The angles between the helical axes are 36°, 60°, 72°, 90°, 108°, 120°, and 144°, with there being helix triplets in which the angles between the axes of each pair of helices are the same and equal to 36°, 90°, 108°, or 120° [11]. Therefore, it is possible to build three-dimensional lattices with edges made of

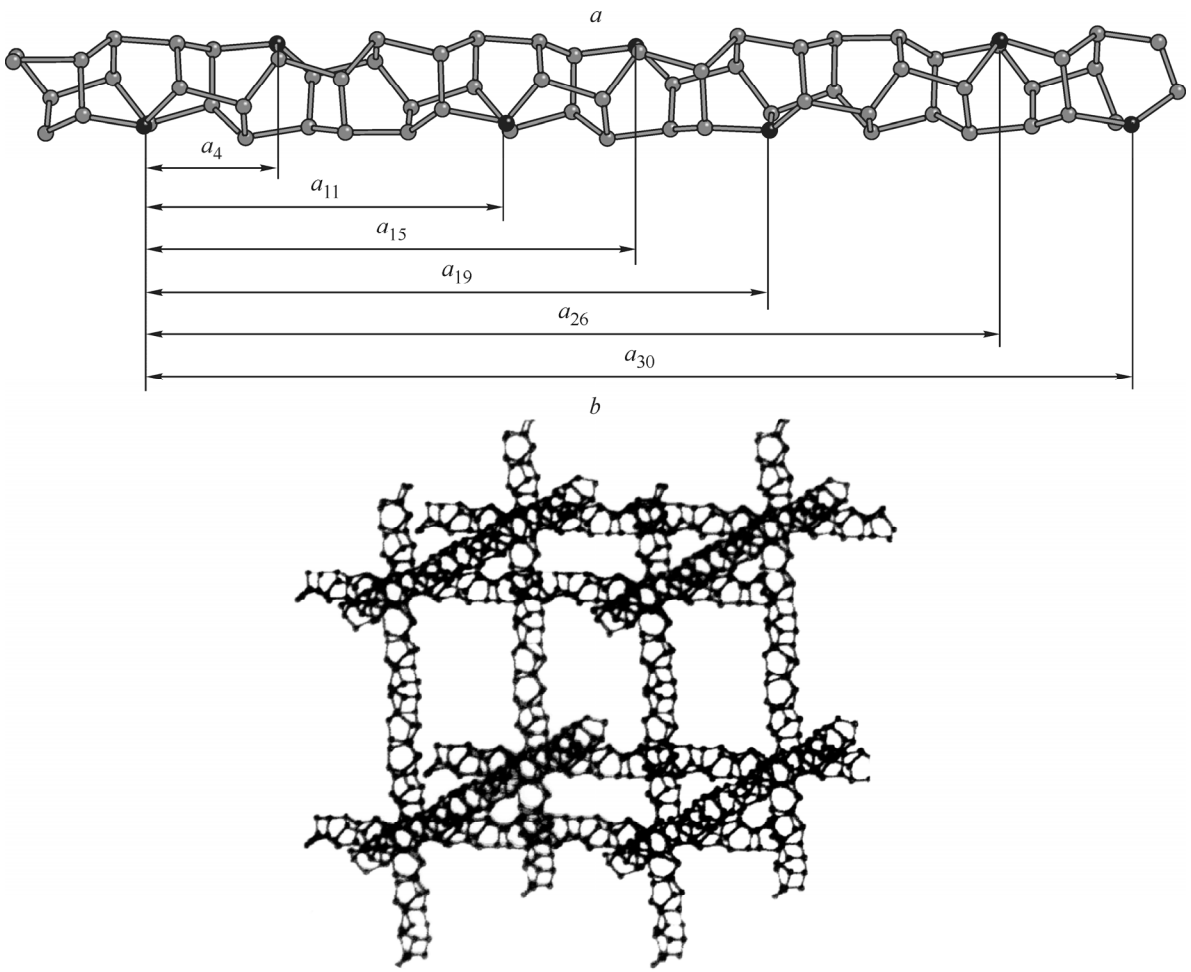

Fig. 1. 30/11 helix and its pseudoperiods (*a*); lattice with edges of 30/11 helices, 90° angles between the edges, and periods of $a = b = c = a_{26}$ (*b*).

30/11 helices and nodes in the form of the corresponding fragments of the *T*-nodes (Fig. 1*b*). The periods of these lattices are equal to one or to the sum of several pseudoperiods of the 30/11 helix, and the angles between the edges will assume the said values of 36°, 60°, 72°, 90°, 108°, 120°, and 144°. There may also be other types of nodes, which connect three 30/11 helices so as two of the angles between the helical axes assume one of the above values and the third angle is $k \times 132°$ modulo 360° (i.e., $132°$, $2 \times 132° - 360° = -96°$, $3 \times 132° - 360° = 36°$, $4 \times 132° - 360° = 168°$, $5 \times 132° - 720° = -60°$, $6 \times 132° - 720° = -72°$, etc.) [11].

It was noted [11] that protein molecules crystallize in a hexagonal, tetragonal, or monoclinic (with an angle of about 108°) lattices whose periods can be expressed, with good accuracy, as a sum of some of the pseudoperiods of the 30/11 helix. Moreover, protein crystals contain a large amount of water (up to 80 vol.%), including structured water, and disintegrate when dried [13]. Therefore, it was suggested [11] that protein crystallization must be accompanied by the formation of the above bound-water lattice with edges made of 30/11 helices. In principle, this lattice, the main parameters of which are the same as those of the unit cell of the protein crystal, may in a sense be discontinual or fractal; i.e., its larger cells may be split into smaller ones by 30/11 helices extending from the main lattice edges through the smaller pseudoperiods. The emergence of this lattice explains the following properties of protein crystals: (a) the possible presence of wide through channels filled with unstructured water; (b) the low density of proteins (20 vol.% or less) filling the lattice cells in biocrystals; and (c) a variety of location patterns for protein molecules, depending on the conditions under which the crystal is grown.

Note that the decomposition of the biocrystal lattice periods into pseudoperiods is subject to their golden ratio metric $a_N = 10\varphi(m + k(1 + \varphi^{-2}))$, where $\varphi \approx 1.618$ is the golden ratio, and is expressed through the lattice pseudoperiods

$a = a_{11} \times m + a_{15} \times k$, with the sum of these pseudoperiods covering the other pseudoperiods $a_{15} = a_4 + a_{11}$, $a_{19} = a_4 + a_{15}$, and $a_{26} = a_{11} + a_{15}$ [11].

The edges of this lattice may be elements of the structure of hydration shells and large molecules as well as small molecules in the solution. Let us consider this assumption, using the structure of the hydration shell of a PEG molecule as an example.

ROLE OF POLYETHYLENE GLYCOL MOLECULES IN FORMING BIOCRYSTALS AND IN IMPROVING THEIR STABILITY

A 30/11 helix of bound-water molecules can be split into two 15/4 helices connected by hydrogen bonds (Fig. 2*a*). As in the case of the 30/11 helix, each repeating unit of a 15/4 helix consists of two water molecules; therefore, there are 30 water molecules and 4 full turns per one period of this helix.

PEG molecules in the crystal state are known to form 7/2 helices with the following characteristics: each repeating unit consists of one monomer ($–CH_2–CH_2–O–$); the period is 7 units; and two full turns are made in one period [14]. If such a helix is untwisted by a factor of 2, there will be 1 full turn and 7 repeating units per 1 period, and this helix will be described by the formula 7/1 = 14/2 = 28/4. Next, if each four-period segment of the helix (28 monomers) is untwisted by another 96°, then the helix will make 4 full turns for 30 units (where unit is a monomer) because, given that there are two more units, the helix will gain the missing $96° = ((4 \times 360° – 96°)/28) \times 2$. Since the distances between the O atoms of the OCCO sequence in the *gauche* conformation are 2.8-2.9 Å and the length of the hydrogen bond between water molecules in the liquid is 2.75-2.85 Å, then the above-described untwisted PEG helix can be substituted for one of the two 15/4 helices making the 30/11 water helix so that the O atom of each monomer ($–CH_2–CH_2–O–$) would occupy the position of the O atom of the water molecules in the 15/4 helix (Fig. 2*b*). In this case, there will be two monomers ($–CH_2–CH_2–O–$) per each repeating unit of two water molecules in the 15/4 helix. The lengths of the hydrogen bonds between the O atoms of the PEG chain and water molecules in the 15/4 helix remain unchanged. A view along the common axis of the PEG and 15/4 water helices is shown in Fig. 2*c*. The inner diameter is 5 Å, and the outer diameter is about 10 Å. The values of the angles of internal rotation around the bonds O–C, C–C, and C–O in one step OCCOCCO of the 15/4 helix correspond to the *TGTGGG* conformation; i.e., the O atoms of the PEG molecule are in the position *G*, like in the crystal.

Adding small portions of PEG into water substantially improves its hydrodynamic properties by reducing turbulence [15]. In particular, a spray from a hose comes at a greater velocity and covers a much greater (several times over) distance. This might be due to the similar structure of the hydrated form of the PEG molecule, stretched by external directional influence: being within the volume of the spray, these ″needles″ increase the laminarity of the flow. Furthermore, PEG molecules may reinforce the surface layer of the spray by embedding themselves, in a similar way, into the structure of the surface water layer consisting of 30/11 helices [16].

Interestingly, the so-called meander conformation of the PEG molecule, whereby the internal rotation angles at OCCO correspond to the *GTG* sequence, is also commensurate with the parameters of the 30/11 helix and the structure of the surface water layer (Fig. 2*d*). In this case, the correspondence between the structures of the surface water layer and meander PEG conformation is such that only 1/4 of the O atoms in the PEG molecules can form hydrogen bonds with the surface layer water molecules (Fig. 2*d*).

PEG molecules with molecular weights ranging from 200 to 8000 and a length of ~7 Å to ~270 Å in such a hydrated state are the most popular precipitants. They appear to be able to stabilize a lattice consisting of 30/11 helices by substituting for one of the two 15/4 helices that form the 30/11 helix.

An example of a protein crystal in which the stabilization of the water structure by PEG molecules appears to play the decisive role may be crystals of human serum albumin (HSA) that were obtained from a solution containing PEG 400 and KH_2PO_4 ([17]; for a corrected model of the structure see [18] and, in part, [19]). These crystals are unique because their mother liquor content can be as high as 78 vol.% and they have a large solvent channel passing parallel to the *c* axis; the

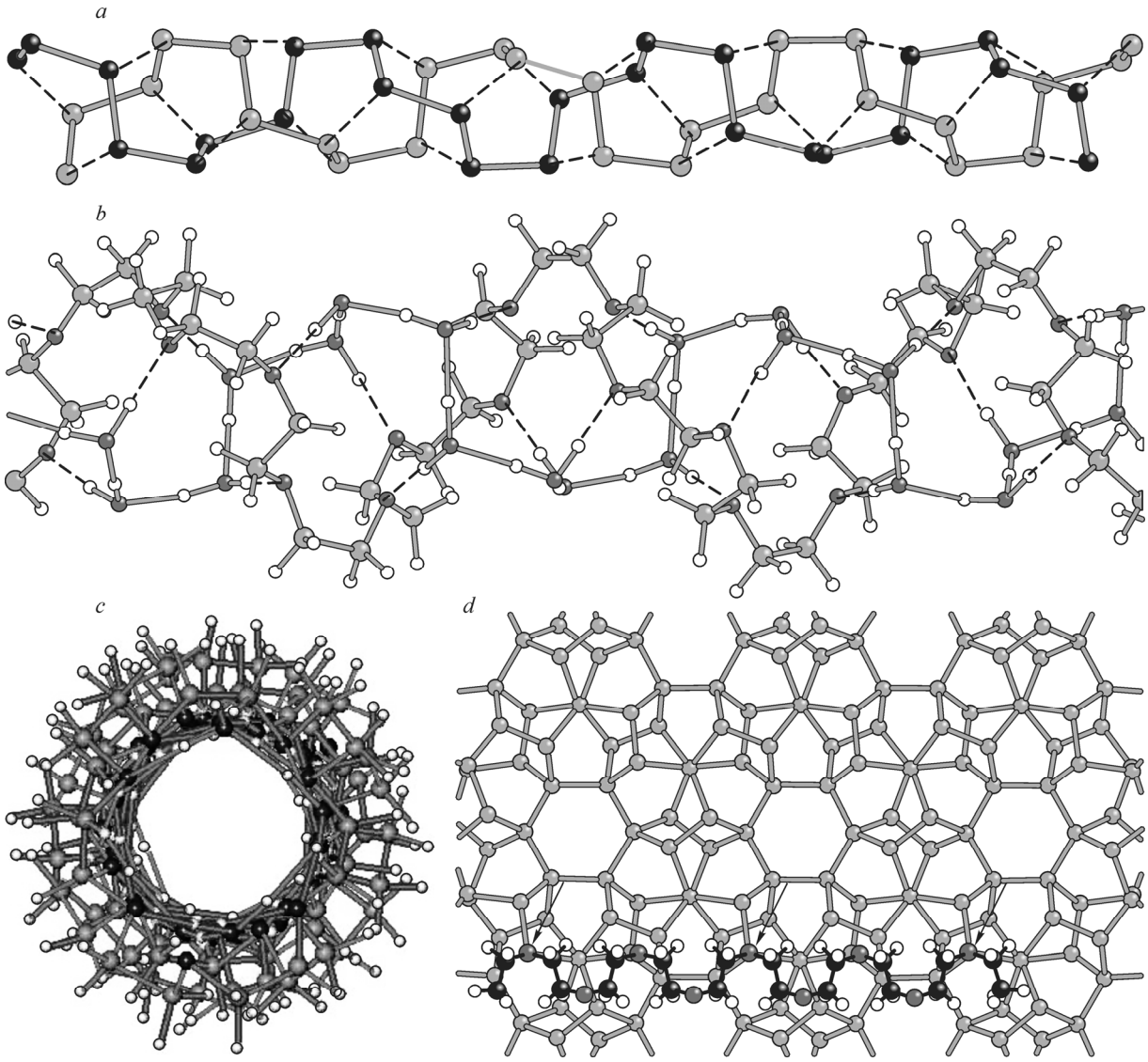

Fig. 2. Decomposition of a 30/11 helix into two 15/4 helices of black and gray atoms; hydrogen bonds between the 15/4 helices are dashed (*a*); substitution of a PEG molecule for one of the 15/4 helices constituting the 30/11 helix (hydrogen bonds between the O atoms of water and PEG molecules are dashed; all the covalent bonds in PEG and water molecules and those between water molecules are indicated by solid lines), view perpendicular to the common axis of the 15/4 helices (*b*); the same, view parallel to the axis of the 15/4 helices (*c*); meander conformation of PEG (black lines) superimposed on a 30/11 helix in the structure of the surface water layer (gray lines); the sites of formation of hydrogen bonds between the O atoms of PEG and water molecules are indicated by arrows (*d*).

channel has a square-shaped cross-section with a side of ~90 Å. The crystals are shaped as tetragonal plates and described by the space group $P42_12$; their unit cell contains 8 protein molecules and has the parameters $a = b = 186.5$ Å and $c = 81.0$ Å. The large solvent channel parallel to the *c* axis is clearly visible in the electron density map (see the scheme in Fig. 3*a*) obtained for these crystals ([17]; the map was placed on the cover of that issue of *Science*).

In our understanding, the packing of the HSA crystal is based on a lattice composed of 30/11 bound-water helices with periods of $a_{lat} = a_{15} \times 6 = 22.06 \times 6 = 133.36$ Å and $c_{lat} = a_{11} \times 5 = 16.18 \times 5 = 80.9$ Å, which correspond to the experimental values $a\sqrt{2}/2 = 186.5 \times \sqrt{2}/2 = 131.9$ Å (with a deviation of $\Delta = 1.1\%$) and $c = 81$ Å ($\Delta = 0.13\%$) (Fig. 3*b*). In Fig. 3*a* the positions of the main edges of this lattice are shown in a schematic representation of the electron density map of the HSA crystals. The continuous channel running along the *c* axis with width of about 90 Å

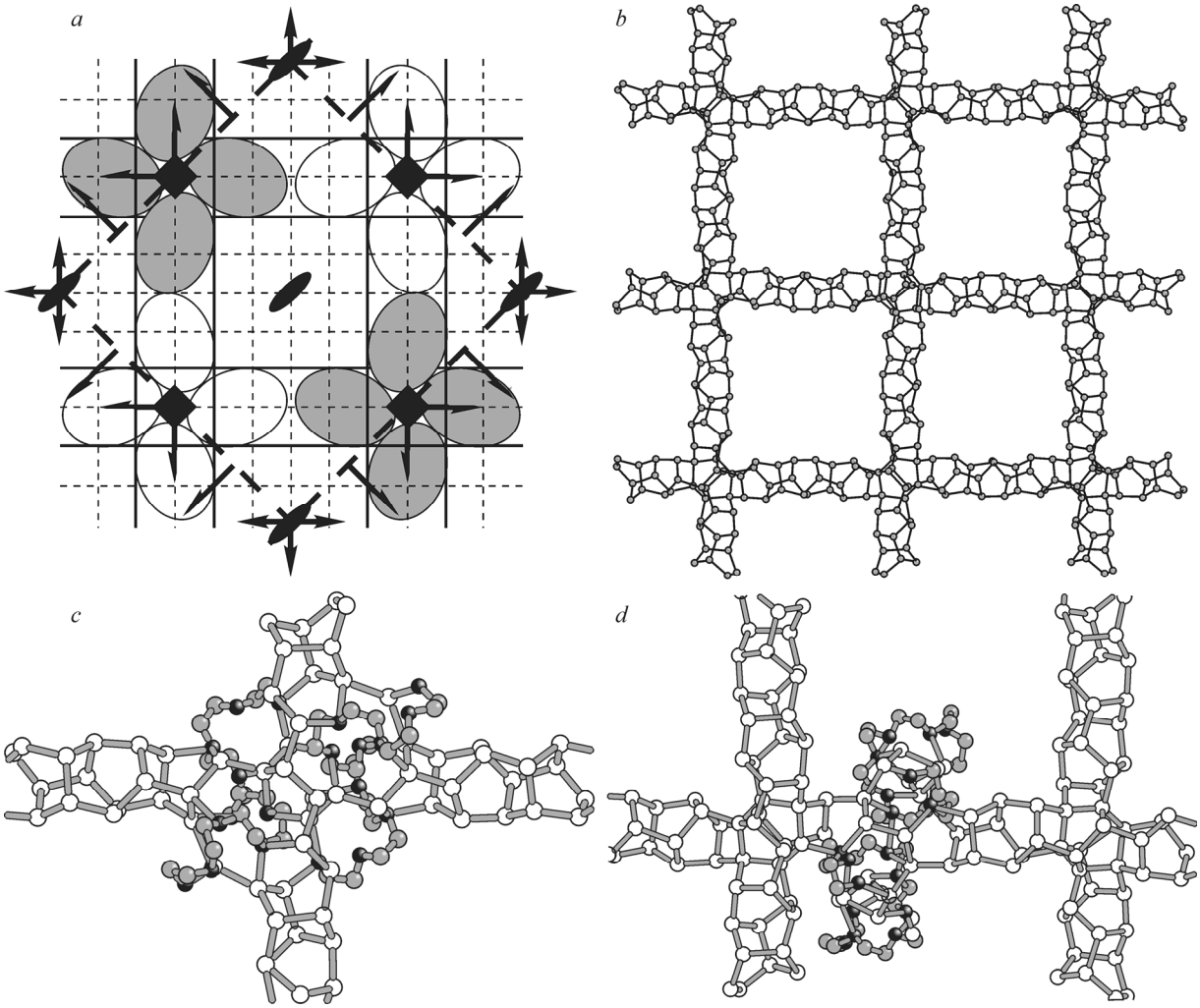

Fig. 3. Electron density map of a HAS crystal with a lattice composed of 30/11 bound-water helices, view along the *c* axis (*a*); fragment of the bound-water lattice with dimensions $a_{15}\times a_{15}$ (*b*); positions of PEG molecules strengthening the node of this lattice (*c*); positions of PEG molecules located at a distance of pseudoperiod a_4 from the lattice node (*d*).

($a_{15} \times 4 = 22.06 \times 4 = 88.24$ Å) is in principle filled with unstructured water; however, the original figure [17, the cover] shows small electron-density peaks on this channel near the nodes of the bound-water lattice. These peaks can only be due to PEG 400 molecules and KH_2PO_4 anions, which cannot occupy a fixed position inside the large channel unless there is something that binds them, which can only be bound-water structures. This suggests that, inside the channel, not all the nodes and edges of the lattice composed of 30/11 helices are broken. This lattice both fixes the positions of PEG molecules (and ions) inside the channel and is, in turn, stabilized by them. Figs. 3*c* and *d* show fragments of the bound-water lattice composed of 30/11 helices and the positions of hydrated PEG molecules corresponding to the peaks in the electron density map. PEG molecules in the positions shown in Fig. 3*c* stabilize the lattice nodes. PEG molecules in the positions shown in Fig. 3*d* can stabilize the additional edges of this lattice that are at distance of the pseudoperiod a_4 from the nodes. A PEG 400 molecule contains 9 monomers; the length of the molecule in the said conformation is approximately 13 Å, which is consistent with the way it is depicted in the electron-density map [17, the cover].

It should be noted that, for crystallization of recombinant human serum albumin (rHSA) in a neutralized PEG 400 solution, researchers have also obtained another, more common crystalline form [19], which is different from the above-described tetragonal one with a large channel along the *c* axis. This form is characterized by the frequent monoclinic space group $P2_1$ and the parameters $a = 58.9$ Å, $b = 38.3$ Å, $c = 60.7$ Å, and $\beta = 101.9°$. The protein molecules are packed much

TABLE. Unit Cell Parameters of Some Forms of BPR Crystals and Their Decomposition into Pseudoperiods of the 30/11 Bound-Water Helix

Name and reference	Crystal lattice parameters: a, b, c, Å, angle β, deg.	Volume per a protein molecule in a cell, Å^3	Lattice period expressed through the pseudoperiods $a_4 = 5.88$ Å, $a_{11} = 16.18$ Å, $a_{15} = 22.06$ Å, $a_{19} = 27.94$ Å, and $a_{26} = a_{11} + a_{15} = 38.24$ Å of the (30/11) helix and deviation from experimental data Δ
Ribonuclease BPR I, [17]	$P2_12_12_1$ ($Z = 4$) $a = 44.63$ $b = 76.3$ $c = 37.6$	32010	$a = 2a_{15} = 44.12$, Δ = 1.2 % $b = 2a_{15} + 2a_{11} = 2a_{26} = 76.48$, Δ = 0.3 % $c = a_{15} + a_{11} = 2a_{11} + a_4 = 38.24$, Δ = 1.7 %
Ribonuclease BPR VI, [17]	$C2$ ($Z = 4$) $a = 70.60$ $b = 38.99$ $c = 51.65$ β = 103.96	34494	$a = 2a_{19} + a_{11} = 72.06$, Δ = 2.1 % $b = 2a_{11} + a_4 = 38.24$, Δ = 1.9 % $c = 2a_{15} + a_4 = 50.00$, Δ = 3.2 %
Ribonuclease BPR XIII, [17]	$P2_1$ ($Z = 4$) $a = 51.35$ $b = 76.0$ $c = 31.56$ β = 106.11	29582	$a = 2a_{15} + a_4 = 50.00$, Δ = 2.7 % $b = 2a_{15} + 2a_{11} = 2a_{26} = 76.48$, Δ = 0.7 % $c = 2a_{11} = 32.36$, Δ = 2.5 %
Ribonuclease BPR III, [17]	$P2_1$ ($Z = 4$) $a = 42.91$ $b = 45.38$ $c = 77.2$ β = 114.31	34250	$a = 2a_{15} = 44.12$, Δ = 2.8 % $b = 2a_{15} = 44.12$, Δ = 2.8 % $c = 2a_{15} + 2a_{11} = 2a_{26} = 76.48$, Δ = 0.9 %
Ribonuclease BPR II, [18]	$P2_1$ ($Z = 2$) $a = 30.13$ $b = 38.11$ $c = 53.29$ β = 105.75	29447	$a = 2a_{11} = 32.36$, Δ = 7.4 % $b = 2a_{11} + a_4 = 38.24$, Δ = 0.4 % $c = a_{15} + 2a_{11} = 54.42$, Δ = 2.1 % or $c = 2a_{19} = 55.88$, Δ = 4.9 %

more densely so that the solvent occupies only about 33 vol.% of the crystal. In this case, the lattice parameters can also be expressed through the pseudoperiods of the 30/11 helix: $a = 2a_{15} + a_{11} = 60.30$ Å (Δ = 2.4 %), $b = 2a_{11} + a_4 = 38.24$ Å (Δ = 0.2 %), and $c = 2a_{15} + a_{11} = 60.30$ Å (Δ = 0.7 %).

ROLE OF PRECIPITANTS IN THE FORMATION AND POLYMORPHISM OF BIOCRYSTALS

Apart from large molecules such as PEG, small molecules or organic substances and other compounds containing tetrahedral PO_4^{3-} - and SO_4^{2-} -type anions, small Cl^-, F^-, and I^- anions, and K^+-type cations can also act as precipitants. The type and concentration of the precipitant have little effect on the biopolymer conformation, leaving it almost the same as in the native form; however, they affect the spatial arrangement and packing patterns of protein molecules. Being affected by different solvents, one and the same weakly structured protein can yield up to two dozens of polymorphs. Biocrystals rarely contain any through inner channels such as the one discussed above for HSA crystals and are usually characterized by a monoclinic or triclinic symmetry.

For example, for the BPR (bovine pancreatic ribonuclease) protein, there is evidence of at least 13 polymorphs [20-22], whose synthesis conditions differ in the type and concentration of the precipitant. The majority of forms of BPR

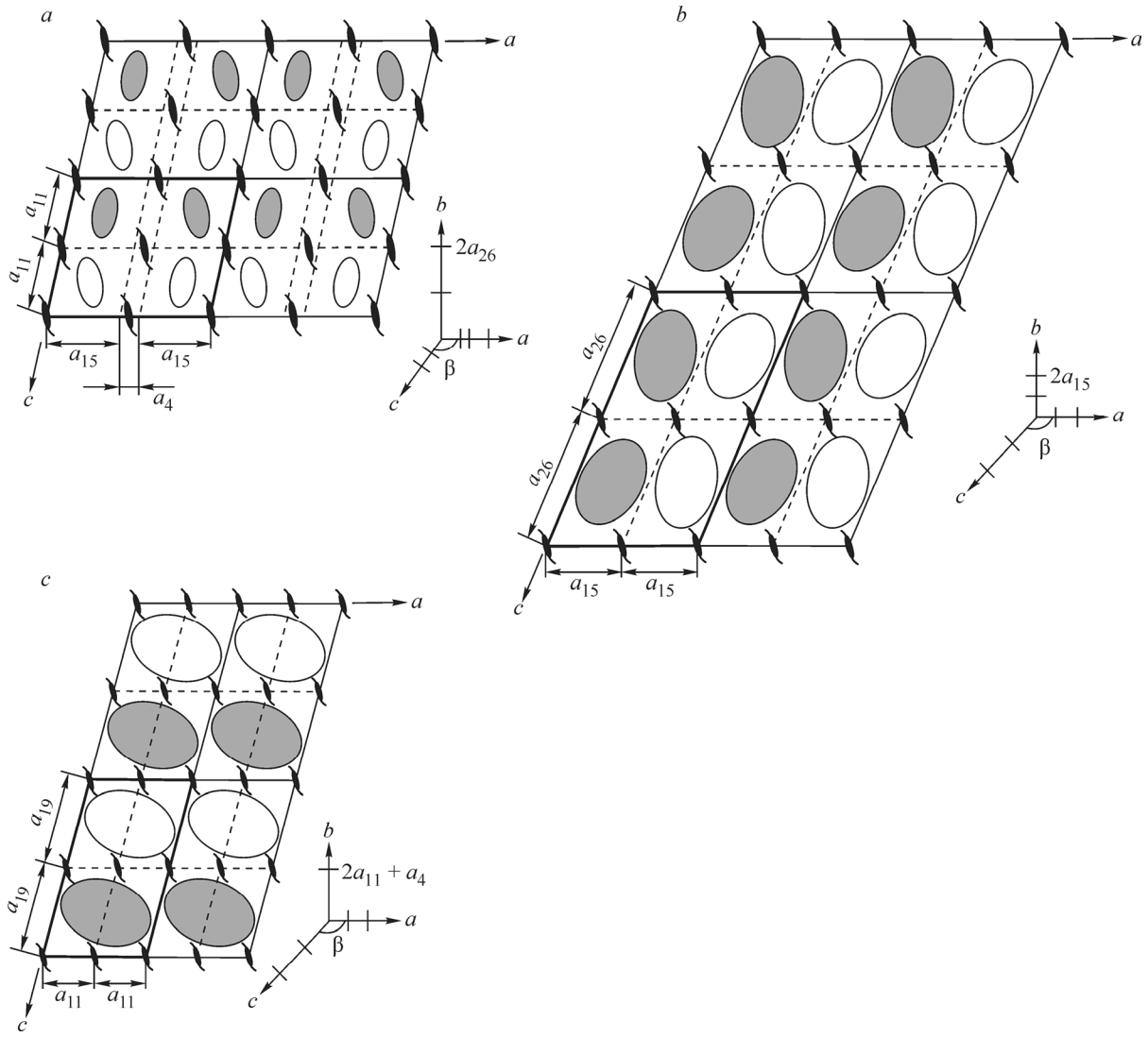

Fig. 4. Possible packing pattern of BPR molecules in a lattice composed of 30/11 bound-water helices for the case of the $P2_1$ spatial group for crystals of BPR XIII (*a*), III (*b*), and II (*c*). The figure shows 4 neighboring unit cells of the crystal in the projection along the *b* axis (the boundaries of one of the cells are shown in bold), the splitting of a unit cell by the edges of the lattice composed of 30/11 helices, and positions of protein molecules in the lattice cell. The molecules shown in gray are shifted by half of the period parallel to the *b* axis in comparison with those shown in white. The small inserts show the splitting of the unit cell into pseudoperiods along all the three axes.

crystals belong to the monoclinic and rhombic crystal systems with the spatial groups $P2_1$, $C2$, and $P2_12_12_1$ and lattice parameters of ~30 Å to ~80 Å. For some of the forms, these parameter values were split into the pseudoperiods of the 30/11 helices (see the table). The packing arrangement of the BPR molecules in the lattice composed of 30/11 bound-water helices for the $P2_1$ spatial group is shown in Fig. 4.

The tetrahedral PO_4^{3-} - and SO_4^{2-} -type anions stabilize, within the space of the cells, the lattices composed of 30/11 helices and occupied by biomolecules by forming hydrogen bonds with these biomolecules and with the water molecules forming the edges of the cells, i.e., 30/11 helices. The total length of the P–O…H–O bonds is about 5 Å, and that of the whole bridge, e.g., between the NH-group of the biomolecule and the bound-water molecule N–H…O–P–O…H–O, is 7-8 Å.

CONCLUSIONS

Previously, it was shown that 30/11 helices consisting of bound-water molecules are the primary structural element of the hydration shells of biomolecules [11]. This fact, as well as the possibility to express the unit cell dimensions of biocrystals as a sum of the pseudoperiods of the 30/11 bound-water helix, is evidence that biocrystals may have a lattice composed of 30/11 bound-water helices, with the distances between the edges of the lattice being equal to these pseudoperiods. This gives grounds to logically explain the polymorphism of biocrystals, which is associated with these lattices being highly diverse and possibly having large cavities and wide channels. The edges (30/11 helices) of the cells occupied by biomolecules are stabilized either by biomolecules themselves or by small precipitant ions; the presence of larger PEG molecules (or the like) is not required. The stabilization of 30/11 bound-water helices in large cavities or wide channels requires the presence of sufficiently long PEG molecules and is achieved through the incorporation of the latter into the 30/11 helix as one of its two constituent 15/4 helices. Thus, the position of a PEG molecule is fixed inside a large cavity or wide channel and becomes visible on the electron density map. The proportion of structured water in the biocrystal is 30% or less; the other water is in a liquid, disordered state.

In biocrystals, due to a high content of the aqueous solvent, the conformation of the biomolecule does not change much compared to that which was formed in the original solution so as to be consistent with the possible structure of the hydration shell. Thus, the structure of bound water in 30/11 helices plays a dual role in the self-organization of biocrystals. It determines both the conformation of biomolecules in solution and their packing arrangement in the crystal, and the biocrystal structure depends not only on the composition, conformation, and size of the biomolecule, but also on the composition of the mother liquor, because precipitants have a considerable impact on the stabilization of the lattice made of 30/11 helices.

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