Molecular dynamics of DNA-binding protein and its 2D-crystals

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Abstract. In this work the dodecamers and the two-dimensional crystals of DNA-binding protein from starved cells (DPS) of Escherichia coli bacteria were investigated. The DPS monomer contains 167 amino acids residues. It can form dimers, trimers, and dodecamers. The versatility of the DPS protein structure can be used to design nanomaterials with structures and functions not found in living nature. The ability of this protein to self-assemble into complex shapes and structures defined on the nanometer scale can make them highly demanded for various technological applications. It was used all-atom classical molecular dynamics simulation on 0.1 microsecond scale to obtain the spatial and energy characteristics of the proteins and the components of the simulation box. The fluctuation mobility of DPS protein at various temperatures was discussed. The diffusion of ions in the presence of dodecamers and 2D crystals was compared. It has been shown that this protein retains its ability to accumulate ions in a wide range of biological temperatures from 277 to 369K. It also retains the mobility of key amino acid residues involved in the formation of nanocrystals and the transport of ions into the cavity, even at low physiological temperatures.

1. Introduction

Fabrication of biotemplated functional nanostructures is one of the important areas in nanotechnology [1]. The dimensions of biomolecules are nanometers. However, they can self-organize into subcellular structures, form cells and whole organisms, exceeding the size of the molecules themselves by several orders of magnitude. A detailed understanding of the processes of proteins functioning and the peculiarities of their structure made it possible to talk about proteins as natural nanosystems. These nanosystems have great potential in the field of nanomaterials, nanobiointerfaces, and nanomedicine. Understanding how these systems self-assemble, how they function during different assemblies, how structural features and other molecules and ions affect them is critical to the development of new synthetic materials. Each of the proteins has a unique structure. Moreover, the structure is repeated from molecule to molecule. Many of the proteins are capable of forming functional complexes or even crystals. In addition, protein molecules can interact with inorganic substances to form stable structures. One example of a protein that combines all of these characteristics is the DNA-binding protein from starved cells (DPS) [2]. This protein is the member of the ferritin superfamily and exists widely in bacteria and archaea. Thus, it can be easily and quickly produced by nanotechnology. DPS in the nature protects the cell from oxidative stress by inactivating the potentially dangerous Fe^{3+} ions and provides intracellular nucleoid crystallization. This protein is capable of accumulating up to 500 iron ions, which forms a ferrihydrite-like core. It protects the DNA under hazardous conditions. It was shown that its concentration reaches 180000 molecules per bacterial cell under stress. This quantity of
protein is necessary for the cell to ensure biocrystallization: a special mechanism for the stabilization of the nucleoid, in which the nucleoid is packed with proteins into a compact, extremely stable structure. DPS can form of DPS–DNA liquid crystal mesophases that depends strongly on the protein dodecameric structure. In this work, we investigate the dynamics of protein at different temperatures. The migration of ions and the fluctuation subspecies of the protein at different temperatures are considered. The principal component analysis is used to assess the difference in protein dynamics in dodecameral and crystalline forms.

2. Materials and methods

2.1. Molecular structures of dodecamers and monolayer crystal of DPS protein

The crystal structure of Escherichia coli DPS was first obtained in 1998 by X-ray diffraction analysis with a resolution of 1.6 Å [3]. It was shown that the DPS protein forms spherical homododecamers with a cavity inside, similar to ferritin. The location of the subunits is such that the protein belongs to the tritetrahedral point symmetry group (the 23rd class, according to international classification). Later it was shown that the DPS protein can exist in the form of a monomer, dimer, trimer, or dodecamer (Fig. 1). The most interesting is the dodecamer form of the protein. In this form, the DPS is spherical with a cavity inside. The outer diameter of the sphere is 9 nm, the inner diameter of the cavity is 5 nm. DPS dodecamers can accumulate ions. Also, this form is capable of forming various crystal structures in the living cell and in vitro. The dodecamer of the DPS protein can be considered as a cube whose faces are formed by two subunits, between which there are two ferroxidase centers. Each of the centers is divided into two Fe$^{2+}$ binding sites.

We obtained the molecular configuration of DPS monolayer from the RSCB Protein Database, entry 6gcm [4]. These molecular crystals were solved by our group at the European synchrotron radiation facility (ESRF). This is an x-ray structure of the DPS protein resolved at an average RMS of 2.45 Å, reconstituted from plasmid expressed in E. coli. Up to 13 N-terminal residues in each chain were unidentified in this experimental structure since they are disordered. These residues play an important role in DPS-DPS and DPS-DNA binding [5-7]. Therefore, they were added to the model using UCSF Chimera package [8].

![Figure 1. Structure of the DPS protein. From left to right: monomer, dimer, trimer, dodecamer (Fe-pore view), dodecamer (side view). Each subunit is coloured with its own colour. C and N letters correspond to C-terminus and N-terminus.](image)

2.2. Molecular dynamics protocol

The systems were prepared for calculation in the Gromacs 2018 program [9]. The DPS dodecamer was placed in a cubic unit cell with an edge 14.7077 nm in length. DPS 2D-crystals consisted of 4 DPS dodecamers placed in the center of box modelling infinite single layer two-dimensional crystal. The box dimensions were 14.7 nm * 17.7 nm * 20.0 nm. To neutralize the charge of the system, 48 Na$^+$ ions were added to the cell as counter ions for each DPS dodecamer. In addition, other q$^+$, q$^-$ and q$^{2+}$ ions were placed to the box at low and physiological concentrations. To solvate the systems, the SPC/E water model was used. The calculations were carried out in the AMBER99-PARMBSIC1 force field [10]. The energy minimization of the systems containing the DPS protein (due to its complex structure with an internal cavity) cannot be carried out with sufficient accuracy by the conjugate gradient method. Therefore, the fastest descent algorithm was used in all the systems. Then, sequential
relaxation of the systems was carried out under conditions of constant volume and pressure of 100 ps duration. After relaxation, the dynamics of the systems was calculated. The calculations were carried out in an NPT ensemble. The constant temperature was maintained using a stochastic thermostat, the constant pressure was maintained using a Parrinello–Raman thermostat in isotropic (for the system of DPS dodecamers) or semi-isotropic manner (for DPS 2D-crystals), a time constant was 2 ps. Fast degrees of freedom were limited using the LINCS algorithm. The cut-off radii for the Coulomb and van der Waals interactions were taken as equal to 1.5 nm. The DPS dodecamer systems were calculated at temperatures from 277 to 369 K with a step of 4 K. The temperature of the 2D-crystal system was 300 K. The integration step was 2 fs, and the trajectory calculation time was 0.1 μs.

3. Results and discussion

The system of the DPS protein and its two-dimensional crystals is relatively large and requires trajectories of the order of 0.1 μs. Internal relaxation of systems, for which the parameters of the calculation box and the dynamic characteristics of protein molecules are balanced, occur at times up to 0.05 μs. We obtained molecular dynamics trajectories for dodecamers of the DPS protein at temperatures from 277 to 369 K and for two-dimensional crystals of this protein at 300 K. The Root mean square fluctuation (1) is shown as a bar plot of residue numbers on Fig. 2. The amino acid residues responsible for DPS binding for the formation of DPS crystals and co-crystals are active even at temperatures close to 0°C (yellow dots, 0-12 residues). This means that DPS crystals must maintain their stability over a wide temperature range. RMSF of several amino acid residues remain high. It is Arg70 in the inner cavity of the DPS, which is responsible for the coordination of ions. Lys105, which apparently stabilizes DPS molecules in crystals. And Lys140, which directs ions into the cavity.

\[
RMSF = \left( \frac{1}{T} \sum_{i=1}^{T} \left( r_i(t_j) - r_{i,\text{ref}} \right)^2 \right)^{1/2}
\]  

where T is the duration of the simulation (every time step), \( r_i(t_j) \) the coordinates of atom \( r_i \) at time \( t_j \) and \( r_{i,\text{ref}} \) is reference position of atom \( r_i \).

One of the capacious characteristics of biological polymers is the radius of gyration (2). For DPS, it increases slightly with increasing temperature (Fig. 3). The maximum values of the radius of gyration are achieved at 325K and 369K.

\[
R_g = \left( \frac{\sum_i ||r_i||^2 m_i}{\sum_i m_i} \right)^{1/2}
\]

where \( m_i \) is the mass of atom \( i \) and \( r_i \) the position of atom \( i \) with respect to the center of mass of the molecule.

**Figure 2.** RMSF per residue of DPS monomers in DPS dodecamers at different temperatures. The legend shows the temperatures of simulation in K.

**Figure 3.** Radii of gyration for DPS protein at different temperatures.
Fig. 4 shows the self-diffusion coefficients of ions near DPS dodecamers calculated from the Einstein relation (3) based on the linear parts of the Mean square displacement (MSD) plots. Ion diffusion coefficient is small compared to the value for an aqueous solution [11-13], and practically does not change with increasing temperature.

\[
\lim_{t \to \infty} \langle \| r_i(t) - r_i(0) \|^2 \rangle = 6Dt
\]

where \( r_i(t) \) the coordinates of atom \( r_i \) at time \( t \) and \( r_i(0) \) is the initial coordinate. For molecules consisting of more than one atom, \( r_i \) was taken as the center of mass positions of the molecules.

**Figure 4.** Calculated self-diffusion coefficients for water (grey circles), Na\(^+\) ion (orange circles), Ca\(^{2+}\) ion (green circles) compared with experimental self-diffusion coefficient of water (blue diamonds) and inter-diffusion coefficient of NaCl-Water (orange diamonds). Red line with red diamonds corresponds to the DPS protein.

Bivalent ions have the lowest diffusion coefficient near two-dimensional DPS crystals, since they have a high affinity for the DPS surface. Monovalent positive ions accumulate in the DPS cavity, so their average diffusion coefficients are low. In this case, the diffusion coefficients of monovalent ions vary from 0 (for ions bound to the surface of the DPS) to 2.5 \( \times 10^{-9} \) m\(^2\)/s, which corresponds to the diffusion of ions in aqueous solutions.

**Table 1.** Self-diffusion coefficients of ions near 2D-crystals of DPS and lateral diffusion coefficients in the plane of 2D-crystal (10\(^{-9}\) m\(^2\)/s) at 300K.

|        | Na\(^+\) | K\(^+\) | Ca\(^{2+}\) | Cl\(^-\) |
|--------|----------|---------|-------------|---------|
| diffusion | 0.50±0.04 | 0.46±0.07 | 0.24±0.15 | 0.81±0.19 |
| lateral diffusion | 0.63±0.06 | 0.65±0.04 | 0.34±0.23 | 1.10±0.25 |

DPS molecules inside crystals are packed very tightly. Crystals are formed in such a way that they provide the distribution of DPS molecules over only two angles of rotation. In this case, the rotational autocorrelation functions reveal an extremely high degree of correlation (more than 0.99) for DPS molecules both in space and relative to the normal axis to the 2D crystal. We observed a slowdown of protein rotational diffusion upon crowding conditions in 2D-crystals. In our previous studies, it was found that DPS molecules accumulate not only ions, but also small molecules (for example, 4-hexylresorcinol), as well as oligonucleotides. This property of DPS molecules with a strict orientation
of DPS molecules inside the crystal can make it possible to create various ordered structures in the composition of co-crystals.

**Conclusions**

The results obtained show that DPS molecules are capable of accumulating various cations, ensuring their strong binding to the surface of the inner cavity at different temperatures. At the same time, DPS crystals remain active both in the accumulation of ions and in the binding of other molecules even at physiologically low temperatures close to 0°C. This property of DPS molecules can make it possible to create new functional materials in the inner cavity of a protein, as well as to obtain new crystalline materials like DPS – DNA liquid crystal mesophases.

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