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Molecular dynamics of three different α-helices in ribosomal protein L25 from *Escherichia coli*

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ABSTRACT

A true native protein state is realized in a water solution where proteins exhibit their dynamic properties important for the functioning. This is way we have analyzed the dynamics of α-helices inside ribosomal protein L25 from *Escherichia coli* in a water solution. The dynamics of only main chain Cα-atoms have been simulated along the five independent trajectories at a total time 200ns. Superposed average dynamics picture of L25 structure coincides very well with the NMR protein structure in a water solution. Dynamic shifts of Cα-atoms of the α-helices are related with a restraint status of the residue side chain. In contrast, Cα-atoms of the β-sheet, which form a hydrophobic core, show very low dynamic motion and higher stability. Dynamic specificity of the main chain of protein L25 could explain its particular features in the complex with 5S rRNA and in the structure of the ribosome.

1. Introduction

α-Helix is one of the basic elements of the secondary structure of proteins [1]. Its dynamics relates the specific structural features of the protein and suggests to be essential for the function. For example, the contribution of dynamics in the signal transfer of the human tyrosine phosphatase PTP1B from an allosteric inhibitor to the active site of the enzyme was shown [2]. There are a limited number of work devoted to systematic study of dynamics of standard structural motifs in globular proteins, particularly, to the study of α-helical fragment [3]. We should mention here at least one exciting paper devoted directly to the study of collective vibrations for long polypeptide α-helix of 20–80 residues [4]. Here all helix models display collective vibration mode of bending type. However, these finding is not related to the globular protein, where the lengths of helices are shorter and include usually 6–12 residues. In many other cases, the authors studied the stability of dynamic structures at the final period of fluctuation time. We have recently studied the dynamics of Cα-backbone of α-helix in poly-L-glutamic acid and in a protein with only one helix in a water solution [5,6]. It was shown that the shifts of the helical Cα-atoms depend on the restraint status of the helical residues: the larger dynamic shift appears in the residue immersed in a water medium. The structure of ribosomal protein L25 includes three different helices: helix1 is located separately from the rest compact globule, while two other helices 2 and 3 participate in forming hydrophobic core of this protein. Internal side of two helices is fixed by the hydrophobic core while the opposite side is immersed in a water solution. It suggests making a difference in the dynamics of two of these sides of the helices. In other words, dynamics of the helix depends on its structural surroundings inside the protein and that has been also observed in our previous publication [6]. Here we considered ribosomal protein L25 with three helices which are different in size and their location inside of the molecule. In this communication we do not consider inherent stability of any definite α-helix. It is much more complicated because of complex behavior of the helical residues in the life span during time period of observation. In fact, the problem could be solved more accurately with the help of advanced quantum simulation approach [7,8]. The main aim of this work is to study the differences in dynamic behavior of three helices which suggest to be correlated with their structural features. It should be noted that this considered case presents a good example of the helical fragments available in huge amount of the protein presented in the Protein Data Bank.

Abbreviations: MDS, molecular dynamic simulation; NMR, nuclear magnetic resonance.

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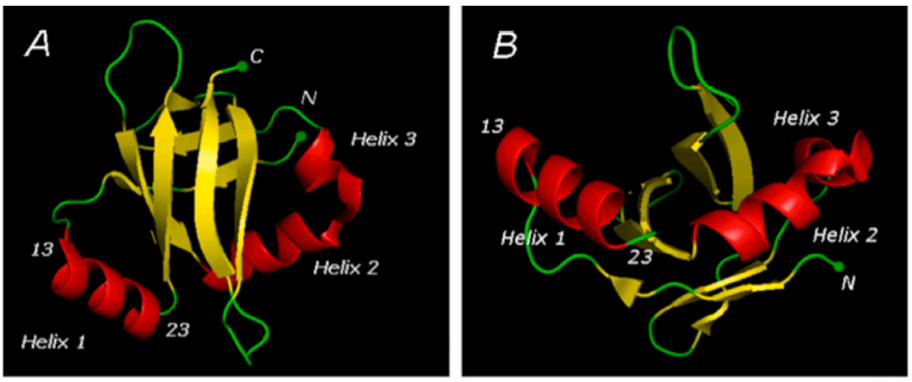


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1          10         20         30         40         50
.          .          .          .          .          .
MFTINAEVRKEQGKGASRRLRAANKFPAIIYGGKEAPLAIELDHDKVMNM
  β1  β2     ----α1-----  ---β3--  -L1-  --β4-  ---α2--

51         60         70         80         90   94
.          .          .          .          .    .
QAKAEFYSEVLTIVVDGKEIKVKAQDVQRHPYKPKLQHIDFVRA
--   -α3-    -β5-   β6   ---β7---  -L2-  ---β8---
```

α1 (13-23), *α2* (44-52), *α3* (55-58) — 11, 9, 4
β1 (3-5), *β2* (7-8), *β3* (25-31), *β4* (38-43) — 3, 2, 7, 6
L1 (33-36), *L2* (81-84) — 4, 4
β5 (62-65), *β6* (68-69), *β7* (72-79), *β8* (86-93) — 4, 2, 8, 8

Fig. 1. *Upper*: Polypeptide Cα-backbone chain of the ribosomal L25 protein from *Escherichia coli* in crystalline state. Projections *A* and *B* are related by horizontal rotation at 90°. *Lower:* Sequence and secondary structure of the ribosomal L25 protein from *Escherichia coli.* Number of residues of the fragment is shown with the bold font.

2. Materials and methods

2.1. Ribosomal protein L25 model

The structure and function of ribosomal protein L25 are of great interest. The structure of L25 protein from *Escherichia coli* in a water solution was determined by NMR-method both in a free state and in a complex with rRNA consisting of the protein and 5S rRNA [9,10]. The crystal structure of complex of L25 protein with E-domain of 5S rRNA was also solved by X-ray method [11]. Later it was shown that two different protein-RNA binding sites are formed by the spatial sign-alternating charge clusters [12,13]. One site is pre-existing in the protein and the other is induced by the RNA molecule in the process of the complex formation. The difference between the L25 protein structures in crystal and in solution is functionally very significant. The protein structure data are: a free state in a water solution (pdb:1b75), the protein from the complex with a fragment of 5S rRNA in a water solution (pdb:1dfk), and the protein from the complex in crystalline state (pdb:1dfu). The protein model for dynamic simulation was built on the X-ray data of L25-rRNA complex. The protein consists of 94 residues. All side chains were completed and the hydrogen atoms were added. Then protein was placed in a water medium with periodic boundary conditions in the rectangular unit cell with 74x62 × 62 Å, which corresponds to a density 1.0 g/cm^3. The model of solution system was adjusted to a neutral pH 6.

2.2. Molecular dynamics simulation

The molecular dynamics simulation was performed similarly to our previous study [6]. Here all the protein atoms were used. The first run of the initial trajectory was performed in time period from 0 to 40ns. The electrostatic Coulomb and van der Waals potentials were controlled. The relaxation process included the refinement of the protein model and the relaxation of the system in the water medium. The relaxation was completed at 1.0ns. Macroscopic quantities such as energy, temperature, and pressure were calculated every 0.1ps, and the trajectory output data were taken every 10ps. A dynamic fluctuation of the protein in the water medium was observed every 1.0ns. To check the reproducibility of the results, we calculated four additional independent trajectories, each during 40ns. All of them started from the end of the first trajectory. Thus, we were able to study the dynamic features over a period of time of 200ns in total.

We have studied the dynamics of only Cα-backbone atoms. The changes of the structure were determined by the Cα-shift diagrams between two selected time points as it has been described in earlier work [6]. Absolute shifts of Cα-atoms are taken from the structures of two time points divided by 1.0 ns. During the dynamic process the protein model is deformed and displaced as a whole. Because of this, the superposition of two structures selected at different times requires place them in the common internal coordinate system determined on bases of the ellipsoid of moments of inertia. In this system the principal axes of both molecules are combining one with other. And its origin is placed in the center of masses of the Cα-atoms. This algorithm was added in the Trajectory Analyzer of Molecular Dynamics TAMD [14–16]. The calculations have been carried out with the help of the program package based on the standard MDS-software PUMA [17–19] and modified PUMA-CUDA [20] which used the parallel programming technologies and compatible with the supercomputer code signs. Force field AMBER [21] was used as well as the program to generate the water medium model TIP3P [22]. All calculations were carried out by the hybrid supercomputer K-60 at the Keldysh Institute of Applied Mathematics, Russian Academy of Sciences using parallel programming technologies.

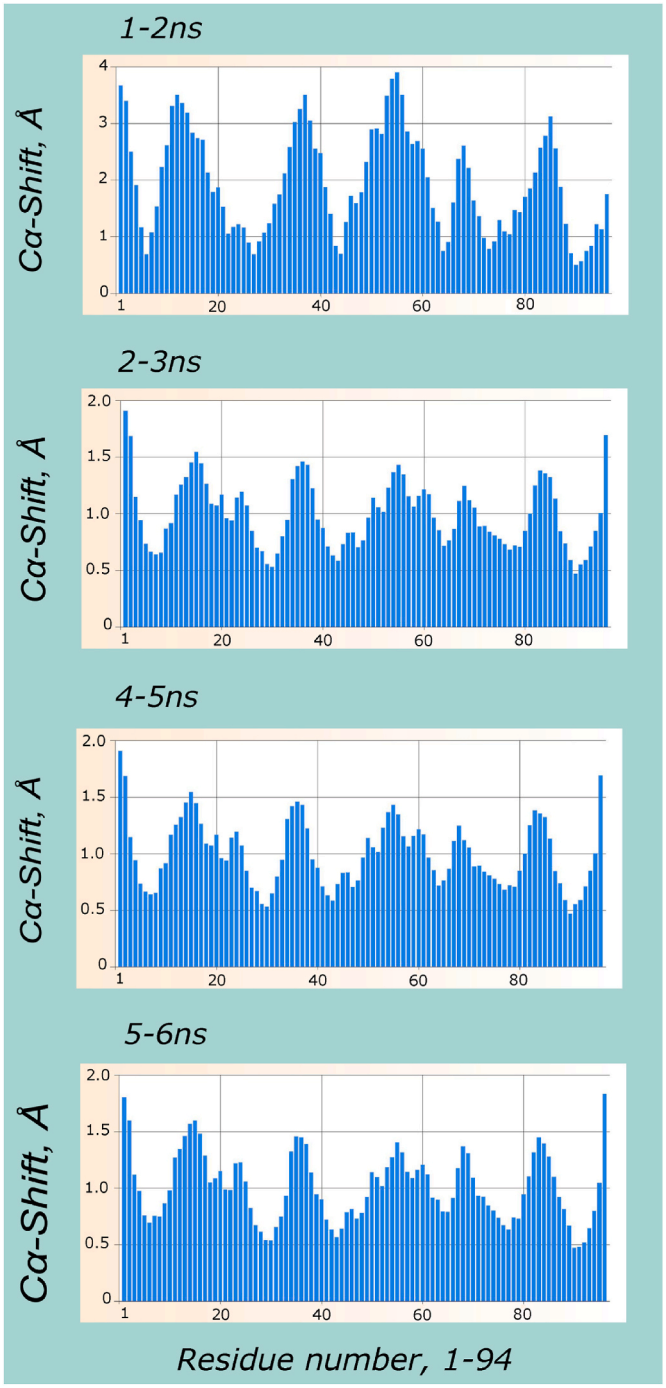

Fig. 2. Examples of dynamic profiles of Cα-shifts of the ribosomal protein L25 at 1–6ns.

3. Results and discussion

3.1. Location of helices inside ribosomal protein L25 from Escherichia coli

The initial model for dynamic study has been built on the bases of X-ray data of the crystalline complex of L25 with a fragment of 5S rRNA (pdb:1dfu). The spatial structure of protein L25, its sequence and secondary structure are presented in Fig. 1. The structure of this protein differs from the classic compact globular protein. The L25 hydrophobic core is surrounded by two twisted β-sheets: β1-β5-β6 and β3-β4-β7-β8. But the helix1 with 11 residues is located outside of the main protein body. Two other helices with 11 and 9 residues are attached to the external side of the sheet cylinder and their ends are faced with a few contacts. This indicates that mobility of these helical backbones differs depending on their positions in the protein molecule.

3.2. Molecular dynamics of ribosomal protein L25 in a water solution

Protein dynamics of polypeptide backbone of L25 in water solution have been analyzed in the period from 1 to 40ns. The Cα-shift diagrams in the whole trajectory shows rather similar dynamic profiles. Examples of four frames of Cα-shift dynamic fluctuations from the first trajectory are presented in Fig. 2. For the fist trajectory main maxima of dynamic Cα-shift are about 1.3 Å with average background value 0.7 Å. During the time period from 1 to 20 ns Cα-shift maxima of helices are decreased as follows: helix1 (from 1.3 to 0.8 Å), helix2 (1.1–0.7 Å), helix3 (1.1–0.9 Å).

Detailed assignment of the maxima on the backbone Cα-shift diagram at 6–7ns is presented in Fig. 3. Here the maximal Cα-shift values, about 1.4 Å, belong to the external loops L1 and L2 of the protein chain. The helixes display high maxima at about 1.2 Å. N-terminal residue of helix1 has maximal Cα-shift 1.3 Å, and the rest residues have lower shifts according their restrained status. Very similar dynamic behavior we can observe for helix2. In contrast, the extended strands β3, β7 and β8 show low values of Cα-shifts about 0.7 Å, as average.

3.3. Fluctuation splashes and restraint status of α-helical residues

Now we observe a common dynamic structure of a protein in a water solution from all five trajectories. Fluctuation of helical Cα-atoms for the first trajectory over the time period of 1–32 ns varies from 1.1 to 0.7 Å. However, at 33 ns the first strong ***fluctuation splash*** in the region of helix1 with maximum about 6.0 Å was observed. Further similar splashes were also observed in four repeated trajectories which have been started after the end of the first trajectory. The total amount of splashes was about 17 % in the full period of observation. The fluctuation splashes suggest appearing due to a Brownian chaotic motion of the water molecules of solution. However, in these fluctuation modes helix1 was displaced *entirely as a whole block*. The dynamic structures of two β-sheets remain stable. Stabilization of helix1 movement is completed at approximately 20ns. However, at the time point of 23ns helix1 is virtually destroyed while helices 2 and 3 remain stable, as seen in Fig. 4. We compared the averaged MDS-structure of L25 with that of NMR-structure [10]. Surprisingly, both dynamic structures are very similar, as seen in Fig. 5, thereby confirming the reliability of the obtained dynamic results.

We have here found that the dynamic behavior of Cα-backbone atoms depends on their restraint status. In other words, the corresponding residue can move easily in case when its side chain immersed in a water solution. And the residue is restricted in the motion when it has ***a restraint status*** being fixed in space due to the interactions with other protein residues, for example, located inside a hydrophobic core. This could explain a different motion of the Cα-helical atoms of L25 during the time as seen in Fig. 4. Analogous dynamic behavior of the helix we have observed in other protein with a single α-helix in the molecule [6].

4. Conclusion

We have presented the dynamic features of the main polypeptide chain of the ribosomal protein L25 in a water solution. Here the novel approach has been used which is based on the analysis of Cα-atom shift diagrams. The dynamic structure of protein shows the significant shifts of Cα-atoms in helix1 and low shifts in helices 2 and 3 of the ribosomal protein L25. The dynamic mobility of β-sheet remains low. The mobility of two external large loops was always high. The important result is that a dynamic MDS-structure of L25 in solution coincides very well with the mobile structure obtained with the NMR-method. However, the MDS-

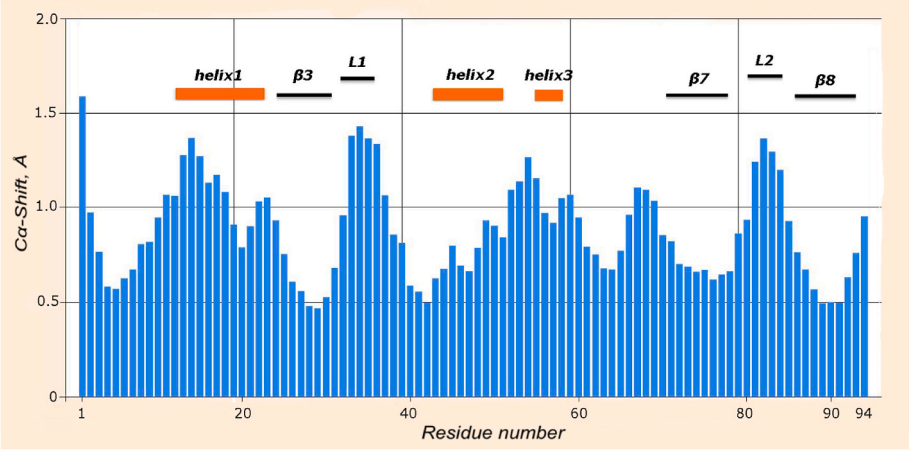

Fig. 3. Dynamic fluctuation of polypeptide backbone of the L25 protein model from *Escherichia coli* at time 6–7ns. Cα-shifts are marked for α-helices with pink color, β-strands and loops - with black strips. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

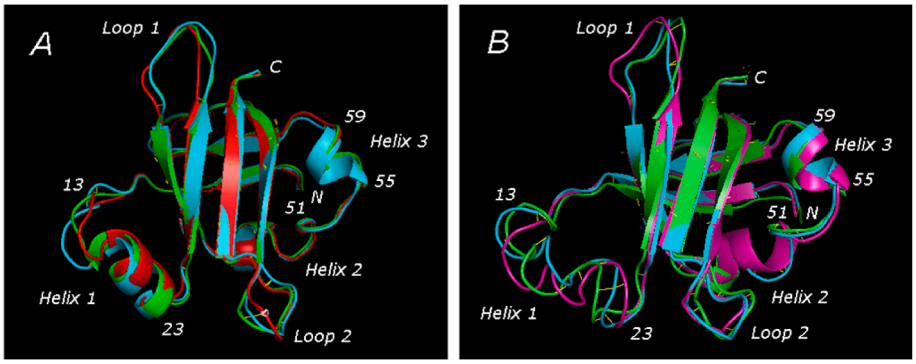

Fig. 4. Dynamic fluctuation of the polypeptide backbone of the L25 protein. *A* - Superposition of the structures at time points 6-7-8ns; *B* - Structures at time points 13-23-33ns. Structures at 8 and 33ns are shown in red, the rest ones - in green colors. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

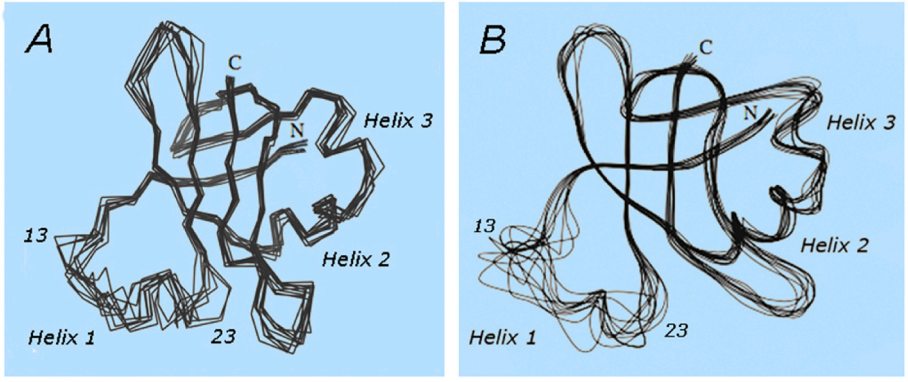

Fig. 5. Integrated pictures of main chain of the protein L25 from *Escherichia coli*. *A*: MDS-structure in water solution, time 21–30ns; *B*: NMR-structure in water solution, calculated with the PDB ID:1d6k. Superposition of ten structures has been used in both cases.

structure shows new dynamic features. For example, we can observe the changes in the structure during the all period of time. We have also found a new phenomenon of fluctuation splashes which relate the dynamic displacement of the large block of the structure during its life span. Thus, the current dynamic study suggests to be very important on the new look on the protein structure at the native condition and gives us a new opportunity to understand the protein function.

Disclosure statement

The authors report no competing interests to declare.

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CRediT authorship contribution statement

Yuri Chirgadze: Writing – original draft, Visualization, Conceptualization. **Ilya Likhachev:** Software, Data curation. **Nikolai Balabaev:** Supervision, Methodology, Formal analysis, Conceptualization. **Evgeniy Brazhnikov:** Visualization, Investigation.

Declaration of competing interest

The authors declare no conflict of interest.

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