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LETTER TO THE EDITOR

Molecular dynamics of α-helix inside a protein with single helix

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1. Introduction

In a crystalline state, the protein molecules often come in contact with each other (Protein Data Bank, Berman et al., 2002). However, the native proteins realize their function mainly in a solution. A molecular dynamic simulation allows us to study in detail the dynamic fluctuation of protein structure in this condition (Rudnev et al., 2021). Today it is clear that the *mobility of protein structure is the basis of their unique functional features*. Recently, the evolutionary conservation of protein dynamics has also been revealed with the help of dynamics simulations of the N-terminal 'peptidase' domain from *Saccharomyces cerevisiae* and three of its homologs (Gaur et al., 2023). Molecular dynamic simulation highlighted conserved motions in all three homologs which are similar to Spt16N protein. This appears to be a very important observation which gives us a new look at the behavior of protein molecules in their native condition. Due to the molecular dynamics, a particular feature of proteins is observed in solution and compared with that in a crystalline state. Thus, the dynamics of standard motifs significantly extend our understanding of the particular feature of the whole protein structure.

There are several studies devoted to the dynamic stability of the protein structures (review of Rudnev et al., 2021), mentioned above the dynamic simulations of three N-terminal 'peptidase' domain homologs (Gaur et al., 2023), and dynamics study of super-secondary αα-corner structure (Rudnev et al., 2021a). We should mention at least one exciting paper devoted directly to the study of collective vibrations for long polypeptide α-helix of 20–80 residues (Zhang et al., 2022). Here all helix models display collective vibration mode of bending type. However, these findings are not related to the globular protein, where the lengths of helices are shorter and include usually 6–12 residues. In many cases, the authors studied the stability of dynamic structures at the final period of fluctuation time. There are very limited attempts to systematical study a variety of standard super-secondary structures inside of proteins. One of the simple standard structures is an α-helical motif. The first studies of that have been devoted to the analysis of the stability of a helical fragment separated from a myoglobin protein molecule by using dynamics simulation. The stability of the 22-residue part of myoglobin, namely a two α-helical fragment, has been studied in a water and in a trifluoroethanol-water solution (Van Buuren & Berendsen, 1993). In a water solution, the fragment of two α-helices quickly unfolds in about 200 ps. This indicates the importance of non-polar interaction of the helical fragment with the hydrophobic core of the protein. This results in an alteration of the helix structure because of the strong influence of the local surroundings of the helix. Recently, we have carried out a dynamic study of the 16 residue fragment of a poly-L-glutamic acid in a helical form in a water solution (Chirgadze et al., 2023). It has been shown that a few intrinsic fluctuation modes, not presented before, appear along the whole dynamic trajectory. Because each helix inside the protein molecule has a unique surrounding, the systematic study of the dynamics of helix fragments is desirable. In this work, we want to find a difference in the dynamics of helical residues immersed in a solution and imbedded in the protein body.

The aim of this work is the dynamic simulation study of the α-helix inside the protein in a solution. Internal side of the helix is fixed by the hydrophobic core while the opposite side is immersed in a water solution (Figure 1). This model suggests making a difference in the dynamics of two of these sides of the helix. The obtained result can be compared with that of the dynamics of helical poly-L-glutamic acid in a water solution (Chirgadze et al., 2023).

2. Method and data

2.1. Search a suitable protein structure

We have studied the protein structure with only one helical fragment. A search has been carried out among the X-ray crystal and NMR solution structures from the Protein Data Bank (Berman et al., 2002) and the PubMed Bank (PubMed Database, 2009) by combining the key words "crystal, dynamic, protein, helix" and others. In addition, we have used the database of protein structural trees (Gordeev et al., 2010). Total of 72 protein structures have been found and inspected. Most of them cannot be suitable as a satisfactory model. For example, most proteins exist as oligomers, and

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we should consider the large structure to be more accurate. In other cases, the selected helical fragment was fixed by S-S bonds or bound with metal ions. Finally, for the first study the DNA-binding protein TTHA1013 from *T. thermophilus* has been chosen as more suitable for our study (Mizohata et al., 2005; Hattori et al., 2005; PDB ID 1wv8). The protein model for dynamic "experiment" includes polypeptide chain with residues from 2 to 66 residues. All residue side chains have been completed and the hydrogen atoms have been added to the structure. Then protein was embedded in a water medium with periodic boundary conditions. The dimensions of the rectangular unit cell were $74 \times 62 \times 62$Å which corresponds to a density 1.0 g/cm^3. The system was then adjusted to be neutral with pH 6. The crystal structure of a protein is shown in Figure 2.

2.2. Molecular dynamics simulation

The molecular dynamics simulation was carried out as follows. The first run of the initial trajectory has been performed from 0 to 37 ns. We have controlled the electrostatic Coulomb and Van der Waals potentials. The relaxation process includes the refinement of the protein model and dynamic relaxation of the whole system in the water medium. The relaxation was completed at 1.0 ns. Recording the macroscopic quantities, such as energy, temperature, and pressure, has been done every 0.1ps, and the output data for trajectory have been taken every 10 ps. We can observe a dynamic fluctuation of the protein in water medium starting from 1.0 ns. To check the reproducibility of the fluctuation process, we have calculated for four other prolonged trajectories, each during 25 ns. All of them started from the end of the first trajectory at 37 ns. Thus, we can study the dynamic feature of the helix inside the protein for a total of 137 ns. In a globular protein, the dynamic movements have mainly affected the atoms of surface side chains. However, here we are studying the dynamics of only Cα-atoms of the helix backbone. For analysis of conformational changes we have used the original method of *time dependence of Cα-shifts* between two sequential pairs of frames (Chirgadze et al., 2022, 2023). An absolute shifts of Cα-atom positions have been taken from two neighbor dynamic structures at two sequential time points divided by 1.0 ns. In the dynamic simulation all the atoms have been used. During the process, the protein model is deformed and displaced. Thus, the *correct superposition of two structures* selected at different times requires putting them on the common internal coordinate system determined from the tensor of moments of inertia. In this system the principal axes of both molecules are obtained by calculating the ellipsoid of inertia, and its origin is placed in the center of masses of the Cα-atoms. This critical procedure needs to be applied for every two sequential points of trajectory for correct comparison. The same procedure for the alignment of two models was also used as implemented in the PyMol program (DeLano, 2000). We have

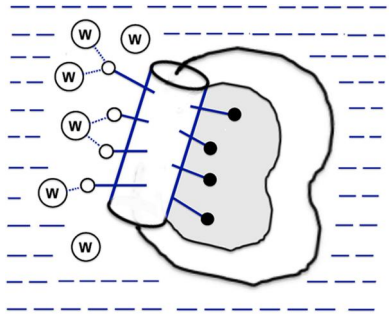

Figure 1. Globular protein with one α-helix in a water medium. Side groups of the residues, fixed by the hydrophobic core, are shown as black spheres. Side groups bound with the waters are shown as white spheres. Water molecules are marked with the letter W.

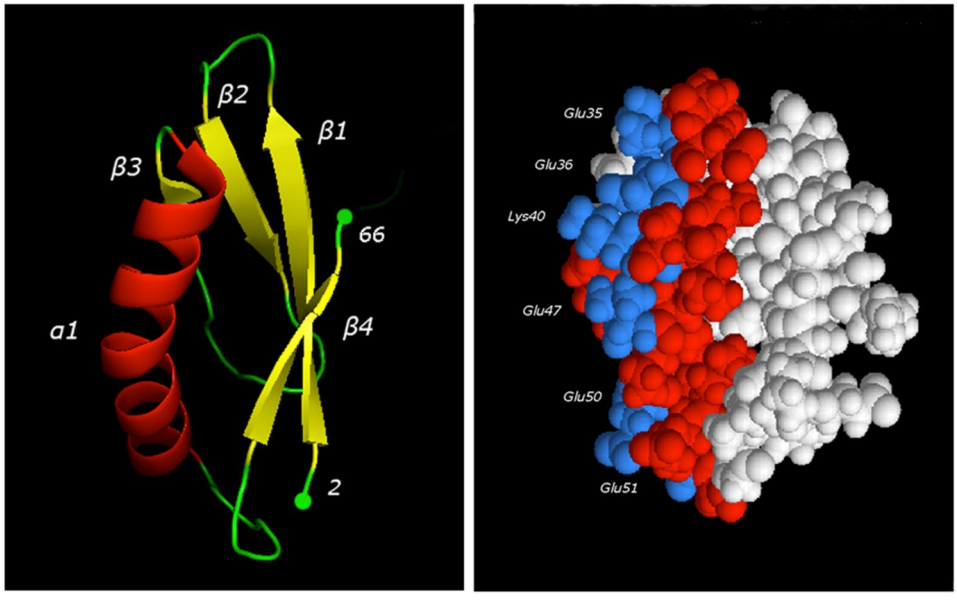

Figure 2. The protein structure TTHA1013 from *Thermus thermophilus* selected for dynamics study. *Left:* Polypeptide backbone chain. *Right*: Space-filling ball model. Helix is painted in red color; charged external surface residues of the helix are marked in blue.

controlled the alterations in the protein structure by checking the Cα-Cα distances inside the backbone of a single chain, which in standard α-helix is 3.80 Å. At time point 7 ns the average Cα-Cα distance was equal to 3.8 Å for the whole peptide chain and 3.6 Å for the helical region, respectively. We also evaluated the angles of internal rotations ϕ and ψ on the peptide map by Ramachandran. The 87.5% residues, from the total 66 residues, are in the allowed regions. The helical region shows 67% residues located in the allowed region. Thus, we can conclude that geometry of α-helix is not drastically changed at the dynamic fluctuations.

The calculations have been carried out with the help of the programs based on the MDS standard software PUMA (Lemak & Balabaev, 1995, 1996) and modified by us PUMA-CUDA which is compatible with supercomputer code signs. Force field AMBER (Wang et al., 2000) was used as well as the program to generate the water medium model TIP3P (Mahoney & Jorgensen, 2000). The resulting trajectories of molecular dynamics were investigated by the Trajectory Analyzer of Molecular Dynamics TAMD (Likhachev et al., 2016; Likhachev & Balabaev, 2007, 2009).

3. Results and discussion

3.1. Molecular dynamics of protein TTHA1013 in a water solution

The considered protein suggested to be a simple representative of large world of globular proteins. The protein is relatively small in size. Its polypeptide fold has simple configuration which includes one bent α-helix and one twisted β-sheet surrounding a hydrophobic core. Rather long helix consists of residues 34–52 as follows:

34 40 47 52

L**EE**LLA**K**LAVMVP**E**LL**EEN**

The external side of the helix contains seven polar residues: 35Glu, 36Glu, 40Lys, 47Glu, 50Glu, 51Glu, and 52Asn marked here in bold letters and shown in Figure 2. Helix includes 19 residues in 5.3 turns. Side chains of polar residues are bound with the mobile water molecules of the solution.

Protein dynamics have been analyzed from 1 to 37 ns. As an example, the first six frames of all Cα-shifts are presented for time periods from 1 to 6 ns in Figure 3. We can see the complicated diagrams of dynamic fluctuations. A sequence of α-helix includes the residues 34–52. While the strands of

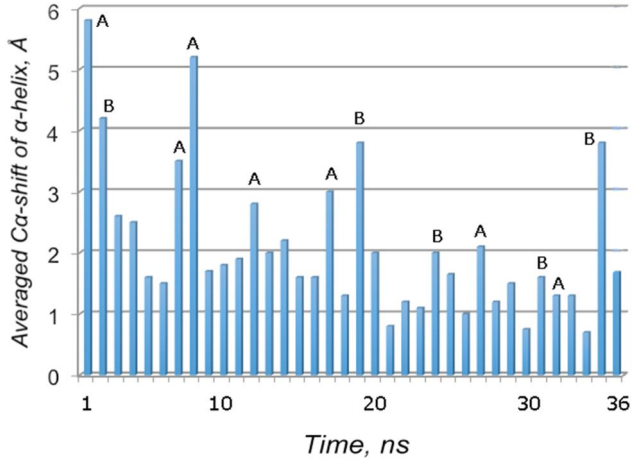

Figure 4. Distinctive fluctuation modes observed as averaged values of Cα-shifts of α-helix, in Å. Averaged shift peaks are marked where fluctuation modes A or B appear.

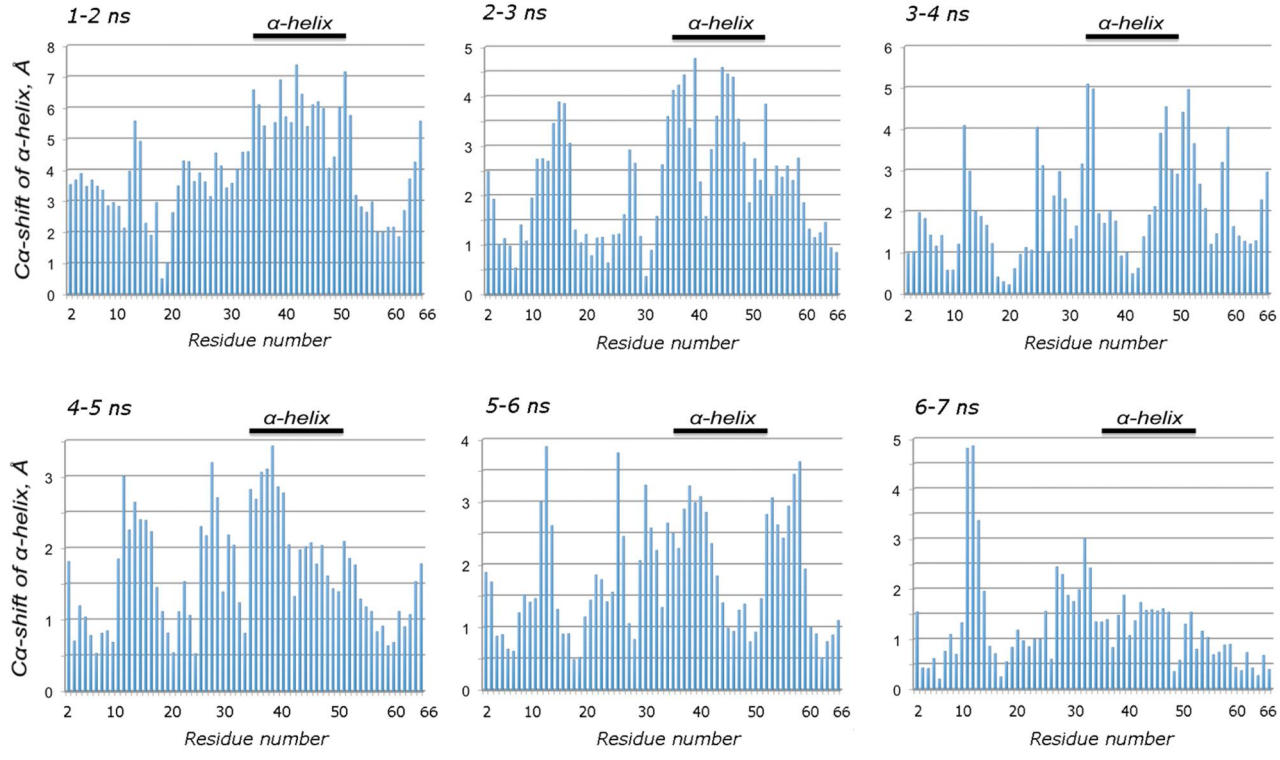

Figure 3. Absolute values of Cα-shifts in protein TTHA1013 for time period 1–6 ns. Sequence related to the α-helix, residues 34–52, is marked with black lines.

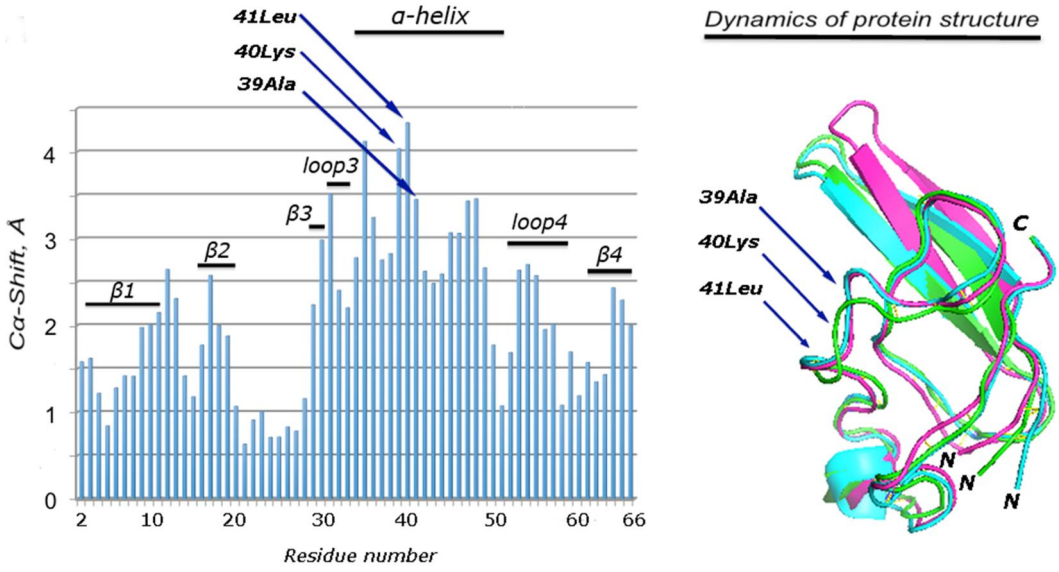

Figure 5. Distinctive dynamic fluctuation mode A of α-helix. *Left:* Mode A, time pair 7–8 ns. Large Cα-shifts directly correspond to the shifts of a group of adjacent residues located at the external site of the protein. *Right:* Three dynamic structures of the protein model at time points 2, 8 and 13 ns. Large Cα-deviation, about 4 Å, corresponds to the Cα-shifts around Cα-Lys40 at the external site of the protein molecule. Arrows show dynamic Cα-shifts and displacements of Cα-atoms.

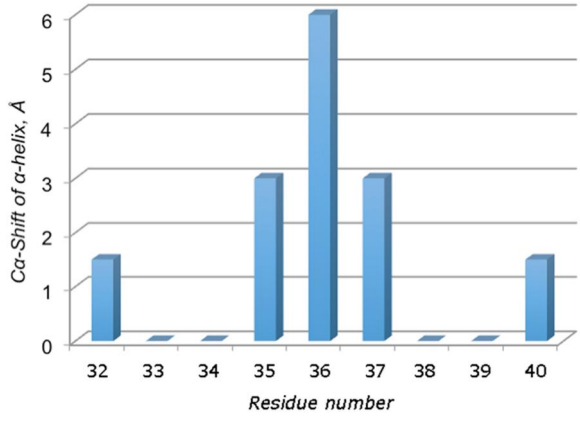

Figure 6. Appearance of different modes of Cα-helix fluctuation for the helical residue 36. Additional shift peaks appear due to interactions of adjacent atoms along the protein chain and along the hydrogen bonds in the different turns.

β-sheet are located in different parts of the protein sequence, as follows: β1 (3–11), β2 (16–21), β3 (30–31), and β4 (61–65). The main feature of any distinctive fluctuation mode is a rather large increase of Cα-shift values. The latter is directly transferred into the displacements of Cα-atoms. It may be related to a secondary structure, a more flexible connecting loops, and a group of N- or C-terminal ends of the chain. We have put more attention here to dynamics of α-helix structure. For the studied protein, a few prominent fluctuation modes have been observed. In order to overview of all helical fluctuation modes we have used the averaged values of all Cα-shifts of α-helix along the whole trajectory (Figure 4). We can see about ten peaks of averaged helix Cα-shifts which appear after time periods from 1 to 36 ns. The fluctuation maxima are decreased along the trajectory from about 5.0 to 1.3 Å with a corresponding decrease of the fluctuation noise values from 1.6 to 0.6 Å (Figure 4). Similar behavior was observed in four other repeated trajectories, total of about 100 ns.

Strong fluctuation modes of the helix appear just after the relaxation of the protein model was finished. For example, two distinctive fluctuation modes are seen in time pairs 1–2 and 2–3 ns in Figure 3. In general, system with the N point masses has 3 N-6 independent movements. Thus, our helix with 19 Cα-atoms has 51 such modes of movement. Most of them seem not to be easily detected due to distribution along the chain of the helix. We have clearly observed a few strong fluctuation modes. First distinctive fluctuation mode of type A presents the large Cα-shifts of all external residues of the helix which are buried in water. This mode is presented in Figure 5. Large Cα-shifts directly correspond to the group of adjacent residues located at the external site of protein molecule contacting with a solution. Second mode of type B, which includes an excitement of some parts of the helix chain. In the modes of types B we have faced to several types of the modes where an Cα-shifts cover different parts of helix: one of the ends, both ends, or parts of the helical region.

3.2. Appearance of other modes of Cα-helix fluctuation

We have found the dynamic fluctuation modes of α-helix inside protein which constantly appear along the time of observation. We describe this phenomenon in more details paying an attention to the fine structure of helix Cα-shifts. The point is the excitement of closest Cα-atoms which appear due to interaction of adjacent atoms along the chain and along the hydrogen bonds in the different turns. Approximate estimates of interactions can be made from comparison of energies of corresponding bonds: nearly 20 kcal/mol for the chemical bonds and 3 kcal/mol for the hydrogen bonds. A tentative diagram of multiple Cα-shifts around the atom with residue number 36 is presented in Figure 6. It clarifies the fine dynamic structure of Cα-shift diagram in the region of α-helix.

Thus, helix backbone fluctuation inside the protein is determined by two rules: Cα-atoms must be free for movement in a water solution, and protein dynamics generate the several shifts of adjacent atoms. An other note concerns the terminal ends of the helix which are connected with the flexible loops 3 and 4 (Figure 5). This explains excitement of the helical ends in the fluctuation of B-modes as seen, for example, in Figure 5 right. Finally, it should be noted that fluctuation depends on the length of a helix (Zhang et al., 2022).

4. Conclusion

We have studied the dynamics of α-helix backbone inside a simple globular protein in a water medium. A single helical fragment displays a few distinctive fluctuation modes. These modes include primarily the dynamic displacements of the helical Cα-atoms which residues immersed in water. We can describe the fluctuation of the helix on the basis of the very simple protein model. Our results confirm that fluctuation modes A and B display a dynamic Cα-movement of external helical residues surrounded by a water medium. Detailed analysis of helical Cα-shifts shows dynamic involvement in the fluctuation the nearest Cα-atoms, both along the chain and through the hydrogen bonds. These shifts are determined by the interactions between adjacent Cα-atoms. At present, we can conclude that fluctuations of the helix backbone inside the protein are determined by two conditions: Cα-atoms must be free for movement, and each shifted Cα-atom gives rise to several shifts of adjacent atoms. A current observation of dynamics for the helical structure inside the protein allows further study of complex super-secondary structural motifs in the proteins, evolutionary conservation of protein dynamics, alteration of protein structure in the catalytic processes, and others.

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