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Structural, dynamical and functional aspects of the inner motions in the blue copper protein azurin

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Abstract

Molecular dynamics was applied to dissect out the internal motions of azurin, a copper protein performing electron transfer. Simulations of 16.5 ns were analyzed in search of coordinated displacements of amino acid residues that are important for the protein function. A region with high conformational instability was found in the 'southern' end of the molecule, far away from the copper site and the binding sites for the redox partners of azurin. By excluding the 'southern' region from the subsequent analysis, correlated motions were identified in the hydrophobic patch that surrounds the protein active site. The simulation results are in excellent agreement with recent NMR data on azurin in solution [A. V. Zhuravleva, D. M. Korzhnev, E. Kupce, A. S. Arseniev, M. Billeter, V. Y. Orekhov, Gated electron transfers and electron pathways in azurin: a NMR dynamic study at multiple fields and temperatures, J. Mol. Biol. 342 (2004) 1599–1611] and suggest a rationale for cooperative displacements of protein residues that are thought to be critical for the electron transfer process. A number of other structural and dynamic features of azurin are discussed in the context of the blue copper protein family and an explanation is proposed to account for the variability/conservation of some regions in the cupredoxins.

Keywords: Azurin; Blue copper proteins; Molecular dynamics; Essential dynamics; Cooperative motions

1. Introduction

Azurin is a small (N=128 residues) copper protein, acting as an electron transfer shuttle in *Pseudomonas aeruginosa* and other bacteria. The presence of the copper ion gives this protein a number of features, including an intense blue color, a high reduction potential and a small parallel hyperfine coupling in the electron spin resonance spectrum [1]. In addition, azurin is fairly resistant to thermal denaturation: the temperature of melting is T_m=86 °C and the conformation stability at 20 °C and pH 7 is ΔG=60 kJ·mol^{-1} [2,3], both values unusually high for a (non-thermophilic) protein of this size. The robustness of azurin is likely related to the presence of the copper ion and necessary to perform its biological role [4]. In fact, the folding of azurin is common with the other proteins of the same family, the cupredoxins, and consists in a two-sheet β-sandwich with eight β-strands arranged in a Greek-key topology. The high β-sheet content provides stiffness for large protein regions and ensures that the coordination of the copper ion in the active site is similar in the reduced and oxidized state, in between the distorted tetrahedral geometry preferred by Cu(I) and the tetragonal geometry preferred by Cu(II). Thus, no significant conformational changes accompany the redox reaction, leading to a small reorganization energy and a consequently high rate of electron transfer.

Because of their peculiar spectroscopic and structural properties, azurin and other blue copper proteins have been intensively studied in the last two decades by using a number of experimental techniques [5], as well as employing computational methods [6]. In particular, Molecular Dynamics (MD) simulation has been used as a tool to investigate the dynamics of azurin with the aim of unraveling the microscopic details of its function [7,8]. Anharmonic inner motions involving single residues that may be important in the electron transfer process were suggested [7]. The basic features of the coordination of the copper ion with the protein matrix, as the latter undergoes large scale motions in the presence of the solvent, were clarified [9].

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Moreover, the comparison of the MD results obtained for azurin and plastocyanin [8] strongly suggests that similarities in the structure, dynamics and function are ultimately related within the entire blue copper protein family.

In this work, the inner motions of azurin are dissected out in connection with the conditions assessing their equilibration. The results show that a few nanoseconds of simulations are sufficient to determine fluctuations of the protein backbone, but not global collective motions. Convergence of sampling for coordinate displacements is obtained by identifying and excluding from the subsequent analysis a protein region with high conformational instability. This region is small and poorly structured and constitutes the 'southern' end of the protein [1], remote from the active site and the binding sites for the redox partners of azurin. After excluding the 'southern' end, the analysis of the inner motions in the 'northern' region around the active site suggests a rationale for the collective mobility evidenced in recent NMR experiments [10,11] that is believed to contribute to the efficiency of the electron transfer process in azurin. Furthermore, from a structural point of view, an explanation is proposed to account for two seemingly-conflicting features of the blue copper protein family: (a) high variability in the relative position of the two β-sheets and (b) strong conservation for the region that is part of the two sheets and extends from the active site to the hydrophobic core.

2. Computational methods

2.1. Molecular dynamics

MD was performed using the GROMACS package [12] with the GROMOS force field 43A1 [13]. The crystal structure of *P. aeruginosa* azurin [14], 4AZU entry in the Protein Data Bank, was used as the starting structure. Only one of the four crystal monomers was considered, together with 80 related crystallization water molecules. Protonation state of the protein residues was adapted to mimic neutral pH. Active site was modeled as previously described [9] using a set of atomic partial charges that takes into account the presence of the Cu ion and employing constraints to fix the bond lengths between the metal and its five native ligands. The protein, together with crystallization waters, was solvated in a rhombic dodecahedron with a nearest image distance of 0.8 nm. The total number of water molecules was 5441 and the simple point charge (SPC) model was used [15]. Periodic boundary conditions were applied.

Residual strain in the system was relieved by 80 steps of steepest descent minimization, using a force constant of 10^3 kJ mol^{-1} nm^{-2} to restrain the position of the protein atoms. Simulations were performed in the NPT ensemble using a time step of 2 fs for integrating the equations of motion. A Berendsen thermostat and barostat were applied [16] with reference temperature of 300 K and pressure of 10^5 Pa and with coupling times of 0.1 and 1 ps, respectively. A twin range cutoff of 0.8 and 1.4 nm was used for nonbonded interactions, updating the neighbour pair list every 5 steps. The SHAKE algorithm [17] was used to constrain the bond lengths of protein and water molecules. Initial atomic velocities were assigned from a Maxwellian distribution at the starting temperature of 250 K. Simulated annealing was performed for 50 ps to gradually increase the temperature up to 300 K. Trajectory data were saved every 0.2 ps during the production run, performed for a total time of 16.5 ns. In addition, three control runs of the same length were performed under slightly different conditions of simulation, by removing the constraint between the Cu ion and, respectively, the His46, His117 or Met121 ligand residues, as previously described [9].

2.2. Data analysis

Correlated atomic motions can be estimated in a straightforward way by using dynamical cross correlations (DCC) [18]. DCC between the position $r_i(t)$ and $r_j(t)$ of the ith and jth atom, respectively, is calculated as:

$$\gamma_{ij} = \frac{\langle r_i r_j\rangle - \langle r_i\rangle\langle r_j\rangle}{[(\langle r_i^2\rangle - \langle r_i\rangle^2)(\langle r_j^2\rangle - \langle r_j\rangle^2)]^{1/2}} \quad (1)$$

where the brackets <..> represent the time average over the simulation trajectory. Correlation can be computed for each protein atom with any other; in our case, only C^α atoms were considered. Thus, DCC directly indicate whether two residues move in the same or opposite directions, though they are not sensitive to perpendicular displacements and to the magnitude of the motion.

In addition, the essential dynamics (ED) technique [19] was also employed. The covariance matrix is calculated from a high-dimensional set of coordinates $x(t)$ of a number of atoms whose collective position determines the protein structure (in our case, the $N = 128$ C^α atoms of azurin were considered). This allows to represent each structure in the simulation ensemble as a point in a $3N$-dimensional space, the conformation space. The covariance of coordinate i and j is defined as:

$$C_{ij} = \langle (x_i - \langle x_i\rangle)(x_j - \langle x_j\rangle)\rangle \quad (2)$$

where the brackets <..> indicate the average over the data points in the trajectory. The corresponding, symmetric matrix **C** can be diagonalized with an orthonormal transformation matrix **R**:

$$\mathbf{C} = \mathbf{R}\ \mathit{\Lambda}\ \mathbf{R}^T \quad (3)$$

where the superscript T denotes the transposed matrix. The matrix **R** contains as columns the eigenvectors, i.e. the principal components, whereas the resulting diagonal matrix **Λ** contains the corresponding eigenvalues, i.e. the mean square positional fluctuations. The eigenvectors can be sorted to decreasing eigenvalues, so that the first principal components correspond to the less constrained degrees of freedom of the protein. The analysis was performed after least squares fitting on the protein C^α atoms of a reference structure to remove the overall molecule rotation. Convergence of the sampled space can be measured in terms of overlap of the fluctuations. The covariance matrix **C**, relative to a given time interval, can be compared with the covariance matrix, indicated as **M**, relative to a different