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ACCELERATED PUBLICATION

Investigation of Formate Transport through the Substrate Channel of Formate Dehydrogenase by Steered Molecular Dynamics Simulations

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Abstract—Steered molecular dynamics simulation has revealed the mechanism of formate transport via the substrate channel of formate dehydrogenase. It is shown that the structural organization of the channel promotes the transport of formate anion in spite of the fact that the channel is too narrow even for such a small molecule. The conformational mobility of Arg284 residue, one of the residues forming the wall of the substrate channel, provides for the binding and delivery of formate to the active site.

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NAD^+-dependent formate dehydrogenases (FDH; EC 1.2.1.2) catalyze the oxidation of formate to carbon dioxide with concomitant reduction of NAD^+ to NADH and are used in industry for the regeneration of cofactors [1, 2]. The crystallographic structures of the apo and the holo form of FDH from *Pseudomonas* sp. 101 have shown that the active site formed by the Arg284, Ans146, Ile122, and His332 residues is deeply buried inside the protein globule and is accessible to the substrate either through the NAD^+ binding site or through the substrate channel [3-6]. The presence of the substrate channel gives an explanation for the random mechanism of substrate and coenzyme binding to prokaryotic FDHs [7]: after NAD^+ has bound, formate still could get to the active site via the channel. The substrate channel opens into the bulk solvent through the outer neck formed by Lys286, Leu257, and Tyr102 residues. The inner neck of the channel serving as the entrance to the active site is formed by Arg284, His332, and Pro97 residues. While the NAD^+ binding site is a wide pocket on the protein surface, the substrate channel is extremely narrow, which could cause difficulties for free diffusion of formate to some extent. To study the mechanism of the transport through the substrate channel, we have carried out molecular dynamics modeling of FDH-formate association. Since the entry of substrate into the enzyme active site is attended with the crossing of appreciable energy barriers, the modeling of this process is hardly probable on the time scale of traditional molecular dynamics simulations. For the purpose of speeding up the transport, a steered molecular dynamics approach was used, when an external force is applied to a ligand to move it in the required direction [8-10].

METHODS OF INVESTIGATION

The preparation of the starting system and the trajectory analysis were performed using the AmberTools 1.2 program package; the energy minimization and molecular dynamics simulations were performed using the Amber 10 package (http://ambermd.org) with the *ff99SB* force field [11, 12]. Quantum mechanical calculations for formate parameterization were carried out with the

Abbreviations: FDH, formate dehydrogenase.

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PC GAMESS/Firefly program (Granovsky, A. A., http://classic.chem.msu.su/gran/gamess/index.html), and the formate partial atomic charges were fitted using the RESP program [13]. Molecular docking was performed with the Lead Finder 1.1.12 program [14]. Parallel computations of molecular dynamics trajectories were carried out using the SKIF-MGU "Chebyshev" supercomputer.

NAD^+ force field parameters were taken from the Amber parameter database [15, 16]. Formate molecule parameters were evaluated in this work. The equilibrium bond lengths and angle values were derived from geometry optimization at the MP2/6-31G* level of theory. The molecular electrostatic potential was calculated at the HF/6-31G* level of theory, and then RESP charges were fitted. The van der Waals parameters and force constants were taken from the *GAFF* force field [17] that is compatible with *ff99SB*.

In the modeling of FDH-coenzyme complex, PDB structure 2nac of the apoenzyme was used [3]. The NAD^+ coordinates were transferred from 2nad structure (ternary complex with coenzyme and inhibitor) superimposed onto 2nac by fitting the coenzyme-binding domains. Hydrogen atoms were added to the protein structure considering ionization of amino acid residues, and then it was solvated by a 12 Å-thick layer of TIP3P water molecules. Prior to 10 nsec molecular dynamics simulation, energy minimization and heating from 0 to 300 K over 50 psec were done. The SHAKE algorithm was used to speed molecular dynamics calculations, and the integration time step was 0.002 psec. In the study of the transport through the substrate channel, formate was docked into the area of the Lys286 residue located at the channel entrance. Then the steered dynamics modeling was carried out with the restraint on the formate–Asn146 distance. A spring was connected to the formate carbon by one end and to the C^α atom of the active site residue Asn146 by the other end. During the simulation the equilibrium length of the spring was decreased to steer the formate to the active site. The spring constant was 150 pN/Å, and the spring rate was 5 Å/nsec.

RESULTS AND DISCUSSION

The model of FDH used in our work was obtained on a basis of 2nac structure at 1.8 Å resolution as a result of 10 nsec molecular dynamics equilibration of the protein molecule in explicit solvent. In the constructed enzyme, the substrate channel has a length of about 15 Å and a width up to 10 Å. The inner neck is the narrowest part of the channel: the cross-section could be described as an ellipse with axes of 5 and 3 Å (for comparison, the narrowest part of the NAD^+ binding site is described as an ellipse with axes of 6.5 and 5 Å). Since the largest dimension of the formate molecule is of 5 Å, the passage of the substrate through the inner neck without conformational rearrangement of the neck residues should be obstructed because it requires the strictly determined orientation of the molecule (parallel to the major axis of the neck).

To elucidate dynamic characteristics of the substrate channel, the formate anion was docked into the model of FDH-NAD^+ complex near the Lys286 residue, and from this point steered molecular dynamics simulation of the transport via the channel was started. The pulling of the formate into the active site revealed an important role of the conformational transition of the Arg284 residue in the mechanism of substrate delivery (figure; see color insert). With the formate approaching the substrate channel entrance as a consequence of diffusion and electrostatic attraction to Lys286, the Arg284 residue turns towards the formate (with 180° rotation of the guanidinium group plane) and subsequently captures it from the bulk solvent. Then Arg284 moves back to its original position and carries the formate to the active site interior, resulting in the formation of the enzyme–substrate complex ready for further catalytic transformations. Thus, the Arg284 residue provides the capture of the formate from the solvent bulk, the subsequent transport through the substrate channel to the active site, and the formation of the Michaelis complex. The electrostatic interaction of Arg284 with the formate is persistent over the whole trajectory excusing the transport of the polar molecule from the formation and the rupture of additional bonds. The work of an external force during the steered simulation (i.e. the costs to overcome transitional interactions with the amino acid residues forming the substrate channel and to remove the hydration shell when binding in the active site) is just 5 kcal/mol, indicating that there are no formidable obstacles for the transport. The high value of the average temperature factor for the Arg284 side chain in the apo form of FDH (44.04 $Å^2$, PDB structure 2nac), which is a measure of atomic movement, confirms the conformational lability of the residue. The use of steered molecular dynamics allowed the modeling of the substrate transport into the active site of prokaryotic FDHs and elucidation of the detailed functioning of the substrate channel, which plays an important role in the catalytic mechanism.

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