

Molecular Dynamics of 3-site Simulation of F₁-ATPase

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Abstract

F₁F₀-ATP Synthase is a membrane enzyme that is responsible for ATP synthesis in almost all living organisms. It is present in the chloroplast thylakoid membrane, mitochondrial inner membrane and bacterial plasma membrane. It is a molecular machine that uses physical rotation of its γ subunit during catalysis. Though several crystal structures have been obtained (1E1Q, 1H8E, 1W0K), the actual mechanism relating ATP synthesis or hydrolysis to rotation is currently unknown. In most crystal structures one catalytic site is empty. The first aim of the project involves analysis of Molecular dynamics (MD) simulations when all three catalytic sites contain Mg-ATP. To date, the simulations have acquired 60ns of data that calculate conformational changes in the protein as a result of the substitution of ATP for ADP at the second site and addition of ATP at the third site. The binding change hypothesis predicts that the 'empty site' will close and the 'ADP sites' will open in a sequential way. The work focuses on the changes in the distances between the Mg-nucleotide complexes at the three catalytic sites and the amino acids that surround the nucleotides. The second aim of the project was to purify chloroplast F₁-ATPase (cF₁) from *Chlamydomonas reinhardtii*. To achieve highly purified cF₁ the enzyme was purified from a strain of *Chlamydomonas* that contains a his-tag on the β -subunit C-terminus. Although the his-tag is required for single-molecule studies to measure the rotation of the motor, it created serious difficulties in obtaining significant quantities of the enzyme. A procedure was developed that enabled cF₁ to be isolated with high purity that had an average yield of 1.2mg of protein from 120-140g wet weight of cells grown in 40L of liquid culture medium.