MS82.O06

Combining neutron crystallography, high-performance computing for enzyme design

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Enzymes continue to expand their role in industry as a "green" option for the synthesis of value-added products. They are targeted for the design of drugs in pharmaceutical applications and also for protein engineering in industry to improve their efficiency, stability, and specificity. Knowledge of the exact mechanisms of enzymatic reactions may provide essential information for more effective drug design and enzyme engineering. For the first time, we are employing a joint X-ray/neutron (XN) protein crystallographic technique in combination with high-performance computing, including QM and QM/MM calculations, MD and Rosetta simulations, to investigate the mechanisms of several enzymes that are important to renewable energy and chemical synthesis. D-xylose isomerase (XI) is an enzyme which can be used to increase the production of biofuels from lignocellulosic biomass and also to synthesize rare sugars for pharmaceutical industry. XI catalyzes the reversible multi-stage sugar inter-conversion reaction facilitated by the presence of two divalent metal cations in its active site. It primarily catalyzes the isomerization of the aldo-sugar D-xylose to the keto-isomer D-xylulose, but can also epimerize L-arabinose into L-ribose, albeit much less efficiently. The reaction involves moving hydrogen atoms between the protein residues, sugar and water molecules, and can only be understood if hydrogen atoms are visualized at each reaction stage. We have obtained a number of joint XN structures of XI complexes representing snapshots along the reaction path with D-glucose, D-xylose and L-arabinose. The suggested reaction mechanism has been verified by QM calculations using the novel O(N) methodology. We are using this structural and mechanistic information to re-design XI to be more efficient on D-xylose and L-arabinose for biofuels and biomedical applications by employing QM/MM, MD, and Rosetta methodologies.

Keywords: xylose isomerase, catalytic mechanism, enzyme design