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Neutron structure of Leishmania mexicana triosephosphate isomerase variant E97Q reveals a possible general acid

Content

Triosephosphate isomerase (TIM) is a key enzyme in glycolysis that catalyses the interconversion of glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP). This simple reaction involves the shuttling of protons mediated by protolysable side chains. The catalytic power of TIM is thought to stem from the ability to facilitate the deprotonation of a carbon next to a carbonyl group to generate an enediolate intermediate. The enediolate intermediate is well mimicked by the inhibitor 2-phosphoglycolate (PGA) and a previous neutron structure showed that E167 was protonated, confirming it as the general base. The identity of the general acid catalyst remains debated. One candidate is K10, where we saw potential indications of deprotonation, but finally concluded that K10 was not deprotonated. To further explore this we mutated E97 that hydrogen bonds to K10 and determined the neutron structure the variant E97Q. Surprisingly the mutation leads to a conformational change in K10 and the inhibitor, explaining a previously observed minority conformation of the inhibitor. We then performed joint neutron-X-ray refinement followed by quantum refinement, but the quantum refinement failed to converge. The reason to this is that K10 is ˜30% deprotonated, which is very challenging to accurately model in quantum chemistry methods. This deprotonation of K10 suggests that it could act as a general acid in at least one direction of the reaction. The inhibitor conformation also suggests that the reaction may involve a conformational change of the substrate that has not been previously postulated.

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