A Structural Study of Quinolinate Synthase, a Key Enzyme in Bacterial NAD+ Biosynthesis

Neela Yennawar, Olga Esakova, Tyler Grove, Alexey Silakov, Allison Saunders, Martin McLaughlin and Squire Booker
Pennsylvania State University

Quinolinate synthase (NadA) catalyzes the synthesis of quinolinic acid (QA), the universal precursor in the biosynthesis of nicotinamide adenine dinucleotide (NAD). NadA contains a [4Fe-4S] cluster cofactor with a unique, non-cysteinyl-ligated, iron ($Fe_a$) implicated in a direct catalytic role. Here, we report several crystal structures of complexes of *Pyrococcus horikoshii* NadA, which provide unique insight into the enzyme's complex mechanism of catalysis. These structures include NadA (i) with its substrate aspartate-enamine (ii) with its substrate dihydroxyacetone phosphate (DHAP) (iii) with a potential reaction intermediate, a condensation product of aspartate-enamine and dihydroxyacetone with the phosphate eliminated (iv) with a similar but not identical reaction intermediate produced with the inactive variant, E198Q (v) and with its product QA. The orientation and binding modes of the substrate, intermediate and the product have been confirmed by Hyperfine Sublevel Correlation (HYSCORE) spectroscopy. Aspartate-enamine preferentially binds to $Fe_a$ in the first step of the reaction, which is followed by formation of a Schiff's base complex between DHAP and the nitrogen of aspartate-enamine. In all structures that contain aspartate-enamine or remnants of the molecule, the C8 carboxylate group is always coordinated to $Fe_a$, suggesting that this coordination is mechanistically important. In the QA-bound structure, the C7 carboxylate and N1 atoms are bound to $Fe_a$ in a bidentate fashion. This orientation suggests that in the progression from the intermediate to the product, a rotation around both the N1–C2 and C2–C3 bonds takes place. Next, the C8 carboxylate dissociates from $Fe_a$ and the C7 and N1 atoms rebind to it. This suggested conformational change is strikingly reminiscent of catalysis by aconitase, the paradigm enzyme for the Fe/S-dependent hydrolyase family of enzymes, which catalyzes the isomerization of citrate to isocitrate via the intermediate, *cis*-aconitate. The binding mode of QA places the C5 hydroxyl group of the postulated final intermediate distal to $Fe_a$ and virtually incapable of coordinating to it. Hence protein residues and not the $Fe_a$ ion are involved in the final dehydration and ring closure steps to form QA. The structure shows that three strictly conserved amino acids, Glu198, Tyr109, and Tyr23, are in close proximity to the bound product. Substitution of these amino acids with Gln, Phe, and Phe, respectively, leads to complete loss of function. The series of X-ray structures and additional binding information from HYSCORE spectroscopy have provided unexpected new insight into NadA's catalytic mechanism, with a particular focus on the role of the Fe/S cluster and active site residues.