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Structure and mechanism of soluble quinoprotein glucose dehydrogenase

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Summary

Dehydrogenases and oxidases participate in a variety of metabolic pathways. Amongst others they convert alcohols into carbonyl compounds. Three distinct classes of such proteins use three different types of organic cofactors: nicotinamides, flavins, and quinone-containing compounds. The three-dimensional structures and catalytic mechanisms of the nicotinamide- and flavin-dependent enzymes have been characterized well. Both classes employ a catalytic mechanism that involves the direct transfer of a hydride anion from the substrate to the cofactor. In contrast, much less is known about how the quinone cofactor-containing enzymes perform catalysis.

This thesis describes the elucidation of the three-dimensional structure and catalytic mechanism of a soluble glucose dehydrogenase (s-GDH) by X-ray crystallography. This enzyme uses pyrroloquinoline quinone (PQQ) as a cofactor to oxidize glucose and a variety of other sugars to their corresponding lactones. In **Chapter 1** the current knowledge of the chemistry of PQQ and PQQ-dependent proteins is reviewed. Furthermore, several possible reaction mechanisms are discussed, which differ in the identity of the reactive moiety of PQQ (C4 or C5 carbonyl group) and in the nature of the intermediate (substrate or hydride addition to the cofactor).

The crystallization, structure determination and refinement of the s-GDH structure are described in **Chapter 2**. The three-dimensional structure of s-GDH was determined using the multiple isomorphous replacement method. The initial experimental phases were improved by a combination of solvent flattening and non-crystallographic symmetry averaging, resulting in an interpretable electron density map. A nearly complete model was built and refined at 1.7 Å resolution.

s-GDH functions as a homodimer. Each subunit has a β -propeller fold, formed by six four-stranded anti-parallel β -sheets, aligned around a six-fold pseudosymmetry axis. One monomer binds three calcium ions. Two are located in the dimer interface, whereas the third is bound in the active site. The discovery of four uncharacterized protein sequences that are homologous to s-GDH are also described in **Chapter 2**. These homologs may have a similar fold and catalyze similar PQQ-dependent reactions. A structure based sequence alignment of the six β -sheets in the s-GDH structure reveals a six times repeated segment, which gives rise to a number of polar interactions with a stabilizing function.

The high resolution structure of s-GDH in complex with the cofactor PQQ and the competitive inhibitor methylhydrazine is presented in **Chapter 3**. It affords a detailed view of the active site with bound cofactor. PQQ binds to the active site calcium in a large solvent accessible cleft in the centre of each monomer. The binding of the cofactor is mainly governed by polar interactions. Methylhydrazine is covalently bound to the C5 atom of PQQ, which is confirmed to be the most reactive moiety towards nucleophilic compounds. Polarization of the C5-O5 bond by the side chain of Arg228 and the calcium ion may enhance the reactivity of the C5 atom. This indicates that the catalytic mechanism most likely involves nucleophilic attack on the C5 atom, thus resolving one of the two controversies in PQQ-assisted enzymatic catalysis.

In **Chapter 4** a complex consisting of s-GDH, reduced PQQ and substrate is described. Glucose docks onto the surface of PQQ and makes hydrogen bonds with several protein side chains. In combination with biochemical and kinetic data, the arrangement of PQQ, calcium, glucose and His144 provides conclusive evidence for the catalytic mechanism: The side chain of His144 abstracts the proton from the O1 hydroxyl group. This occurs in a fast step together with the transfer of a hydride from the substrate to the PQQ C5 atom. In this step, the product and a PQQ intermediate are formed. In the remaining steps of the reaction, the cofactor intermediate is converted into reduced PQQ. For s-GDH this resolves the second controversy about the catalytic mechanism.

As for s-GDH, several crystal structures of PQQ-dependent methanol dehydrogenase (MDH) have been previously determined, including a complex of this enzyme with its natural substrate methanol. On the basis of this latter complex, however, a completely different catalytic mechanism has been proposed, even though several lines of biochemical and kinetic evidence are strikingly similar for both enzymes. To resolve this discrepancy, we have compared the two enzyme-substrate complexes in **Chapter 5**. In both complexes, PQQ, the calcium ions, the basic amino acid residues and the substrates glucose and methanol are all bound at similar positions. This suggests that MDH and s-GDH catalyze the oxidation of their substrates through an identical reaction mechanism. After a detailed analysis of all structural and biochemical data we propose that this mechanism proceeds as described above for s-GDH. Thus, the PQQ-dependent enzymes MDH and s-GDH use a mechanism that is similar to that of the nicotinamide- and flavin-dependent enzymes. Hence, it appears that hydride transfer is a common mechanism in enzymatic dehydrogenation reactions.

Dehydrogenases en oxidases
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dehydrogenases en oxidase
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wordt. De structuur is na
(propellerbladen), die net al
draai-as van pseudosymme
raakvlak van de monomeren