X-ray crystallographic studies of two bacterial quinoproteins. Methylamine Dehydrogenase and Quinohaemoprotein Alcohol Dehydrogenase

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
1994

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Summary

For the catalysis of reactions involving the transfer of electrons, enzymes utilize either metal ions or special organic compounds called cofactors. A group of enzymes, known as quinoproteins, makes use of cofactors that contain a quinone function. This quinone function enables quinoproteins to oxidize a wide range of substrates, including amines, alcohols, and sugars. During catalysis two electrons are transferred from the substrate to the quinone cofactor.

Until recently, all quinoproteins were thought to contain the cofactor pyrroloquinoline quinone (PQQ). However, a number of recent discoveries have shown that besides PQQ at least two other cofactors containing a quinone function are used by quinoproteins. A review of recent developments in the field of quinoproteins and a summary of the current knowledge about this class of proteins is presented in Chapter 1.

In subsequent chapters the results of X-ray crystallographic investigations performed on two quinoproteins are reported.

Chapters 2 to 4 deal with the enzyme methylamine dehydrogenase (MADH) isolated from the bacterium *Thiobacillus versutus*. MADH consists of four subunits: two identical light subunits containing 131 amino acids and two identical heavy subunits containing 395 amino acids. The quinone cofactor of MADH has a unique structure. It consists of two indole rings that are covalently linked. One of these indole rings contains an ortho-quinone function. A remarkable aspect of this cofactor is the fact that it arises through chemical modification of the side chains of two tryptophan residues that are located in the polypeptide chain of the light subunit. Since the cofactor is made up of two tryptophan side chains it has been named tryptophyl tryptophanquinone (TTQ).

The work of Fred Vellieux formed the basis for the structure determination of MADH. However, since the amino acid sequence of MADH as well as the identity of its cofactor were determined only recently, the structure determination could only now be completed. This resulted in the crystallographically refined structure of MADH at a resolution of 2.2 Å, which is described in Chapter 2.

The determination of the structure of MADH makes an analysis of the structure-function relationships of this enzyme possible. The focus of this study was the mechanism of the enzymatic reaction of MADH which involves the degradation of methylamine to formaldehyde and ammonia.

Because a quinone function consists of two carbonyl groups a prominent question arises: which of the carbonyl groups is involved in substrate binding? As is described in Chapter 3, we used hydrazines to address this question. Many hydrazine compounds irreversibly inhibit MADH by reacting with the TTQ cofactor. Two structures of MADH, each inhibited by a different hydrazine compound were determined. The active site electron densities of both structures clearly indicated that the carbonyl group at position 7 of the tryptophan-quinone ring is the reactive one. Apart from the identification of the reactive carbonyl group, an unexpected result was obtained from the inhibitor binding studies. Although two inhibitors of differing sizes, namely methylhydrazine and trifluoroethylhydrazine were employed, the observed electron densities for both inhibitors were similar in size and much smaller than was expected. Apparently, partial breakdown of the inhibitors after binding to the protein had occurred.

The reactive carbonyl group of the TTQ cofactor is located next to a small pocket. In this pocket the catalytic reaction takes place. Most of the residues surrounding the pocket are contributed by the light subunit. One side of the pocket is, however, closed off by the side chain of a phenylalanine from the heavy subunit. In Chapter 2 a putative reaction mechanism is discussed in the context of the active site environment. It is argued that the carbonyl moiety of an aspartate residue located at position 76 in the light subunit is involved in a key step of the reaction mechanism, namely the abstraction of a proton from the carbon atom of the substrate.

The TTQ cofactor accepts two electrons from the substrate during the catalytic reaction. These electrons are subsequently transferred to the copper-containing protein amicyanin.

Two atoms of the tryptophyl part of the TTQ cofactor are exposed to the bulk solvent. Close to these atoms several hydrophobic residues are present on the surface of the protein. It seems likely that electron transfer to amicyanin takes place at this position. In order to obtain more information on the MADH:amicyanin complex involved in electron transfer, we tried to grow co-crystals of these proteins. Unfortunately these crystallization experiments did not yield co-crystals suitable for a high resolution structure determination. A collaboration with Dr. Scott Mathews and colleagues of the Washington University in St. Louis resulted, however, in the structure of a complex of MADH and amicyanin both isolated from *Paracoccus denitrificans*. The structure determination of this complex is described in Appendix C and involved the molecular replacement method using the structure of MADH from *T. versutus* as the search model. The structure of the complex...
reveals that amicyanin does indeed bind to MADH at the position mentioned above and interacts with both the heavy and the light subunit. The shortest distance from the TTQ cofactor to the copper ion of amicyanin is about 9 Å.

Although the crystallisation experiments of MADH and amicyanin from *T. versutus* did not yield suitable co-crystals, we did obtain a new crystal form of MADH. The structure determination of this new crystal form is described in Chapter 4. An analysis of the potential binding sites for amicyanin shows that these are blocked by crystal contacts. Test exposures on a synchrotron X-ray beam line in Daresbury (England) indicated that this crystal form is well suited for a high resolution structure determination, since its diffracting power is superior to the diffracting power of the 'old' crystal form of MADH.

The last two chapters cover investigations of the enzyme quinohaemoprotein alcohol dehydrogenase (QH-ADH). This enzyme contains a PQQ cofactor as well as a covalently bound haem group and needs calcium ions for catalytic activity. QH-ADH catalyses the breakdown of primary alcohols to aldehydes, but is also capable of converting aldehydes to carboxylic acids.

In Chapter 5 the crystallization of QH-ADH is reported. Although crystals grew readily, it proved very difficult to obtain a sufficient number of crystals suitable for X-ray diffraction experiments. Fortunately, the few suitable crystals that were obtained diffracted well, enabling the collection of high quality diffraction data to a resolution limit of 2.5 Å.

The QH-ADH crystals of have unique optical properties. When viewed under linearly polarized light the color of the crystals varies from brightly orange to completely colorless, depending on the crystal orientation. This optical effect was exploited to determine the approximate orientation of the haem group with respect to the crystallographic unit cell.

The progress towards the structure determination of QH-ADH is described in chapter 6. To obtain phases, the molecular replacement method as well as the isomorphous replacement method was used. The structure of the PQQ binding domain from the quinoprotein methanol dehydrogenase, which is about 34% homologous to the N-terminal domain of QH-ADH, was used as the search model. A clear solution was obtained for the rotation and translation functions. However, the haem binding N-terminal domain of QH-ADH, which makes up about 25% of its structure, is not present in the structure of methanol dehydrogenase. This has an adverse effect on the quality of the molecular replacement phases.

A screening of heavy atom compounds resulted in a trimethyllead acetate derivative. The phases obtained from this heavy atom derivative showed significant correlation with the phases calculated from the molecular replacement model, which is a strong indication for the correctness of our results. Moreover, a solvent mask calculated from the heavy atom phases pointed out a likely position for the haem binding domain. The quality of the electron density map was, however, not sufficient for an interpretation at the atomic level. Therefore additional phase information will be needed to complete the structure determination of QH-ADH.

The appendices contain, besides the article describing the previously mentioned complex of MADH and amicyanin, two more publications that resulted from our collaboration with Dr. Mathews and colleagues. Appendix A, published shortly after the identity of the MADH cofactor was discovered, shows that the active site electron densities of both *T. versutus* and *P. denitrificans* MADH are well compatible with the structure of the TTQ cofactor. Appendix B describes the structure determination by molecular replacement of MADH from *P. denitrificans* using the structure of *T. versutus* MADH as the search model.