Three-dimensional structure of quinoprotein methylamine dehydrogenase.

Vellieux, Frédéric Marie Daniel André Philippe

Summary

In this thesis, the crystal structure determination of quinoprotein methylamine dehydrogenase to a resolution of 2.25 Å is described. The structure of this enzyme, complete with its PQQ-related quinone cofactor is presented, and relevant observations concerning this structure are discussed.

In the introduction (chapter I), the current state of knowledge concerning the PQQ cofactor and quinoproteins in general, and of the enzyme methylamine dehydrogenase in particular, is discussed. This part of the thesis focusses first on a historical overview of the facts having led to the discovery of the cofactor PQQ and of PQQ-related cofactor containing enzymes, called quinoproteins. Later, a summary of the known properties of the PQQ cofactor and of those of quinoprotein methylamine dehydrogenase is given.

Chapter II is divided into two parts. The first part (part A) describes the successful crystallization of quinoprotein methanol dehydrogenase (MDH) from ***Pseudomonas maltovidia***, and the preliminary crystallographic investigation of the crystals obtained. The crystals, of space group P1, probably contain one homodimeric MDH molecule in the asymmetric unit. A heavy atom derivative search yielded four potential heavy atom derivatives which could be used in a crystal structure determination, and rotation function calculations revealed the orientation of a non-crystallographic 2-fold axis of symmetry, proposed to relate the two identical subunits to each other. In part B, the purification, crystallization and preliminary crystallographic investigation of quinoprotein methylamine dehydrogenase (MADH) from ***Thiobacillus versutus*** are discussed. After purification of the enzyme from the bacterium, crystals of MADH were grown by the hanging drop method. Preliminary X-ray investigations of these crystals revealed that they had space group $P3_121$, and that they were suitable for high resolution crystal structure analysis.

In chapter III the crystal structure determination work leading to the solution of the structure of MADH is described. The structure was solved by a combination of three crystallographic methods, namely multiple isomorphous replacement including anomalous scattering information (MIRAS), phase extension by solvent flattening, and phase combination with phases obtained from incomplete models after refinement by molecular dynamics procedures. This led to a 2.25 Å resolution electron density distribution for the native enzyme which could be interpreted in the absence of any sequence information to provide an atomic model for this enzyme.

Chapter IV provides a description of the structure of MADH. The enzyme is a tetramer of two types of subunits, namely a heavy (H) and a light (L) subunit, which are arranged as a flat parallelepiped of dimensions 76*61*54 Å. The overall structure can be best

described as a dimer of (HL) dimers. The H subunit consists of an N terminal extension, which 'embraces' the L' subunit of a (H'L') dimer, and of a disc shaped main body. The latter is made up of seven 4-stranded anti-parallel β-sheets, all with 'W' topology, which are arranged circularly with approximate seven-fold symmetry. This circular arrangement of 4 stranded 'W' β-sheets is reminiscent of the structure found in influenza virus neuraminidase, possibly suggesting an unexpected evolutionary relationship between the two proteins. The smaller L subunit is made up by a scaffold of three β-sheets comprising a total of seven β-strands, which are interconnected by loops and segments of irregular structure. The density in the active site region of the enzyme suggests that the cofactor as found in the native structure is not integral PQQ, but that it might be instead a precursor of PQQ, which was named "pro-PQQ".

In chapter V, the electron density in the cofactor region of MADH is reexamined. Model building studies led to a proposal for the structure of the MADH cofactor, which agrees with all available chemical information. The proposed structure is made up by a nucleus containing the catalytically important ortho-quinone group, which consists of a tyrosine derived quinone indole bicyclic structure. This part of the cofactor is covalently linked to the side chain of the putative glutamate 57 of the L subunit, which is in turn connected by a peptide bond to the N-ϵ atom of the putative arginine 107 of the same subunit. With the elements of this proposed structure being derived from tyrosine, glutamate and arginine residues, it has been named "TGA pro-PQQ".

This thesis is completed by two appendices. In appendix A, a method permitting the alignment of weakly diffracting crystals on precession cameras is described. This method is based on the use of screened still photographs, to be taken after a preliminary orientation of the crystal using screenless still photographs. This method was very useful for the structure determination work described in this thesis, since it allowed a rapid alignment of quinoprotein crystals on precession cameras, which was required both for space group characterization and during the search for heavy atom derivatives.

Appendix B gives a reciprocal space analysis of two widely used density modification methods, namely solvent flattening and non-crystallographic symmetry averaging. Similar "molecular replacement equations" are derived for the two methods. These equations express the value of each structure factor as the weighted sum of all structure factors. In practice, these relationships do not extend over the whole reciprocal space, since only a limited number of structure factors contribute significantly to the value of each structure factor. The implications of these equations to carry out phase improvement and phase extension by electron density modification are then discussed.