Structural studies of a soluble monomeric quinohemoprotein alcohol dehydrogenases from *Pseudomonas putida* HK5

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Introduction

A family of alcohol dehydrogenases (ADHs) that are independent of pyridine nucleotides and contain noncovalently bound pyrroloquinoline quinone (PQQ) as a redox cofactor is found in many aerobic bacteria [1]. One class of ADH (called type II) is composed of monomeric, soluble quinohemoproteins of ~70–75 kDa that contain PQQ and covalently bound heme, and is found in several strains of *P. putida* [2] and in *Comamonas testosteroni* [3]. Sequence analysis of the *C. testosteroni* enzyme indicates that the first 60 kDa are homologous to methylamine dehydrogenase, and the remaining 15 kDa appear to form a c-type cytochrome domain. One form of type II ADH, ADH-IIB, can be isolated from *P. putida* strain HK5 cells grown on 1-butanol. It is estimated to have a molecular weight of 69 kDa on the basis of SDS-PAGE and has been shown to be monomeric [2]. Its amino acid sequence is unknown. *In vitro*, potassium ferricyanide can serve as an artificial electron acceptor for ADH-IIB and is used in a dye-linked assay. An azurin isolated from the same organism has been shown to be an efficient electron acceptor for ADH-IIB [4] suggesting that it acts *in vivo* as an electron transfer mediator in a PQQ-dependent alcohol respiratory chain.

Methods and Materials

ADH-IIB was prepared as described previously [2]. Crystals are obtained with 22% PEG8000, 200 mM sodium citrate, 100 mM HEPES-HCL (pH = 7.5), and 6% 2-propanol [5]. The crystals are triclinic with cell parameters a = 54.8 Å, b = 57.4 Å, c = 67.5 Å, $\alpha = 89.6^o$, $\beta = 69.4^o$, $\gamma = 68.4^o$ and contain one molecule in the unit cell. X-ray data were collected at 100 K from a cryoprotected crystal to 1.9 Å resolution (Rmerge = 6.4%, 87% completeness).

The data were analyzed by molecular replacement (MR) with AMORE [6] using methanol dehydrogenase (MDH) from *Methylophilus methylotrophus* W3A1 as the search molecule. An anomalous difference electron density map based on the MR phases contained two prominent peaks. One peak (14σ) is ~20 Å from the PQQ position in the MDH search molecule and was attributed to iron, and the other (9σ) is close to the position of the calcium ion in MDH that bridges PQQ to protein side chains and is attributed to calcium.

Two compounds, $K_3UO_2F_5$ and CH_3HgCl showed 12–15% isomorphous structure factor changes. Multiple isomorphous replacement (MIR) data were collected in St. Louis and multiple wavelength anomalous diffraction (MAD) data from the mercury and UO_2 derivatives were collected at the Stanford Synchrotron Radiation Laboratory (SSRL) and Advanced Photon Source (APS) synchrotrons, respectively. Protein phases were calculated with SHARP [7]. Using maps based on combinations of MIR and MAD phases, the quinoprotein and the cytochrome domains could be traced. Since the ADH-IIB sequence was unknown, we fit the sequence of the *C. testosteroni* ADH quinoprotein domain wherever possible and polyalanine elsewhere. The structure was partially refined using CNS [8] (R = 31%, Rfree = 39%, 40–3 Å resolution).

Results and Discussion

The current model of ADH-IIB consists of 664 amino acid residues. Residues 1–556 comprise the PQQ-containing eight-bladed β-propeller domain, residues 582–664 comprise the cytochrome domain and residues 557–581 form a 55-residue linker segment connecting the two domains. The current electron density provides a reasonably accurate representation of the path of the polypeptide chain through the structure although the model is limited by the lack of sequence information. However, the *C. testosteroni* ADH is likely to be reasonably homologous.

The PQQ is located in the funnel of the β-propeller that forms a depression in the top of the domain. As in MDH, the PQQ is sandwiched between a tryptophan side chain on the bottom and a vicinal disulfide group on the top. The calcium ion is in the plane of the PQQ and is liganded by atoms O5, N6, and O7A of PQQ plus a glutamic acid and a gultamine side chain.

The cytochrome domain is very small, containing three α-helices in 83 residues. The heme group is covalently attached to Cys596 and Cys599 via thioether linkage to the two heme vinyl side chains while His600 and Met636 are coordinated to the iron atom. The propionic acid side chains of the heme are located on the surface of the protein and project into solution. Among bacterial cytochromes, the cytochrome domain of ADH-IB most closely resembles the cytochrome subunit of *p*-cresol methylhydroxylase [9]. Both of these proteins contain three α-helices rather than the usual four and have solvent-exposed propionate groups. The heme iron atom and C5 atom of PQQ are separated by about

20.5 Å and the distance between the two rings is about 16 Å. The heme and PQQ planes are tilted by about 70º to each other.

The PQQ and heme group are linked by a covalent chain of four or five amino acids (residues 99–102 or 103) that may provide a pathway for electron flow between the two prosthetic groups. The path involves a through-space jump of about 4 Å from the plane of PQQ to the Cys102–Cys103 vicinal disulfide, travel along the backbone chain from residue Cys102 to Phe99, and a through-space jump of 3.7 Å to Cys599 (which forms one of the covalent attachment sites of the heme).

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