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Crystal structures of *Toxoplasma gondii* pterin-4a-carbinolamine dehydratase and comparisons with mammalian and parasite orthologues☆

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Abstract

The enzyme pterin-4a-carbinolamine dehydratase (PCD) is important for the recycling of pterins within eukaryotic cells. A recombinant expression system for PCD from the apicomplexan parasite *Toxoplasma gondii* has been prepared, the protein purified and crystallised. Single crystal X-ray diffraction methods have produced a high-resolution structure (1.6 Å) of the apo-enzyme and a low-resolution structure (3.1 Å) of a complex with a substrate-like ligand dihydrobiopterin (BH_2). Analysis of the hydrogen bonding interactions that contribute to binding BH_2 suggest that the ligand is present in an enol tautomeric state, which makes it more similar to the physiological substrate. The enzyme can process (*R*)- and (*S*)-forms of pterin-4a-carbinolamine and the ligand complex suggests that His61 and His79 are placed to act independently as general bases for catalysis of the individual enantiomers. Comparisons with orthologues from other protozoan parasites (*Plasmodium falciparum* and *Leishmania major*) and with rat PCD, for which the structure is known, indicate a high degree of sequence and structure conservation of this enzyme. The molecular determinants of ligand recognition and PCD reactivity are therefore highly conserved across species.

Keywords: Apicomplexa; Crystal structure; Dehydratase; Kinetoplastida; Pterin; Tautomer; *Toxoplasma*

1. Introduction

Tetrahydrobiopterin (BH_4) is an important cofactor in essential metabolic pathways of both eukaryotic and prokaryotic organisms. The chemical properties of this cofactor are exploited in the hydroxylase reactions that convert phenylalanine or tryptophan to tyrosine, by nitric oxide synthase, and in the biosynthesis of neurotransmitters such as serotonin, dopamine and noradrenaline [1]. Little is known about the pterin content and metabolism of parasitic protozoa although putative orthologues of several enzymes involved in pterin metabolism in higher eukaryotes have been identified [1]. Research in this area, in large part driven by access to genomic data, is likely to provide a rich vein of new biological data in particular on organisms in the phylum Apicomplexa, including *Plasmodium falciparum* and *Toxoplasma gondii*, the causative agents of malaria and toxoplasmosis, respectively.

In higher eukaryotes BH_4 is synthesised *de novo*, but many parasitic organisms are pterin auxotrophs. These include the parasitic protozoa of the class Kinetoplastida, which acquire pterins through a specific transporter (e.g. BT1 in *Leishmania*) [2]. Recycling is important in these organisms for the regeneration of BH_4 from the pterin-4a-carbinolamine formed when the molecule acts as a cofactor [3–5]. Pterin-4a-carbinolamine dehydratase (PCD, EC 4.2.1.96) is key to this process and catalyses the conversion of pterin-4a-carbinolamine to quinoid-dihydrobiopterin (q-BH_2, Fig. 1a). This PCD catalysed step prevents the spontaneous rearrangement of the unstable pterin-4a-carbinolamine to less useful 7-substituted pterins [4]. An NADH-dependent quinoid-dihydrobiopterin reductase then reduces q-BH_2 to BH_4. The function of the protein encoded by the *pcd* gene (GenBank accession number: DQ223777) in *T. gondii* has been verified by a complementation assay [5] using an *Escherichia coli* tyrosine auxotroph lacking PCD, but carrying

Abbreviations: BH_4, tetrahydrobiopterin; BH_2, dihydrobiopterin; q-BH_2, quinoid-dihydrobiopterin; PCD, pterin-4a-carbinolamine dehydratase; *Rn*, *Rattus norvegicus*; *Tg*, *Toxoplasma gondii*.

☆ Coordinates and structure factor data are deposited with the Protein Data Bank under accession codes 2V6S, 2V6T and 2V6U.

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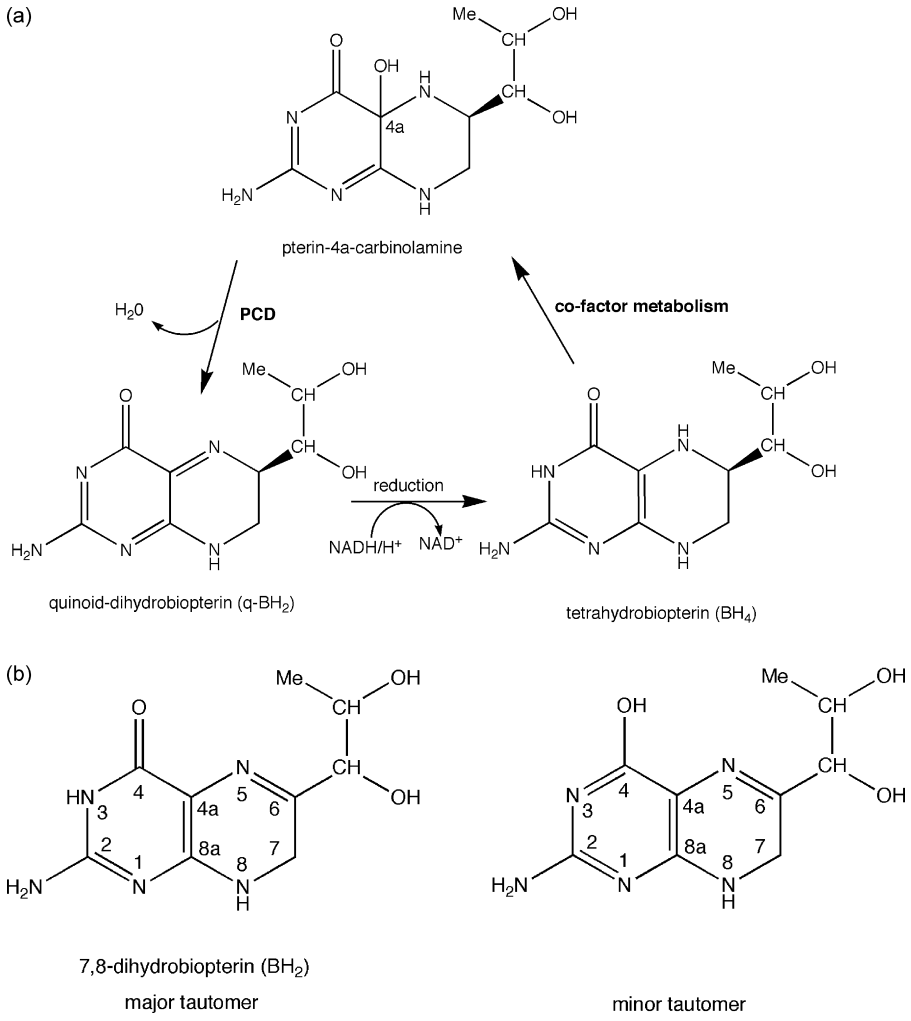

Fig. 1. Pterin salvage and ligand structures. (a) Pterin recycling from metabolised BH_4, which generates pterin-4a-carbinolamine through q-BH_2 and back to BH_4. (b) Chemical structure and atomic numbering of the major and a minor tautomer of BH_2.

a quinoid-dihydrobiopterin reductase (DHPR). When a phenylalanine hydroxylase is co-introduced, pterin recycling (Fig. 1a) can occur, and so tyrosine production is enabled in the presence of the introduced PCD.

Rat liver PCD (*Rattus norvegicus*, *Rn*PCD), which is identical in sequence to human PCD, has been biochemically characterized [6,7] and crystal structures determined [8–11]. A second structure is derived from the Gram-negative eubacterium *Thermus thermophilus* (Protein Data Bank (PDB) codes 1USO and 1USM) however no publications are associated with these entries. Intriguingly, mammalian PCD has a second function assigned to it. It is thought to activate transcription by forming a stable complex with Hepatocyte Nuclear Factor 1 (HNF1) that facilitates DNA binding [11]. This gives PCD the alternative name of DCoH (Dimerisation Cofactor of HNF1). The precise mechanism by which transcription is activated is not yet established. The absence of a detectable HNF1 homologue in *T. gondii* or indeed any other protozoan suggests that either such a second function is unlikely in these organisms or that a different partner protein is involved.

Here we report the preparation of a recombinant protein expression system, purification and crystallisation of *T. gondii* PCD (*Tg*PCD), X-ray crystal structures of the apoenzyme and of the complex with 7,8-dihydrobiopterin (BH_2), which is a product analogue. Structural comparisons with *Rn*PCD and sequence comparisons with orthologues from two other protozoan parasites, *P. falciparum* and *Leishmania major* are presented. These comparisons allow us to assess the potential of this enzyme as a target for the development of inhibitors to assist the development of reagents useful to further dissect pterin metabolism and that might underpin novel therapeutic approaches for diseases caused by protozoan infections.

2. Materials and methods

2.1. Cloning, expression and purification

The TimeSaver cDNA synthesis kit (Amersham Biosciences) was used to generate a cDNA library from total RNA extracted from *T. gondii* (RH strain) parasites. Briefly, first strand synthesis, to make a full-length cDNA copy from mRNA, was performed using an oligo$(dT)_{12–18}$ primer and Moloney murine leukaemia virus reverse transcriptase. This