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A structural model for FOXRED1, an FAD-dependent oxidoreductase necessary for NADH: Ubiquinone oxidoreductase (complex I) assembly

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ABSTRACT

The biogenesis of mitochondrial respiratory chain components is complex. Mammalian complex I (NADH:ubiquinone oxidoreductase) contains 44 different subunits, an FMN and seven iron–sulfur centers. Its assembly involves at least twelve additional proteins, called assembly factors. One of these is FOXRED1, a 486-amino acid FAD-dependent oxidoreductase. FOXRED1 is a member of the D-amino acid oxidase (DAO) family. A structural model of FOXRED1 reveals a large substrate-binding cavity and a putative oxygen-binding site. These features strongly suggest that FOXRED1 is catalytically active as an oxidoreductase. A metabolic role for FOXRED1 in the biogenesis of complex I should be considered.

1. Introduction

Each of the five major mitochondrial complexes of oxidative phosphorylation depends on additional non-subunit, proteins for its assembly. These additional proteins are often called assembly factors and have functions that include protein folding, post-translational modification, cofactor biosynthesis and cofactor insertion. FOXRED1 (FAD-dependent oxidoreductase) has been identified as an NADH:ubiquinone oxidoreductase (complex I)-specific assembly factor. It is a 486-amino acid FAD-containing member of the D-amino acid oxidase (DAO) family and mutations in it lead to infantile onset encephalomyopathy and Leigh syndrome (Fassone et al., 2010). In two affected individuals, the levels of residual complex I activity in skeletal muscle and fibroblasts were less than 10% (Fassone et al., 2010; Calvo et al., 2010).

How FOXRED1 mediates its role in complex I biogenesis remains undetermined. Very little experimental evidence is available to support any mechanism. The protein is likely synthesized as a precursor protein and cleaved to a mature form after import into mitochondria (Fassone et al., 2010). FOXRED1 was recently found in association with complex I assembly intermediates (Andrews et al., 2013).

In this study, I generated a molecular model of FOXRED1 and investigated the structural features that might offer insight into a catalytic function. If FOXRED1 is functional as an oxidoreductase, it would be expected to form an active site near the FAD cofactor and this active site would contain evolutionarily conserved amino acids. FOXRED1 should also share structurally conserved features with other oxidoreductases for which we have experimentally-determined structures.

A previous model of FOXRED1 was proposed based on the structure of a *Bacillus* monomeric sarcosine oxidase (MSOX) (PDB ID: 2gb0), a protein with a covalent flavin cofactor (Fassone et al., 2010; Trickey et al., 1999). The human genome contains four DAO flavoproteins closely related to FOXRED1: peroxisomal sarcosine oxidase (PIPOX), sarcosine dehydrogenase (SARDH), dimethylglycine dehydrogenase (DMGDH) and pyruvate dehydrogenase phosphatase regulatory subunit (PDPR) (Lemire, submitted). The FAD in MSOX is covalently attached via C315; PIPOX has a conserved cysteine, C319, which aligns with MSOX C315. SARDH and DMGDH also have covalent flavins, but their cofactors are attached via histidine residues H108 and H91, respectively (Fig. 1). PDPR is a non-covalent flavoprotein with a dissociable FAD (Lawson et al., 1997).

Abbreviations: CPS, carbamoyl phosphate synthase; DAO, D-amino acid oxidase; DMGDH, dimethylglycine dehydrogenase; DMGO, dimethylglycine oxidase; FOXRED, FAD-dependent oxidoreductase; HADHA, hydroxyacyl dehydrogenase; MDH, malate dehydrogenase; MSOX, monomeric sarcosine oxidase; mtDNA, mitochondrial DNA; MTHFD, methylenetetrahydrofolate dehydrogenase; NDUFAF, NADH dehydrogenase (ubiquinone) complex I, assembly factor; NDUFS, NADH ubiquinone oxidoreductase iron–sulfur protein; NUBPL, nucleotide-binding protein-like; PCK2, phosphoenolpyruvate carboxykinase; PDPR, pyruvate dehydrogenase phosphatase regulatory subunit; PIPOX, peroxisomal sarcosine oxidase; RMSD, root mean square deviation; SARDH, sarcosine dehydrogenase; SCO, synthesis of cytochrome *c* oxidase; SHMT, serine hydroxymethyltransferase; TMEM, transmembrane protein; TSOX, heterotetrameric sarcosine oxidase; UQCC, ubiquinol-cytochrome *c* reductase complex chaperone.

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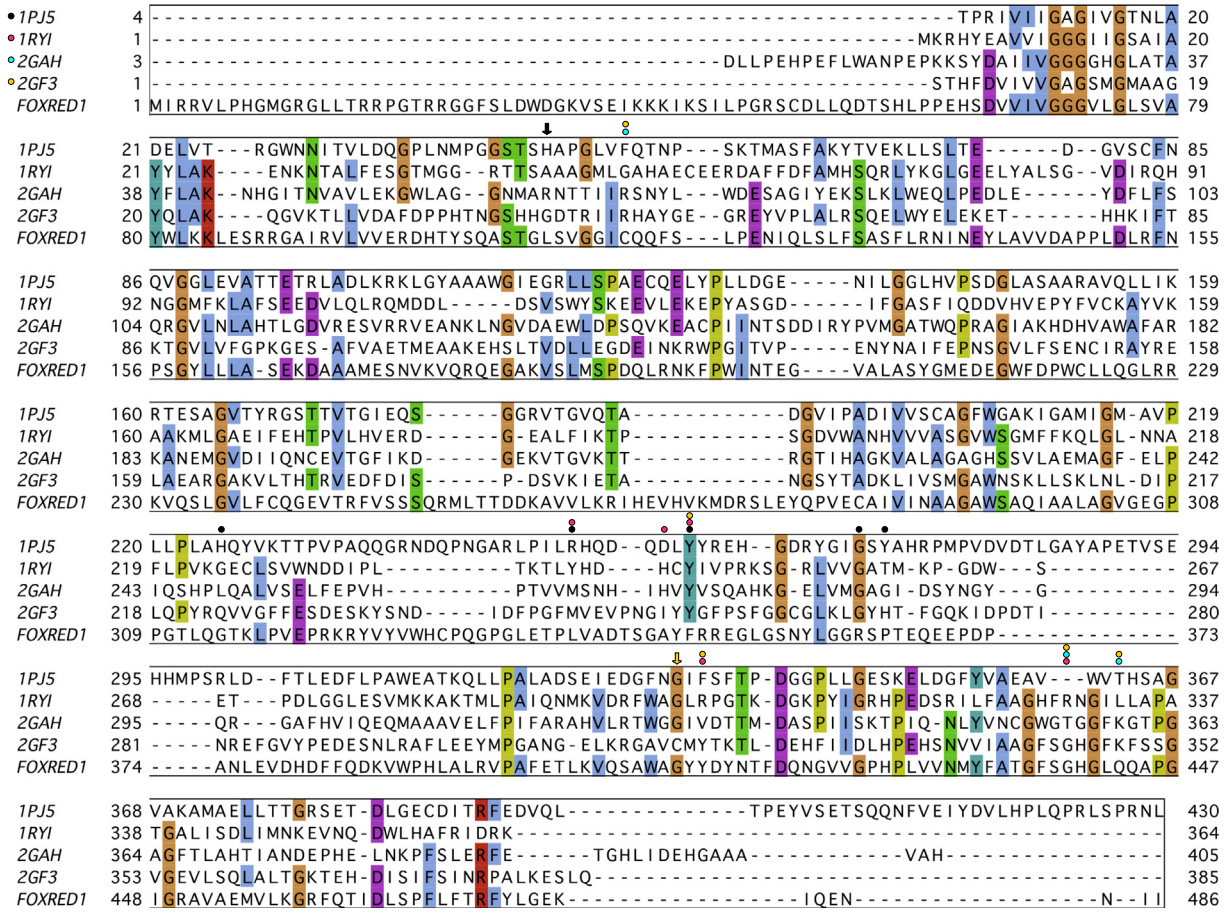

Fig. 1. Alignment of human FOXRED1 with four modeling template sequences. 1PJ5, 1RYI, 2GAH and 2GF3 are structures of the *Arthrobacter globiformis* dimethylglycine oxidase (DMGO), *Bacillus* glycine oxidase, heterotetrameric sarcosine oxidase (TSOX) and monomeric sarcosine oxidase (MSOX), respectively. Only DMGO residues 1–430 were used in the alignment; the C-terminal domains were deleted. Residues conserved in at least three of the five proteins are colored (ClustalX coloring scheme; Jalview v14.0) (Waterhouse et al., 2009). The black and orange arrows indicate the sites of covalent FAD attachment in 1PJ5 (H48) and 2GF3 (C315), respectively. Active site and substrate binding residues discussed in Section 3.2 are indicated with colored dots: black, 1PJ5; red, 1RYI; cyan, 2GAH and orange, 2GF3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

FOXRED1 is a non-covalent flavoprotein; it lacks an appropriately located residue to undergo covalent flavinylation (Fig. 1). In the model of FOXRED1 I generated, there are no His, Tyr or Cys residues near the 6 or 8α positions of the FAD isoalloxazine ring, which are the usual positions for covalent attachment (Heuts et al., 2009). In generating a structural model for FOXRED1, I included non-covalent flavoprotein structural templates.

2. Materials and methods

Comparative model building relies on fold assignment using at least one known template structure, sequence alignment with one or more templates and model building based on the chosen templates. MODELLER is a widely used multi-component, comparative structure modeling program (Eswar et al., 2003). A structure based multiple sequence alignment with four templates was performed with SALIGN, then aligned human FOXRED1 using ALIGN2D (Braberg et al., 2012). A model was computed with MODEL_MULT and evaluated with EVALUATE_MODEL. Loops with higher energy scores (computed with MODEL_ENERGIES) were refined with LOOP_MODEL. Finally, the FAD and a glycolate inhibitor from the glycine oxidase template were inserted into the loop-refined model with MODEL_MULTIPLE_HETERO. At each step, 5 models were calculated and the one with the lowest energy was used. Protein structure images were generated using UCSF Chimera (v1.10) (Yang et al., 2012).

3. Results and discussion

3.1. Template identification, homology modeling and model quality

To identify modeling templates, I submitted the human FOXRED1 protein sequence (NCBI RefSeq NP_060017.1) to Phyre, a protein homology/analogy recognition engine that uses profile–profile matching algorithms to predict protein structure from amino acid sequence (Kelley and Sternberg, 2009). 120 templates with greater than 99% confidence were identified. I chose four of these to create a molecular model using the modeling package MODELLER (Eswar et al., 2003; Šali and Blundell, 1993). PDB ID: 1PJ5 is the structure of the *Arthrobacter globiformis* dimethylglycine oxidase (DMGO), a covalent flavoprotein (Leys et al., 2003; Basran et al., 2006). PDB ID: 1RYI is the structure of a noncovalent flavoprotein, the glycine oxidase from *Bacillus subtilis* (Mörtl et al., 2004). PDB ID: 2GAH is the structure of another non-covalent flavoprotein, heterotetrameric sarcosine oxidase (TSOX) (Chen et al., 2006). PDB ID: 2GF3 is the structure of a monomeric sarcosine oxidase (MSOX) with a covalent FAD (Wagner et al., 2000). These four templates all have a D-amino acid oxidase fold; only residues 1–430 of DMGO were used. An alignment of the four template sequences and FOXRED1 is presented in Fig. 1.

The quality of the model was further assessed with Dali, a program for protein structure comparison that uses a weighted sum of similarities of intra-molecular distances (Holm and Rosenstrom, 2010). When