Structure of the Membrane Proximal Oxidoreductase Domain of Human Steap3, the Dominant Ferrireductase of the Erythroid Transferrin Cycle

Iron plays an integral role in many biochemical processes essential for life. However excess iron leads to the production of highly reactive hydroxyl radicals by Fenton chemistry (1). These free radicals are deleterious to cells as they react indiscriminately with proteins, DNA and lipids. Hence, iron homeostasis is a highly regulated process and is critical to human health (2). Disorders in iron metabolism, are however, surprisingly common. Iron deficiency affects more than one billion people worldwide (3,4), while iron overload disorders (hereditary hemochromatosis) are among the most frequent single gene disorders in humans. For example occurrence of disease associated allele, HFE^{C282Y}, is as high as 10% in individuals of Northern European descent (5).

In humans, 80% of the daily iron need is required for hemoglobin synthesis and erythroid precursor cells utilize the transferrin cycle to import the needed iron. In this cycle, 2 molecules of holo-transferrin (Tf), each loaded with 2 ferric (Fe^{3+}) ions, bind the dimeric transferrin receptor (TfR) on the cell surface. The Tf_2:TfR_2 complex is endocytosed into the early endosome. Following acidification, Tf releases the Fe^{3+}, which is then reduced to Fe^{2+} by Steap3. Divalent metal iron transporter 1 (DMT1) then transports the Fe^{2+} across the endosomal membrane into the cytosol for use in cellular processes.

Recently, Ohgami et al. and Fleming have demonstrated that Steap3 (six transmembrane epithelial antigen of the prostate 3) is the major erythroid ferrireductase (6,7). Other Steap family members are also important in human health. Steap1 and Steap2 are found at particularly high levels in prostate cancer (10), making Steap1 an appealing target for cancer immunotherapy (9-12). The $Steap4^{-/-}$ knockout mice develop spontaneous metabolic disease (13). Steap proteins generally contain a C-terminal six transmembrane helical domain that co-ordinates a transmembrane heme (7,8). Steap2-4 also contain an oxidoreductase domain that lies on the cytosolic side of the membrane (8), allowing them to serve as a transmembrane reductase. Outside the Steap family, the oxidoreductase domain shows greater sequence similarity to an archaeal protein $F_{420}H_2$:NADP$^+$ Oxidoreductase (FNO, 28% identity) than it does to other human proteins. The archaeal FNO utilizes a 5′ deazaflavin that is unknown in mammals (14). Thus, the FNO-like domain in the mammalian Steap family is likely to use more common flavins such as FMN or FAD. The

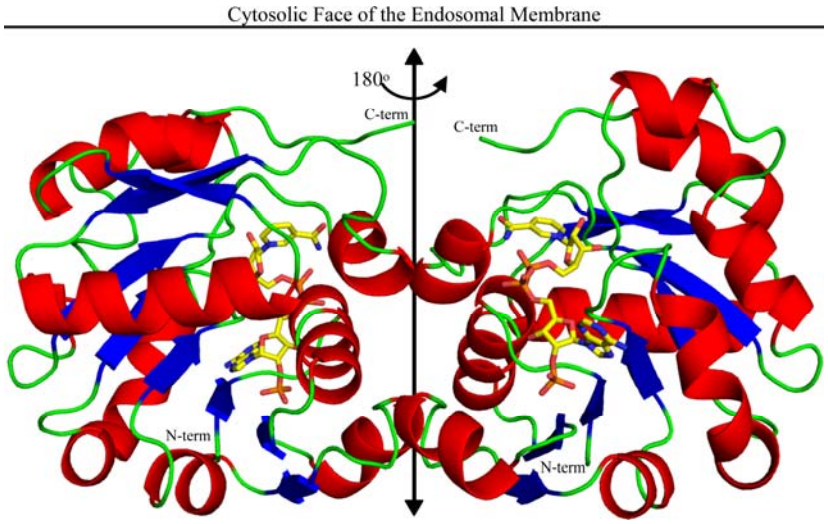

Figure 1:
The structure of the oxidoreductase dimer is depicted with helices in red, strands in blue, connecting loops in green, and the 2-fold axis running vertically (black double headed arrow). The truncated C-termini, which must connect to the C-terminal transmembrane domain, are in green at the top of the structure. NADPH (C, yellow; N, blue; O, red; and P, orange) runs up the front side of the right subunit (back side of the left subunit) with the adenine-ribose-2′-phosphate moieties near the bottom and the nicotinamide ring near the top.

reduction of iron is thought to occur by the sequential, transmembrane transfer of electrons from cytosolic NADPH to endosomal Fe^{3+} via the flavin and the intramembrane heme (6-8).

The structure of Steap3 oxidoreductase domain was solved using data collected at SSRL beamline 9-2. Crystals of Steap3 (apo-Steap3) and co-crystals of Steap3 with NADPH (Steap3-NADPH) diffracted to a resolution of 2 Å. The phases for the apo-Steap3 structure (PDB ID: 2vns) were determined by multiple isomorphous replacement with anomalous scattering, using data from Pt and Hg derivatives. The apo-Steap3 structure was used as the starting model to solve the Steap3-NADPH structure (PDB ID: 2vq3) by molecular replacement.

These structures lacked interpretable density for residues 1 to 28 and the N-terminal His_6-tag. However, the disordered N-terminus is poorly conserved between Steap2-4, and hence may not be essential for the oxidoreductase activity. Since the oxidoreductase domain is N-terminal to transmembrane domain, the C-terminus of the domain can be expected to orient towards the membrane with the dimer axis perpendicular to membrane (Figure 1). The oxidoreductase domain consists of 2 subdomains, the first is the classical dinucleotide binding domain composed of 2 Rossmann folds; the second is a C-terminal subdomain composed of 2 antiparallel β-strands with connecting α-helices.

Comparing the substrate-free and NADPH-bound structures showed no significant structural differences due to ligand binding. The C_α atoms in chain A of apo-Steap3 superpose on chain A of the Steap3-NADPH structure with a root mean square deviation (RMSD) of 0.26 Å. Both structures showed the presence of a two fold symmetric dimer in the asymmetric unit (Figure 1).

The Steap3 core superposes on the archaeal FNO (PDB ID: 1JAX) structure with an RMSD of 1.44 Å. However, there are critical differences, particularly with regard to the position of the dimer interface. In Steap3, the dimer interface is repositioned, which allows the Steap3 oxidoreductase domain to closely approach the membrane for electron transfer to the flavin and the transmembrane heme moiety. The NADPH is bound with the adenine ring on the membrane distal side, and the nicotinamide moiety on the membrane proximal side, facilitating electron transfer across the membrane (Figure 1,2A). This suggests that the intermediate electron acceptor, presumably a flavin, will bind "above" the nicotinamide ring, near the cytosolic face of the lipid bilayer. The similarities between the NADPH binding site of Steap3 and that seen in FNO (PDB ID: 1JAY) and Human Biliverdin IX Beta Reductase

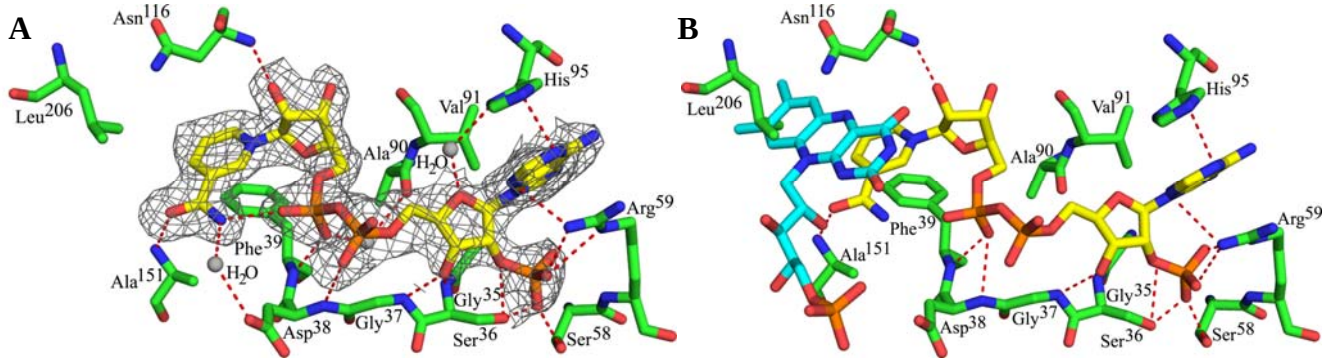

Figure 2:
(A) The Steap3 fold and active site structure reveal a nicotinamide ring that is bound in an unusual orientation with the amide moiety solvent exposed. The NADPH is colored as in Fig. 1. (B) FMN from biliverdin IX-beta reductase (PDB ID: 1HE4) is docked to the Steap3-NADPH structure by superposition of the BVR-B nicotinamide ring onto that of the Steap3-NADPH structure. The docked FMN clashes with the side chain of Leu^{206}. FMN is colored similar to NADPH, but with carbons in cyan.

(BVR-B, PDB ID: 1HE4) allows for superposition of the flavin within the Steap3 active site (Figure 2B). However, the docking results in a clash between the conserved Leu^{206} and FMN (Figure 2B). Because Leu^{206} is the last ordered residue in the Steap3 structure, the clash might be an artifact of the truncated protein. The presence of the C-terminal domain might prevent the residue from crashing down into the active site of the oxidoreductase domain. Alternatively, the clash may suggest that a conformational change is required for the flavin to bind, and might represent a possible gating mechanism to facilitate electron transfer into the endosome only in response to the presence of ferric ions (Fe^{3+}) awaiting reduction. Binding of Steap3 to other players in the Tf-cycle, like TfR or DMT-1, might promote the required conformational change. Interestingly, the dimeric Tf_2-TfR_2 complex carries 4 Fe^{3+} ions, a cargo that is nicely complimented by the Steap3 dimer with 2 molecules of NADPH (4 electrons). Furthermore the proximity of the binding site to the dimer interface suggests the oligomeric state of Steap3 might also influence the binding of the flavin molecule.

The Steap protein family clearly plays important roles in human health. Thus, a deeper understanding of the structure-function relationships in these proteins may aid the design of pharmaceuticals that specifically target processes in which Steap proteins are involved. For example, Steap specific inhibitors might inhibit intestinal iron uptake in iron overloaded individuals. In this light, these studies reveal the unique structural aspects of the Steap oxidoreductase domain, and suggest several strategies for targeting the Steap family of metallo-reductases with high specificity, a certain requirement for successful pharmacological intervention.

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