involve a proton relay mechanism via chemical exchange of hydrogen nuclei along a precise chain of water molecules.

Keywords: inorganic pyrophosphatase, neutron macromolecular crystallography, catalytic mechanism

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Structural insights into the enzyme catalysis of dissimilatory sulfite reductase

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Sulfite reductase mediates the reduction of sulfite to sulfide in sulfate-reducing bacteria. The existence of multi-forms of enzymes for the sulfite reduction and the details of the catalytic mechanism that can lead to a distribution of three products— trithionate ($S_3O_6^{2-}$), thiosulfate ($S_2O_3^{2-}$) and sulfide (S^{2-}) had been a subject of puzzle and controversy. The crystal structures of two active forms of dissimilatory sulfite reductase (Dsr) from *Desulfovibrio gigas*, Dsr-I and Dsr-II, are compared at 1.76 and 2.05 Å resolution, respectively [1]. The dimeric $\alpha_2\beta_2\gamma_2$ structure of Dsr-I contains eight [4Fe-4S] clusters, two saddle-shaped sirohemes and two flat sirohydrochlorins. In Dsr-II, the [4Fe-4S] cluster associated with the siroheme in Dsr-I is replaced by a [3Fe-4S] cluster. This structural feature allows Thrβ145 to position itself closer to the [3Fe-4S] in Dsr-II to replace the role of the Cysβ188 that ligates the [4Fe-4S] in Dsr-I. Electron paramagnetic resonance (EPR) of the active Dsr-I and Dsr-II confirm the co-factor structures, whereas EPR of a third but inactive form, Dsr-III, suggests that the siroheme has been demetallated in addition to its associated [4Fe-4S] cluster replaced by a [3Fe-4S] center. In Dsr-I and Dsr-II, the sirohydrochlorin is located in a putative substrate channel connected to the siroheme and capped by a dynamic loop from the ferredoxin domain. Cysβ198, which is located between the two types of porphyrins, exhibits double conformations. The γ-subunit C-terminus is inserted into a positively charged channel formed between the α- and β-subunits, with its conserved terminal Cysγ104 side chain covalently linked to the CHA atom of the siroheme in Dsr-I. In Dsr-II, the thioether bond is broken, and the Cysγ104 side chain moves closer to the bound sulfite at the siroheme pocket. Moreover, the γ-subunit C-terminus reveals another arrangement with an interaction between Cysγ93 and Cysγ104 in both Dsr forms. A second sulfite, interacting with the conserved Lysγ100, has been identified, implicating this site as the entry into a second putative substrate channel. These different forms of Dsr offer structural insights into a mechanism of sulfite reduction that can lead to $S_3O_6^{2-}$, $S_2O_3^{2-}$ and S^{2-} [1].

[1] Y.-C. Hsieh, M.-Y. Liu, V. C.-C. Wang, Y.-L. Chiang, E.-H. Liu, W. Wu, S.I. Chan, C.-J. Chen *Mol. Microbiol*. **2010**, *78*, 1101-1116.

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Structural insights into function of heterodimeric prenyltransferase

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Over 50,000 structurally diverse isoprenoids, which are built from C_5 isoprene units, are widely distributed in nature. Many kinds of isoprenoids, such as vitamins, hemes, and membrane lipids, are essential components of the cellular machinery of all organisms. Prenyltransferases, the so-called prenyl diphosphate synthases, catalyze consecutive head-to-tail condensations of isopentenyl diphosphates (IPP, C_5) on an allylic-substrate, such as dimethylallyl diphosphate (DMAPP, C_5) or farnesyl diphosphate (FPP, C_{15}), to form linear prenyl diphosphates with various chain-lengths. The linear prenyl diphosphates are common precursors of the carbon skeletons for all isoprenoids. According to the geometry of the newly formed double bonds of the products, prenyltransferases can be divided into two major classes, *trans*- and *cis*-prenyltransferases. Furthermore, the *trans*-prenyltransferases can be divided into two sub-classes, homo- and heterooligomeric enzymes. Compared to the homooligomeric enzymes, the structure-function relationships of heterooligomeric enzymes are not really investigated. The heterooligomeric enzymes are comprised of two different subunits, large and small subunits. The large subunits have been considered to participate in the substrate binding and the condensation reactions because the subunits possess the catalytic motifs conserved in the homooligomeric enzymes. In contrast, the small subunits do not possess such motifs and show very low amino-acid sequence identities with the homooligomeric enzymes. Thus, the function of the small subunits has not yet been clearly understood. In order to elucidate the molecular mechanism of heterooligomeric *trans*-prenyltransferases, particularly with respect to the role of the small subunits, we have determined the crystal structures of heterodimeric hexaprenyl diphosphate (HexPP, C_{30}) synthase from *Micrococcus luteus* B-P 26 (*Ml*-HexPPs) both in the substrate-free form and in complex with 3-desmethyl FPP (3-DesMe-FPP), an analogue of FPP [1].

The structure of the large subunit HexB is composed of mostly antiparallel a-helices joined by connecting loops. Despite the very low amino-acid sequence identity and the distinct polypeptide chain-lengths between the small subunit HexA and HexB, the structure of HexA is quite similar to that of HexB. Two aspartate-rich motifs and the other characteristic motifs in HexB are located around the diphosphate part of 3-DesMe-FPP. The aliphatic-tail of 3-DesMe-FPP is accommodated in a large hydrophobic cleft starting from the two substrate-binding sites of HexB and penetrating to the inside of HexA. Residues in both subunits participate in forming the wall of this cleft. These structural features suggest that the large subunit HexB catalyzes the condensation reactions and the small subunit HexA is directly involved in the product chain-length control in cooperation with HexB.

[1] D. Sasaki, M. Fujihashi, N. Okuyama, Y. Kobayashi, M. Noike, T. Koyama, K. Miki, *J. Biol. Chem.* **2011**, *286*, 3729-3740.

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Crystal structure of ferredoxin-NAD(P)$^+$ reductase from *Rhodopseudomonas palustris*

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