Structure of Heterotetrameric Sarcosine Oxidase (TSOX) at 1.85 Å Resolution

Zhi-wei Chen[a], F. Scott Mathews[a], Alshaimaa Hassan-Abdulah[b], Gouhua Zhao[b] Marilyn Schuman Jorns[b], *[a]Washington University School of Medicine, St. Louis, MO, USA [b]Drexel University College of Medicine, Philadelphia, PA, USA.* E-mail: zchen@biochem.wustl.edu

Heterotetrameric Sarcosine Oxidase is a bacterial flavoenzyme isolated from *Pseudomonas maltophilia*. It contains three coenzymes (FAD, FMN and NAD^+ and comprises 4 different subunits (α, 103 kDa; β, 44 kDa; γ, 22 kDa; δ, 11 kDa; total MW 180 kDa). TSOX catalyzes the oxidation of sarcosine (N-methylglycine) to yield hydrogen peroxide and formaldehyde. In the presence of tetrahydrofolate (THF), the oxidation is coupled to the formation of 5,10-methylenetetrahydrofolate (5,10-CH_2-THF). Sequence analysis suggests that NAD^+ as well as the 5,10-CH_2-THF synthase site are located in the α subunit whereas the covalent FMN site and the noncovalent FAD site, where sarcosine oxidation and peroxide formation take place, are located in the β-subunit.

The structure of selenomethionine-substituted TSOX was determined at 2.0 Å resolution by MAD phasing at three energies from data collected at the Biocars beamline 14ID of the APS. Location of 28 selenium sites with SOLVE and phasing with SHARP allowed automatic fitting of the solvent leveled map using Arp/Warp. Native TSOX was then solved at 1.85 Å resolution using MOLREP.

As predicted, the NAD^+ and putative folate binding sites are located in the α-subunit and the FAD binding site is in the β-subunit. The FMN is bound between the α and β subunits. Unexpectedly, a zinc ion was discovered bound to the δ-subunit and coordinated by 3 cysteine and 1 histidine side chain.

Keywords: flavoenzymes, channelling, MAD phasing