improved with the use of solvent flattening and averaging over the twofold non-crystallographic symmetry and the sequence fit to the resulting electron density. A single data set from homobasisthine HMG-CoA reductase was also collected. Difference Fourier clearly showed the methionine position, confirming the chain tracing. The HMG-CoA reductase monomer is composed of two mixed α/β domains. The large domain contains both the amino and carboxyl terminal ends, while the small domain is composed of an internal 100 residues. The large domain structure shows a central 24 residue α-helix surrounded by three sides by all-α-helical, anti-parallel α-sheets. The small domain is composed of 4 anti-parallel β strands and two connecting α-helices.

**PS-03.05.33** The study of Cu(I) and Cu(II). Dopamine β Hydroxylase (DBH) model complexes by X-ray absorption and diffraction


DBH is a tetrameric glycoprotein of 603 amino acids per monomer, a copper enzyme which catalyzes the transformation of dopamine into norepinephrine. According to the hydroxylation reaction mechanism proposed by Kilman (1982), the two copper atoms are involved and play different roles: one is an electron transfer while the other one, located near the substrate fixation site, directly acts on the dopamine hydroxylase via a Cu(II) species. Recently EXAFS studies on the DBH active site brought new insights on the active site by revealing the presence of a Cu-oximine bond coordinated to Cu(I), not present in the deuterated Cu(I) form of DBH (Scott, Sullivan, Doehring, 1998). In our laboratory, a series of Cu-complexes which are supposed to mimic the structure and the properties of the DBH active site, including N, O and S atoms have been prepared.

X-ray Absorption of nine Cu(I) and Cu(II) model complexes has been measured on XAL-1 station at LURE (Orsay, France). EXAFS and XANES spectra (Fig. 1) exhibit differences in accordance with the Cu ligand atom nature (N, O, S) and the Cu electronic state.

**Fig. 1.** EXAFS and XANES experimental spectra of Cu(I) and Cu(II) complexes.

The interpretation of our results is in progress and is based on the crystallographic structure obtained for two complexes of this series, an example is given on Fig. 2.

**Fig. 2.** EXAFS simulation and fit to the experimental.

The molecular simulation of our model with different force fields (MM2, GROMOS, AMBER) is also used to predict their conformation with some compatibility with regard to the X-ray diffraction and absorption data.


**PS-03.05.34** CRYSTAL STRUCTURE OF THE NONFUNCTIONAL CHORISOMATE MUTASE FROM BACILLUS SUBTILIS

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Chorismate mutase (CMase) is an enzyme in the pathway leading to biosynthesis of aromatic amino acids (shikimate pathway). It is the only characterized example of an enzyme in primary metabolism that catalyzes a pericyclic process, the rearrangement of chorismate to prephenate. An X-ray of this enzyme-catalyzed reaction remains a mystery. The simplicity of this rearrangement and the existence of a readily occurring uncatalyzed rearrangement might provide much insight for understanding enzyme-catalyzed rate-accution. Furthermore, catalytic antibodies with chorismate mutase activity have been prepared (Hilvert et al., 1988, and Jackson et al., 1988) and comparison of the active sites in the enzyme and antibodies will be a novel study to understand catalysis. CMase from Bacillus subtilis, Mr=14,5KD, is the smallest characterized natural chorismate mutase. The size of the enzyme, its Michaelis-Menten kinetics and absence of other associated enzyme activities (Gray et al., 1990) results in a simpler experimental system than other bifunctional and more complex chorismate mutases (e.g. from E. coli and yeast) for structural analysis to understand catalysis. This enzyme crystallizes in space group P2₁ with 12 monomers in the asymmetric unit. The crystal structure of CMase was determined using multiple isomorphous replacement method (derivatives - Fig. 1, Cs and a Selenomethionine protein with 72 Selenium in the asymmetric unit) the figure of merit is 0.710 for reflections at 3.0-3.5Å. A polyalanine trace of approximately 76 residues per monomer was made using the MIR map. This constitutes all secondary structure elements and 60% of the asymmetric unit. One round of phase combination using this partial polyalanine model improved the quality of the electron density significantly, especially the side chains and loop regions. The trace of the structure at 2.7Å is almost 87% complete. Upon completion of the trace, phase extension and refinement will be done to 1.9Å.

Data of complexes with a transition state analog and with the product, prephenate, are also available. Difference Fourier map have revealed the location of the active site. The structural analysis of the active site should provide some long-awaited for mechanistic information on the catalysis by chorismate mutase.

**References**

