ESRF	**Experiment title:** SAP: Serum Amyloid P component	**Experiment number:** LS-1533
Beamline: ID14-EH3	**Date of experiment:** from: [redacted]er 1999 to: 21 November 1999	**Date of report:** Feb 2000
Shifts: 6	**Local contact(s):** Soichi Wakatsuki	*Received at ESRF:*

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Report:

Serum amyloid P component (SAP) is a glycoprotein that binds a variety of ligands including other proteins, glycosaminoglycans and DNA. SAP is universally associated with the amyloid deposits in all forms of amyloidoses including Alzheimer's disease. The X-ray structure of SAP has been determined as has that of a structural homologue, namely C-reactive protein (CRP). CRP is a plasma protein, the level of which is elevated in acute inflammatory conditions and myocardial infarction. Small molecule ligands that displace SAP from amyloid fibres and thereby expose the fibres to proteolytic clearance mechanisms hold potential as therapeutic agents for the prevention and treatment of amyloidoses. We are currently refining the structure of a complex between SAP and a ligand which has potential to assist design of therapeutic agents.

Crystals of the SAP complex as well as crystals of a calcium free form of SAP were flash cooled and brought to ESRF for data collection at 100K on ID14-EH3. Here data were collected to 3.2 Å resolution on the SAP complex and 4.0 Å resolution on the calcium-free crystals. These datasets was processed using DENZO and MOSFLM. The SAP complex has an R_{merge} of 8.6 % and the calcium-free dataset (which is rather weak) has an R_{merge} of 41.9 %. These datasets have allowed the structure of the SAP complex to be solved by molecular replacement using the program MOLREP in the BLANC suite and refinement is in progress. Molecular replacement studies of the calcium-free dataset are in progress.

During the same experiment data were collected on a crystal of the apical domain of the chaperone cpn60 from *T. aquaticus*. This mini-chaperone was cloned and expressed since it may have advantages of greater thermal and denaturation tolerance over the equivalent domain of the *E. coli* chaperonin groEL. The purified protein yielded crystals which were initially solved at 2.5 Å resolution by molecular replacement. In the experiment at ESRF data were collected at 100K to 1.8 Å resolution and are currently being analysed.

5-aminolaevulinate dehydratase (ALAD, porphobilinogen synthase) is a key early enzyme of the porphyrin and corrin biosynthetic pathways which catalyses the condensation of two 5-aminolaevulinic acid (ALA) molecules to form the pyrrole porphobilinogen (PBG). The hereditary deficiency of functional dehydratase in humans is associated with the genetic disease Doss or ALA dehydratase porphyria, a disease with severe neurological symptoms. ALAD is extremely sensitive to inhibition by lead ions which is one of the major manifestations of acute lead poisoning which often leads to neurological disturbances. Succinylacetone (SA) accumulates in the blood of patients with tyrosinaemia and is known to be a potent inhibitor of ALAD. The SA molecule binds to ALAD by forming a Schiff base with Lys 263 of yeast ALAD. We have solved the complex of yeast ALAD with succinylacetone and defined its binding site. Unfortunately there is one major loop in the vicinity of the active site which exhibits significant disorder in the complex. SA binding also causes a substantial structural rearrangement of one of the other active site loops, the significance of which is not yet clear. To improve definition of these loop regions we have collected several datasets on this complex with the aim of getting highly redundant data. To this end a dataset to 1.8 Å was collected in available time during the experiment run. This was processed with MOSFLM and the CCP4 suite and combined with the other data giving an R_{merge} 19.1 %. This data has been included in our on-going refinement of the complex. Structural studies of this and other complexes (in progress) will help to piece together a picture of the substrate binding modes and catalytic mechanism.