<table>
<tr><td> </td><td>Experiment title:
SOUTHAMPTON / PORTSMOUTH BAG</td><td>Experiment number:
LS-1674</td></tr>
<tr><td>Beamline:
ID14-EH2</td><td>Date of experiment:
from: 8/04/00 to: 10/04/00</td><td rowspan="2">Date of report:
Aug 00
Received at ESRF:</td></tr>
<tr><td>Shifts:
6</td><td>Local contact(s):
DUMON Laurence</td></tr>
<tr><td colspan="3">Names and affiliations of applicants (* indicates experimentalists):

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Report:

5-Aminolevulinate dehydratase (ALAD), sometimes referred to as porphobilinogen synthase, catalyses the second step in the biosynthesis of tetrapyrroles involving the condensation of two 5-aminolaevulinic acid (ALA) molecules to form the pyrrole porphobilinogen (PBG). We have determined the X-ray structure of yeast ALAD showing for the first time that the enzyme forms a large homo-octameric structure in which each subunit adopts the TIM barrel fold with an extended N-terminal arm. The monomers associate to form compact dimers and four of these interact, principally via their arm regions, to form the octamer. In the active site of each subunit are two lysine residues, one of which forms a Schiff base link to the substrate. The enzyme has a well defined zinc binding site at the catalytic centre formed by 3 cysteines (α-site). The enzyme is strongly inhibited by lead ions and we have shown that these replace the zinc ion bound at the active site. The structure of *E. coli* ALAD was then determined at high resolution revealing for the first time the nature of a regulatory metal binding site (β-site) possessed by many ALADs. Our work revealed how known clinical mutations of human ALAD, some of which are harmful, would affect the structure of the enzyme. Work on

analysis of active-site directed mutants and complexes with inhibitors that form a Schiff base complex with the enzyme has yielded important information on the substrate binding sites and catalytic mechanism.

During the experiment at ESRF, data were collected on 3 complexes of yeast ALAD. These included 5-chlorolaevulinic acid which is a potent inhibitor. This dataset extended to a resolution of 1.7 Å and had an R-merge of 7.5 %. There have been claims that this inhibitor acts by alkylation of cysteine residues at the active site and more recent claims that alkylation of an active site lysine takes place. However the structure analysis of the complex with the yeast enzyme shows that the inhibitor binds only to the enzyme's Schiff base lysine residue which allows published biochemical data to be re-evaluated. Likewise the data collected on a complex with a dye that has been used to titrate one of the active site lysines (2-hydroxy-5-nitrobenzaldehyde (HNA); resolution = 2.0 Å, R-merge = 18.2 %) did not yield any convincing difference electron density for this molecule. More promising results were obtained with the inhibitor bromo-5-imidazolyl propionic acid (BIP). The latter complex yielded data to 2.0 Å with an R-merge of 8.4 % and showed that this molecule has bound to the triple-cysteine cluster at the active site. The interpretation of the map for this complex is in progress.

Atomic resolution datasets were collected on two complexes of the aspartic proteinase endothiapepsin. The aspartic proteinases are a family of enzymes involved in a number of important physiological and pathological processes. Catalytic action stems from two aspartate groups at the active site. Current proposals for the catalytic mechanism are largely based on X-ray structures of inhibitor complexes but these proposals differ in the assignment of protonation states to the catalytic groups during the reaction. We are attempting to collect atomic resolution X-ray data on complexes with various classes of transition state analogue to aid definition of the protonation states of residues at the active site and the putative role of low-barrier hydrogen bonds in the mechanism. In this respect, the atomic resolution structures of the phosphinic acid analogue (PD-130,328) and the reduced bond analogue (H-256) are of great interest. Data on both complexes were collected to a resolution of 0.88 Å and had R-merge values of 7.9 % for the H-256 complex and 10.0 % for the phosphinate analogue. The structures are currently being refined with SHELX-97.

During the same experimental run datasets were also collected on serine hydroxymethyl transferase to around 2.0 Å resolution and the acute phase protein serum amyloid P-component (SAP) to around 4.0 Å resolution. Analysis of these data is in progress.