PS04.01.45 CRYSTAL STRUCTURE OF A NON-PEPSIN-TYPE ACID PROTEINASE, *Aspergillus niger* PROTEINASE A. Hiroshi Sasaki[1], Atsushi Nakagawa[2], Tomonari Muramatsu[3], Kenji Takahashi[4], Masaru Tanokura[1], [1]Biotechnology Research Center, University of Tokyo, Bunkyo-ku, Tokyo 113, [2]Faculty of Science, Hokkaido University, Sapporo, Hokkaido 060, [3]Biophysics Division, National Cancer Center Reseach Institute, Chuo-ku, Tokyo 104, [4]School of Life Science, Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo 19203, Japan

The molecular structure of *A. niger* proteinase A was solved by the MIR method at 2.0 Å resolution.

Proteinase A has been regarded as a non-pepsin-type acid proteinase, because of its properties. It is insensitive to pepstatin, which is a common and specific inhibitor for the pepsin-type aspartic proteinases. Moreover, for the lack of the consensus sequence, Asp-Thr/Ser-Gly, around the catalytic aspartic acid residues in the pepsin-type aspartic proteinases, it remained to be elucidated which residues participate in the catalysis and how the mechanism operates.

The crystal of proteinase A was grown by the hanging-drop vapor diffusion method with ammonium sulfate as a precipitant. Its space group was $P2_12_12_1$, with unit cell dimensions of a=54.7Å, b=70.4Å, and c=38.0Å. On the assumption of one enzyme molecule per asymmetric unit, the solvent content (Vsol) was estimated to be 26%, indicating that the value was among the smallest for protein crystals. Intensity data were collected by using the Weissenberg camera for macromolecular crystallography at the BL6A in the Photon Factory, National Laboratory for High Energy Physics. By using three heavy atom derivatives, the MIR phases were calculated and then density modification was applied. Density modification program from York University (dm) was very effective for improving the phases.

In the preliminary analysis, the overall shape of the molecule was similar to that of pepsin-type acid proteinases which form a croissant shape. The secondary structure element of the folded ß sheets, however, was unique. Now the refinement is in progress and more precise structure and potential active site will be discussed.

PS04.01.46 STRUCTURAL INVESTIGATION OF REGULATION IN *BACILLUS STEAROTHERMOPHILUS* PYRUVATE KINASE. Simon C Lovell[1], Daniel Ungar[2], Abdul H Mullick[2] and Hilary Muirhead[2]. [1]Biochemistry Dept, University of Bath, Claverton Down, Bath; [2]Biochemistry Dept, University of Bristol, University Walk, Bristol

A variety of techniques are being used to study enzyme regulation using the glycolytic enzyme pyruvate kinase, using the moderate thermophile *Bacillus stearothermophilus* as a model system. Our approach involves the use of X-ray crystallography, site directed mutaganesis, fluorescence and both steady state and transient kinetics.

Pyruvate kinase is a tetrameric enzyme and catalyses the reaction:

PEP + Mg.ADP ——————> Pyruvate + Mg.ATP + H+

We have demonstrated the existence of at least four distinct conformations for *B stearothermophilus* pyruvate kinase, which is activated by ribose-5-phosphate (1). These structures correspond to the unliganded, phosphoenolpyruvate (PEP) bound, activator bound and fully liganded species. Structures have been published for the unliganded enzyme from *E coli* (2) and the constitutively activated forms from muscle (3,4). In order to understand the regulation of this complex enzyme it will be necessary to know the structure of all of the above conformations - preferably from the same species.

We have determined the structure of the unliganded of the *B stearothermophilus* enzyme at a resolution of 3.2 Å. The evidence for this enzyme having a T-state like conformation is presented and the differences between it and the *E coli* structure are described. *B stearothermophilus* pyruvate kinase has a 113 amino acid C-terminal extension when compared to all other pyruvate kinases of known sequence (5). Surprisingly the removal of this extension has very little effect on either activity or regulation. At the current resolution and state of refinement this extension cannot be seen in the crystal structure, although a cavity of the appropriate size and position exists.

1 Lovell, Mullick and Muirhead, in preparation.
2 Mattevi et al (1995) *Structure* **3** 729
3 Muirhead et al (1986) *EMBO J* **5** 475
4 Larsen et al (1994) *Biochemistry* **33** 6301
5 Sakai and Ohta (1993) *Eur J Biochem* **211** 851

PS04.01.47 X-RAY STRUCTURE OF Mn-PHOSPHATE COMPLEX OF YEAST INORGANIC PYROPHOSPHATASE AT 2.4 A RESOLUTION. B. Vainshtein, E. Harutyunyan, I. Kuranova, Institute of Crystallography, Moscow, Russia V. Lamzyn, Z. Dauter, K. Wilson, EMBL Outstation, Hamburg, Germany

The three-dimensional crystal structure of the manganese-phosphate complex of inorganic pyrophosphatase from Saccharomyces cerevisiae has been refined to an R factor of 19.O % at 2.4 A resoluion. 21721 unique reflections ($I > \sigma$) with average redundancy 3.4 and 80 % completness were measured from a single crystal using synchrotron radiation and imaging plate. Atomic model of homodimeric molecule including 4496 protein atoms, 8 Mn and 4 phosphate groups and 222 solvent molecules were refined without non-crystallographic symmetry restraints. The estimate of the r.m.s. coordinate error is O.4 A using either the σ_A plot or the superposition of the two crystallographically independent subunits. The active site in each subunit contains four manganese ions and two phosphates. The manganese ions are coordinated by the side chains of aspartate and glutamat residues. The phosphate groups, identified on the basis of their local stereochemistry, interact either directly or via water molecules with manganese ions and lysine, arginine and tyrosine side chains. The phosphates are bridged by two of the manganese ions. The outer phosphate is exposed to solvent. The inner phosphate is surrounded by all four manganese. The ion binding sites are related to the order of binding previously established from kinetic studies. A hypothesis for the transition state of the catalytic reaction is put forward.