PS04.01.26 CATALYTIC CONFORMATION OF *PSEUDOMONAS* 7A GLUTAMINASE-ASPARAGINASE (PGA): CRYSTAL STRUCTURE OF THE PGA-SO_4^{2-}-NH_4^+ COMPLEX AT 1.7 Å RESOLUTION. C. Jakob[1], M. LaCount[2], K. Lewinski[3], J. Roberts[4], L. Lebioda[2] [1]Chemistry, Davidson College, Davidson, NC 28036, USA, [2]Chemistry and Biochemistry, Univ. of South Carolina, Columbia, SC 29208,USA, [3]Chemistry, Jagiellonian Univ., Cracow, Poland, [4]Pharmacy, Univ. of South Carolina, SC 29208, USA

Pseudomonas 7A Glutaminase-Asparaginase (PGA) catalyzes the hydrolysis of D- and L- isomers of glutamine and asparagine. Type-1 crystals of PGA have been obtained from high salt concentrations. The space group is $C222_1$ with unit-cell dimensions a = 78.62, b = 135.80, and c = 137.88Å. X-ray diffraction data set was collected on an R-Axis IV area detector and is 86% complete to 1.71Å with 68,971 reflections and R_{merge} = 7.5%. The molecular replacement method with *Escherichia coli* L-Asparaginase model was employed to solve the crystallographic structure of PGA at 1.7Å resolution. The resultant high resolution electron density maps enabled us to introduce minor revisions to the amino acid sequence. Each subunit of PGA has an active site consisting of a relatively rigid region and a flexible loop. The catalytic triad, which is analogous to those of serine proteases, has been previously assigned to residues Thr100, Asp101, and Lys173 and are located in the relatively rigid portion of the active site. Earlier published structures of PGA report the flexible loop (residues Thr20-Gly40) in either an open conformation or as a partially disordered region. In the PGA structure reported here, the active site flexible loop is in the closed conformation in both subunits and excellent electron density is observed. This conformation is induced by the presence of sulfate and ammonium ions in the active site. This suggests that the process of loop closure is driven by electrostatic interactions.

PS04.01.27 ANALYSES OF DIFFERENT BINDING MODES OF LIGANDS IN THREE TYPES OF CRYSTALS OF L-ASPARAGINASE FROM *E.coli* A1-3KY3598. N. Nandhagopal, M. Hirokawa, N. Tanaka, T. Senda and Y. Mitsui, Department of BioEngineering, Nagaoka University of Technology, Nagaoka, Niigata, 940-21, Japan

Amidohydrolases from *Echerichia coli* and *Erwinia chrysanthemi* exhibit a relatively high specificity for asparagine and are referred to as asparaginases. The monoclinic crystal structure of L-asparaginase II from *Escherichia coli* K12 has been determined at 2.3Å resolution by Swain *et al.*(1993)[1].

The asparaginase from *E.coli* A-1-3KY3598[2] was used for crystallization in the present analysis. The molecule is composed of four subunits and the molecular weight is *ca.* 136 Kda. Three types of crystals, all belonging to a space group $P2_12_12_1$, have been obtained, one of them as glutamic acid complexes (Glu-complex) and the other two as aspartic acid complexes (Asp-complex1 and Asp-complex2). For the three types of crystals, the cell parameters were different from each other by up to 15%. In the Glu-complex the loop region in the active site is not clearly resolved in the electron density map. Crystallographically there are two binding sites in each asymmetric unit. In the Asp-complexes, equivalent types of aspartate binding were observed for each of the two binding sites. This may be related to the presence of rigid loop region in each of the two binding sites. Surprisingly, in the Glu-complex, each of the bound glutamate ligands was found to have a different comformation. Detailed analyses of the three types of crystals are in progress.

Comparative studies of the three types of crystal structures and the result of the analyses aiming at clarifying the structural basis of their preferred specificity for asparagine (rather than glutamine) will be presented.

1. Swain, A. *et al.* (1993) *Proc. Natl Acad. Sci. USA*, **90**, 1974
2. Mitsui, Y. *et al.* (1978) *Acta Crystallogr.*, **A34**, 560.
3. Hirokawa, M. *et al.* (1994) *Protein Eng.*, 7, 1165

PS04.01.28 KETOACID REDUCTOISOMERASE: FIRST STRUCTURE OF A POTENTIAL HERBICIDE TARGET. Valerie Biou, Eva Pebay-Peyroula, Claudine Cohen-Addad, Institut de Biologie Structurale, Grenoble, France; Renaud Dumas, Dominique Job & Roland Douce, Laboratoire Mixte CNRS-Rhône-Poulenc, Lyon, France.

Ketoacid Reductoisomerase (KARI) takes part in the synthesis of Valine and Isoleucine in plants and micro organisms. KARI is a dimer of 60 kDa per monomer, and it has several exciting characteristics: it specifically recognizes two different substrates with a micro molar affinity, and the reaction requires the presence of NADPH and two Mg ions. This biosynthesis pathway is absent from animals and therefore this enzyme is a good potential target for a rational search for herbicides. Two molecules have been found to inhibit KARI and have herbicidal effects at high doses. They both are analogues of the reaction intermediate and are competitive of the substrate. We present here the structure at 1.65Å resolution of the spinach enzyme overexpressed in E. coli, crystallised in the presence of one inhibitor, IpOHA, and of NADPH and Mg ions.

The structure was solved using the Multiple Isomorphous Replacement method and non crystallographic symmetry averaging. The electron density map clearly shows the presence of two hexa-liganted magnesium ions in the active site. It also reveals interactions between the inhibitor, the protein and the NADPH. Details of those interactions will be presented, with some insight as to how the reaction is performed by KARI.

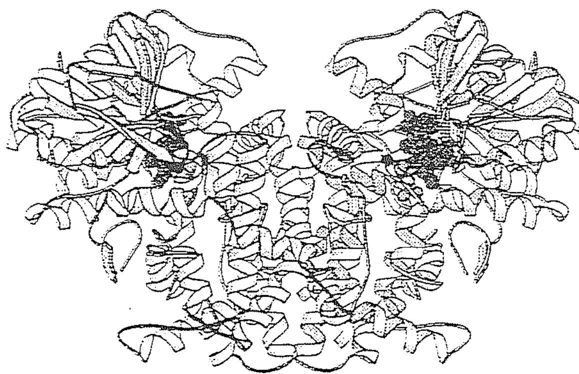

Figure 1: KARI dimer. The ligands are represented with spherical atoms: NADPH in dark grey, Mg^{++} in black and IpoHa in lighter grey. Program Molscript (P. Kraulis, J. Appl. Cryst. (1991), 24, 946-950) was used to generate the figure.

PS04.01.29 CRYSTALLIZATION OF CALPAIN A Ca^{2+} DEPENDENT CYSTEINE PROTEASE. Zongchao Jia, Qilu Ye, Peter L. Davies and John S. Elce. Department of Biochemistry, Queen's University, Kingston, Ontario, K7L 3N6 Canada

The calpains (EC 3.4.22.17) are a family of Ca^{2+} dependent cysteine proteases found in the cytosol of animal cells. Their precise physiological role is uncertain, however it is likely that they are involved in cell signalling and in cytoskeletal modifications. The ubiquitous calpains have a large catalytic subunit (80 kDa) composed of 4 domains, and a small regulatory subunit (30 kDa) composed of 2 domains. The enzymes are activated by Ca^{2+}, and then undergo autolysis. In order to understand the structural basis for Ca^{2+} dependent calpain activation and provide a molecular explanation for autolysis, we are interested in determining the structure of calpain II by X-ray crystallography. To avoid autolysis and oxidation which present great problems during recombinant protein production and crystallization, an inactive C105S active-site

mutant (80k/21k)has been used. After testing a wide range of crystallization conditions, we obtained crystals of this mutant with size of approximately 0.25x0.17x.03 mm, which were rather fragile and diffracted only to low resolution. Under somewhat different crystallization conditions, the mutant also crystallized in the presence of Ca^{2+}, although this was accompanied by heavy Ca^{2+} induced aggregation. Crystallization parameters were varied so that initial nucleation takes place before substantial precipitation, which is difficult to control. We are currently optimizing the conditions to improve crystal quality. (Supported by MRC of Canada and PENCE, Canada)

PS04.01.30 CRYSTAL STRUCTURE OF SUBTILISIN BPN' FOLDED WITHOUT THE PRODOMAIN. Orna Almog, D. Travis Gallagher, Maria Tordova, Joel Hoskins, Phillip Bryan, Gary L. Gilliland. Center for Advanced Research in Biotechnology of the University of Maryland Biotechnology Institute and the National Institute of Standards and Technology, 9600 Gudelsky Drive, Rockville, MD 20850

Secreted proteases such as subtilisin are usually synthesized as inactive proenzymes that are then processed into an active form. Once denatured, mature subtilisin BPN' refolds very slowly to the native state, but the refolding can be catalyzed by the addition of the prosegment [Bryan et al., (1995) *Biochemistry* **34**, 10310-10318]. Deletion of the high-affinity calcium-binding site (A-site; residues 75-83) from subtilisin BPN' can accelerate the refolding of the mature protein to its native form. To help substantiate that refolding without the prodomain produces a native-like fold, Sbt70, a subtilisin BPN' variant, was cloned and expressed in an *E. coli* system without the prodomain. Sbt70 has, besides the deletion of the calcium binding site (residues 75-83), six stabilizing mutations, K43N, M50F, A73L, Q206V, Y217K and N218S, were introduced to restore lost thermal stability. The active-site serine was also replaced by alanine (S221A) to prevent autolysis. The isolated variant was folded without the prodomain by dialysis against phosphate buffer and crystallized. Crystals of Sbt70 belong to the space group $P2_12_12_1$ with unit cell parameters a=60.67 Å, b=84.02 Å, c=54.17 Å. The structure was solved by molecular replacement and refined using 2.2 Å data. The final R factor is 0.17. The structure comparison with other high-resolution subtilisin BPN' structures shows that the conformation of Sbt70 is virtually identical to that of natural mature subtilisin BPN'. An analysis of three previously unreported stabilizing mutations, K43N, A73L, and Q206V, will also be described along with the interactions with the enzyme of the tetra-peptide ALAL that was unexpectedly found bound in the active site.

PS04.01.31 CRYSTAL STRUCTURE OF THE ASPARTIC PROTEINASE FROM *Rhizomucor miehei* at 2.15 Å. Jian Yang*, Alexei Teplyakov# and J. Wilson Quail*. *Department of Chemistry, University of Sasakatchewan, Saskatoon, Saskatchewan, Canada S7N 5C9, and #EMBL, Hamburg Outstation, Hamburg, Germany

The crystal structure of the aspartic proteinase from *Rhizomucor miehei* (RMP, EC. 3.4.23.23) has been refined to 2.15 Å resolution to a crystallographic *R*-factor of 21.5% and a *R*-free factor of 28.1%. The protein contains two domains, which consist predominantly of β-sheets. The C-terminal domain is less rigid than the N-terminal domain due to the crystal packing. A large substrate binding cleft is clearly visible between the two domains and the catalytic residues Asp38 and Asp237 are located in the middle of the cleft with a water molecule bridging these two carboxyl groups. We will report the results of the surface electrostatic potential calculations of RMP and other aspartic proteinases' active sites that show the pH optimum of each aspartic proteinase is determined by the electrostatic potentials of the two carboxyl groups of the two catalytic aspartates. The electrostatic potentials of the two aspartates' carboxyl groups are, in turn, determined by the active-site environment, especially residues 19 and 332. The protein is glycosylated at Asp79 and Asp188. The glycosylations are believed to stabilize the protein by sterically inhibiting the attack of other proteinases and contributing to the protein's high thermal stability. Three-dimensional structure alignment and sequence alignment of RMP with other aspartic proteinases have shown that RMP is structurally similar to *Mucor pusillus* aspartic proteinase (MPP). RMP and MPP are as distinct from other fungal enzymes as they are from the mammalian enzymes. This suggests that RMP and MPP diverged from the main stream of aspartic proteinases at an early stage of evolution.

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PS04.01.32 CRYSTAL STRUCTURES OF THE GLU[18], GLN[18], ASP[18] AND ASN[18] VARIANTS OF TURKEY OVOMUCOID INHIBITOR THIRD DOMAIN COMPLEXED WITH *STREPTOMYCES GRISEUS* PROTEINASE B AT VARIOUS pHs. K. Huang[1], M. Qasim[2], Wuyuan Lu[2], Michael Laskowski, Jr[2]. and Michael N.G. James[1]. [1]MRC Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, AB T6G 2H7 Canada; [2]Department of Chemistry, Purdue University, West Lafayette, Indiana 47907-1393.

The ionizable P_1 residues Glu18I and Asp18I of turkey ovomucoid inhibitor third domain (OMTKY3) variants display unusually high pK_a values (8.7 and 9.4 respectively) when complexed with *Streptomyces griseus* Proteinase B. The complexes remain associated even after the carboxyl groups of the P_1 residues are ionized. The 1.8 Å crystal structures of the Glu^{18} and Asp^{18} variants of OMTKY3 have been determined in complex with SGPB at pH 6.5 (the neutral form) and at pH 10.7 (the ionized form). The crystal structures of the Gln^{18} and Asn^{18} variants of OMTKY3 have been determined at pH 6.5 (the Gln18 variant also at pH 10.7). Surprisingly, a potassium ion has been identified in the pH 10.7 structures of the Glu^{18} and Asp^{18} variants. Apparently it is recruited to the S_1 pocket to balance the negative charge of the P_1 residue at pHs above its pK_a. This K^+ ion forms a saltbridge with the carboxyl groups of the P_1 side-chains. Comparisons among the seven structures determined here show that they are remarkably similar except for the solvent structure in their S_1 pockets. Within the S_1 pocket of these complexes the solvent structure varies both with the size of the side-chain (Asp vs Glu) and with side-chain polarity (e.g. Glu^0, Glu^- and Gln).

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