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 $P2_122_1$, 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.

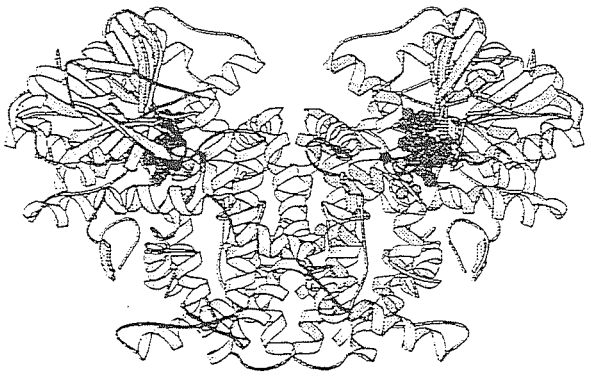

Figure 1: KARI dimer. The ligands are represented with spherical atoms: NADPH in dark grey, Mg^{++} in black and IpoHa in lighter grey. Program Molscript (P. Kraulis, J. Appl. Cryst. (1991), 24, 946-950) was used to generate the figure.

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