PS04.01.26 CATALYTIC CONFORMATION OF
PSEUDOMONAS 7A GLUTAMINASE-ASPARAGINASE
(PGA): CRYSTAL STRUCTURE OF THE PGA-SO42-·NH4 +
COMPLEX AT 1.7 Å RESOLUTION. C. Jakob1, M. LaCount2,
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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 C2221 with unit-cell dimensions
a = 78.62, b = 135.80, and c = 137.88 Å. X-ray diffraction data set
was collected on an R-Axis IV area detector and is 86% complete
at 1.71 Å with 68,971 reflections and Rmerge = 7.5%. The molecular
replacement method with Escherichia coli L-Asparaginase model
was employed to solve the crystallographic structure of PGA
at 1.7 Å 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 glutamate ligands, is 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-KY35982, N. Nandhagopal,
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Amidolydrolases from Escherichia 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 2.3 Å resolution by Swain
et al.(1993). The asparaginase from E.coli A1-KY35982 was used for crystal
pisation in the present analysis. The molecule is composed of four sub-
units and the molecular weight is ca. 156 Kda. Three types of crystals, all
belonging to a space group P212121, have been obtained, one of them as
promiscuous crystals (Fig. 1a) 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
aspartate ligands was found to have a different conformation. 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.


PS04.01.28 KETOACID REDUCTOISOMERASE: FIRST
STRUCTURE OF A POTENTIAL HERBICIDE TARGET.
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nic Job & Roland Douce, Laboratoire Mixte CNRS-Rhône-
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Ketoacid Reductoisorase (KARI) takes part in the synthe-
sis 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
inhibit KARI and have herbicidal effects at high doses. They
both are analogues of the reaction intermediate and are competi-
tive of the substrate. We present here the structure at 1.65 Å reso-
olution 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 averag-
ing. The electron density map clearly shows the presence of two
hexa-ligated 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
946-950) was used to generate the figure.

PS04.01.29 CRYSTALLIZATION OF CALPAIN A Ca2+-
DEPENDENT CYSTEINE PROTEASE. Zongchao Jia, Qilu Ye,
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The calpains (EC 3.4.22.17) are a family of Ca2+- dependent
cysteine proteases found in the cytosol of animal cells. Their pre-
cise 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 Ca2+, and
then undergo autolysis. In order to understand the structural basis
for Ca2+ dependent calpain activation and provide a molecular
explanation for autolysis, we are interested in determining the struc-
ture 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