Title	Crystal Structure of Asparagine Synthetase Reveals a Close Evolutionary Relationship to Class II Aminoacyl-tRNA Synthetase (MOLECULAR BIOFUNCTION-Functional Molecular Conversion)
Author(s)	Nakatsu, Toru; Kato, Hiroaki; Oda, Jun'ichi
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Crystal Structure of Asparagine Synthetase Reveals a Close Evolutionary Relationship to Class II Aminoacyl-tRNA Synthetase

Toru Nakatsu, Hiroaki Kato, and Jun'ichi Oda

The crystal structure of *E. coli* asparagine synthetase has been determined by X-ray diffraction analysis at 2.5 Å resolution. The overall structure of the enzyme is remarkably similar to that of the catalytic domain of yeast aspartyl-tRNA synthetase despite low sequence similarity. These enzymes have a common reaction mechanism that implies the formation of aminoacyl-adenylate intermediate. The active site architecture and most of the catalytic residues are also conserved in both enzymes. These enzymes have probably evolved from a common ancestor even though their sequence similarities are small.

Keywords : X-ray crystallography/ Evolution/ Structural similarity/ Aspartyl-tRNA synthetase/ Amino acyl-adenylate intermediate/ Enzymatic reaction

Asparagine synthetase (AsnA) from *E. coli* catalyzes the synthesis of L-asparagine from L-aspartic acid and ATP in the presence of a magnesium ion using ammonia as a nitrogen source [1]. The crystal structure of the *E. coli* AsnA (amino acids 4 - 330) complexed with L-asparagine was determined by X-ray crystallography using multiple isomorphous derivatives at 2.5 Å resolution. The structure exists as a dimer of identical subunits. Each monomer consists of a core eight-stranded, mostly anti-parallel β-sheet that is flanked by two long and eight shorter α-helices. A small lobe composed of a three-stranded β-sheet (Fig. 1) completes the protein fold. L-Asparagine is bound in a rather open and solvent-exposed cleft located on the surface of the eight-stranded β-sheet.

The overall structure of *E. coli* AsnA is remarkably similar to the catalytic domain of yeast aspartyl-tRNA synthetase (AspRS) (Fig. 1). Their core structural elements (175 α-carbon atoms) can be superimposed with a root-mean-square distance of 1.9 Å. This value is similar with that derived from superinposition among class II aminoacyl-tRNA synthetases in which AspRS is involeved.

There is low sequence similarity between *E. coli* AsnA and the catalytic domain of yeast AspRS**,** however, their sequence comparison based on the crystal structure indicates that most of the structurally and catalytically important residues in AspRS are conserved over

MOLECULAR BIOFUNCTION — Functional Molecular Conversion —

Scope of research

Our research aims are to elucidate structure-function relationships of biocatalysts in combination with organic chemistry, molecular biology and X-ray crystallography, and to design and generate a novel biocatalysis for use as a tailor-made catalyst for organic reations. Major subjects are (1) Design and synthesis of transition-state analogue inhibitors of ATP-dependent synthetases, (2) Time-resolved X-ray crystallographic study of glutathione synthetase, (3) X-ray diffraction analysis of tropinone reductase II, luciferase and asparagine synthetase, (4) Design and preparation of catalytic antibodies for chemiluminescence, (5) Overexpression and purification of pyruvate phosphate dikinase from Maize, and (6) Characterization of an activation protein of Pseudomonas lipase.

Prof
ODA, Jun'ichi
(D Agr)

Assoc Prof
HIRATAKE, Jun
(D Agr)

Instr
KATO, Hiroaki
(D Agr)

Assoc Instr
NAKATSU, Toru
(D Agr)

SHIBATA, Hiroyuki (DC)
AOYAGI, Amane (DC)
KATOH, Makoto (DC)
SAWA, Masaaki (DC)
YAMASHITA, Atsuko (DC)
INOUE, Makoto(DC)
IRIE, Takayuki (MC)
OOKI, Yasushi (MC)
KOIZUMI, Mitsuteru (MC)
ENDO, Masaharu (MC)
FUJII, Ryota (MC)
NAKANISHI, Hidemitsu (MC)
TOKUTAKE, Nobuya
(RF, D Agr)

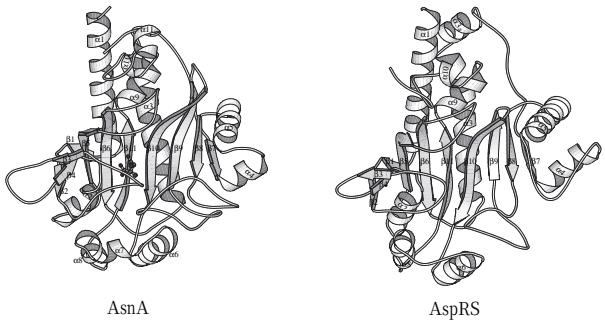

Figure 1. Ribbon diagram ofAsnA from *E. coli* and the catalytic domain of AspRS from *S. cerevisiae*. The ball and stick model in AsnA shows the ligand L-asparagine.

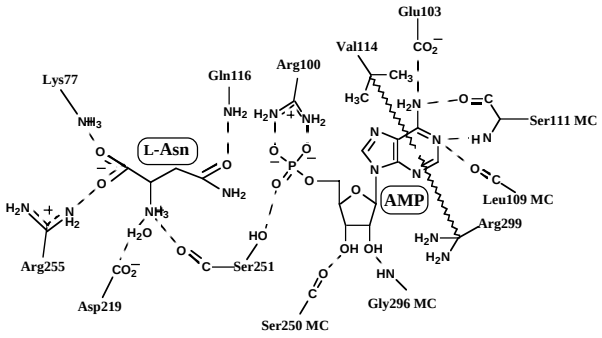

Figure 2. Schematic drawing of the interaction of the ligands and the residues of asparagine synthetase in the catalytic region. Dashed and waved lines correspond to polar and hydrophobic interactions, respectively.

the entire length of AsnA. A proline residue, Pro35, is conserved and seems to be important for subunit-subunit interaction in AsnA as well as in AspRS. There is a small cluster of conserved residues (GGGIG, 292—296). This sequence motif is a part of the highly conserved residues, called motif 3 in the class II aminoacyl-tRNA synthetases, allowing the formation of a cavity that can accommodate bound ATP [2]. In addition, the other conserved residues are also catalytically critical, and interact with the substrates ATP or L-aspartic acid in yeast AspRS. AspRS uses two conserved arginine residues, corresponding to Arg100 and Arg299 in AsnA, to coordinate the α- and γ-phosphates of ATP, respectively. The serine residue of AspRS, which interacts with the α-phosphate of ATP, is conserved as Ser251 in AsnA. Finally, the three AspRS residues which recognize L-aspartic acid by forming a network that interacts with its β-carboxylate group correspond with Lys77, Glu120 and Arg255 in AsnA.

To confirm the function of these conserved residues in the *E. coli* AsnA, the locations of its ligands, L-asparagine and AMP in the three dimensional structure were determined at 2.2 Å resolution (Fig. 2). The crystallographically observed positions of these conserved residues described above are superposable with the related side chains in AspRS. Indeed both enzymes are expected to proceed through the formation of an aminoacyl-adenylate intermediate in their reaction.

The largest differences between AsnA and AspRS were found in their recognition of the reactive carboxyl group and an amino group in the substrate L-aspartic acid (Fig. 3). In the ternary complex structure of *E. coli* AsnA, the amino group of Gln116 interacts with the β-carbonyl group of the bound L-asparagine while the side chain carboxyl group of Asp219 interacts with the amino group of the ligand through a water molecule. Thus, both residues are likely to facilitate the recognition of the substrate L-aspartic acid with a productive binding mode. Structural and mutagenic studies implicated that yeast

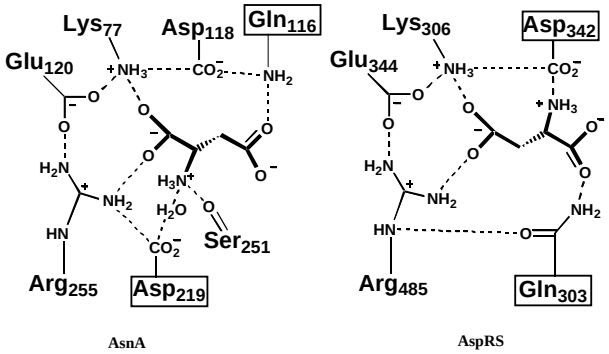

Figure 3. Schematic drawing of the substrate L-aspartic acid binding site of asparagine synthetase and aspartyl-tRNA synthetase.

AspRS utilizes the side chain carboxyl group of Asp342 and the amino group of Gln303 to interact with the amino group and α-carboxyl group of the substrate, respectively. Those glutamine or aspartic acid residues are found at different positions in their crystal structures, however, the role of the glutamine and aspartic acid residues in the substrate recognition are similar.

Crystal structure of *Thermus thermophilus* AspRS [3] has been solved and has provided the finding that it has an extra domain between the helices, aligned with helix 4 and 5 of *E. coli* AsnA, nevertheless the other part of the structure is superimposable with the yeast enzyme. The amino acid sequence of *E. coli* AsnA is less similar to that of *T. thermophilus* or *E. coli* AspRS than that of yeast one. In addition, amino acid sequence of archaean AspRS in the catalytic domain region is similar to the yeast enzyme than *E. coli* one, and there is no additional domain found in the *E. coli* enzyme. These evidence may suggest that this archaean AspRS could be a reliable candidate of the ancestor protein for AsnA.

References

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