*Acta Cryst.* (1996). D52, 604–606

# Crystallization and preliminary crystallographic study of asparagine synthetase from *Escherichia coli*

TORU NAKATSU, HIROAKI KATO AND JUN'ICHI ODA* *at Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan*

*(Received 7 August 1995; accepted 3 January 1996)*


## Abstract

Crystals of *Escherichia coli* asparagine synthetase have been obtained from 45% saturated ammonium sulfate solution at pH 7.5 by using its Cys-free (Cys51→Ala, Cys315→Ala) mutant. The crystals belong to space group $P2_1$, with cell dimensions $a = 52.90\,(4)$, $b = 126.2\,(2)$, $c = 52.8\,(1)$ Å and $\beta = 105.3\,(1)°$. The self-rotation function specifies that there is one dimer in the asymmetric unit and the subunits are related by a non-crystallographic twofold axis. Complete data sets up to 2.7 Å resolution have been collected on an R-AXIS IIc imaging-plate system.


## 1. Introduction

Asparagine synthetase [L-aspartate: ammonia ligase (AMP-forming) E.C. 6.3.1.1] catalyzes the synthesis of L-asparagine from L-aspartate and ammonia, with hydrolyzing ATP to AMP and PPi (Meister, 1974). This enzyme is distributed in prokaryotes as *Escherichia coli* (Cedar & Schwartz, 1969*a*) and *Klebsiella aerogenes* (Reitzer & Magasanik, 1982), and encoded by the *asnA* gene. On the other hand, asparagine synthetase using glutamine as amido nitrogen donor [L-aspartate: L-glutamine amido-ligase (AMP-forming) E.C. 6.3.5.4] is found not only in prokaryotes (Humbert & Simoni, 1980; Reitzer & Magasanik, 1982) but also in eukaryotes (Andrulis, Chen & Ray, 1987; Gantt & Arfin, 1981; Hongo & Sato, 1981; Luehr & Schuster, 1985), and encoded by the *asnB* gene. Studies of steady-state kinetic mechanisms for both enzymes have demonstrated that the mechanism of the ammonia-dependent enzymes is different from that of the glutamine-dependent enzymes (Cedar & Schwartz, 1969*b*; Hongo & Sato, 1985; Mehlhaff, Luehr & Schuster, 1985).

Both types of asparagine synthetase genes have been cloned and sequenced from *E. coli* (Nakamura *et al.*, 1981; Scofield, Lewis & Schuster, 1990), and *asnB*-type from the human enzyme as well (Andrulis, Chen & Ray, 1987). The *asnA* gene from *E. coli* coded for a polypeptide of 330 amino-acid residues with a molecular weight of 36 700, and the *asnA* enzyme exists as a dimer of identical subunits (Nakamura *et al.*, 1981; Sugiyama, Kato, Nishioka & Oda, 1992). Each subunit contains two Cys residues, Cys51 and Cys315, and their chemical modification causes inactivation of the enzymatic activity (Cedar & Schwartz, 1969*a*).

A high degree of amino-acid-sequence similarity has been shown between the glutamine-dependent enzymes (*asnB*-type enzyme from *E. coli* and the human enzyme), whereas no significant similarity has been found between the *asnA* and the *asnB* sequences derived from the same origin, *E. coli* (Scofield, Lewis & Schuster, 1990). On the other hand, sequence comparison between the *asnA* enzyme and aspartyl- or lysyl-tRNA synthetases has detected a short homologous region in a glycine-rich region near the C-terminal residue. In the light of their enzymatic mechanism, it has been proposed that the conserved region participates in the binding of aspartic acid or in the catalytic mechanism necessary to form an aspartyl-AMP intermediate (Gatti & Tzagoloff, 1991; Hinchman, Henikoff & Schuster, 1992). Hinchman *et al.* (1992) have found that Arg299 in the region of the *asnA* enzyme, which aligns with an invariant arginine residue in all reported class II aminoacyl-tRNA synthetases, possesses a functional importance in the asparagine synthetase activity using site-directed mutagenesis. Both asparatyl- and lysyl-tRNA synthetases are members of the class II aminoacyl-tRNA synthetases. Whereas the class I aminoacyl-tRNA synthetases attach their amino acid to the 2′-hydroxyl group, the class II synthetases bind their amino acid to the 3′-hydroxyl group of the ribose of the terminal adenosine of tRNA (Eriani, Deralue, Poch, Gangloff & Moras, 1990). Each class enzyme shows different structural features detected by primary sequence analysis and visualized by crystallography (Cusack, 1995).

To compare the function based on the structure of *asnA* asparagine synthetase with that of the class II aminoacyl-tRNA synthetases, especially aspartyl- (Ruff *et al.*, 1991), lysyl- (Onesti, Miller & Brick, 1995) or seryl-tRNA synthetases (Cusack, Berthet-Colominas, Härtlein, Nassar & Leberman, 1990), we have undertaken the X-ray crystal structure analysis of the *asnA* asparagine synthetase from *E. coli*. This is the first time that asparagine synthetase has been crystallized.

## 2. Experimental

### 2.1. *Mutagenesis*

Cys-free (Cys51→Ala, Cys315→Ala) mutant was produced by the method of Kunkel (1985) using the mutagenic oligonucleotide 5′-CTTGTCGGGCGCTGAAAAAGCG-3′ (Cys51→Ala), and 5′-CCAGGTTCAGGCCGGCGTATGGC-CAGC-3′ (Cys315→Ala). The resulting DNA sequence was checked by dideoxy sequencing (Sanger, Nicklen & Coulson, 1977). The mutant gene was subcloned into the expression vector, pUNAd37 (Sugiyama *et al.*, 1992).

### 2.2. *Overexpression and purification*

The overexpression in *E. coli* and the purification were carried out by the method described previously (Sugiyama *et al.*, 1992), followed by hydrophobic interaction chromatography on a Phenyl-TOYOPEARL 650 column. The last step of purification was as follows. The active fraction from Blue Cellulofine chromatography with 20% saturated ammonium sulfate was applied to a Phenyl-TOYOPEARL 650 column equilibrated with the buffer containing 0.8 *M* $(NH_4)_2SO_4$, 20 m*M* Tris–HCl (pH 7.5), 10%(*w*/*v*) glycerol and 5 m*M* β-mercaptoethanol. After the column had been washed with 200 ml of the same buffer, elution was performed with a linear gradient from 0.8 to 0 *M* ammonium sulfate in the buffer. The

enzyme was eluted at an ammonium sulfate concentration of approximately 0.5 *M*. The purity of the preparations was judged by SDS–poly(acryl amide) gel electrophoresis, and the final preparations ran as a single band with a molecular mass of approximately 37 kDa.

### 2.3. *Crystallization*

The purified protein solution was concentrated to 30 mg ml$^{-1}$ using an Amicon YM 10 membrane and dialyzed against 20 m*M* HEPES (pH 7.5), 10%(*w*/*v*) glycerol and 5 m*M* $\beta$-mercaptoethanol. Crystals of asparagine synthetase were grown in a CYSCHEM MVD/24 plate (Supper) by sitting-drop vapour diffusion at 293 K. Each drop consisted of 2 µl of the protein solution plus 2 µl of the reservoir solution containing 45% saturated $(NH_4)_2SO_4$, 22 m*M* Asn, 88 m*M* $MgCl_2$, 50 m*M* HEPES (pH 7.5), 10%(*w*/*v*) glycerol and 5 m*M* $\beta$-mercaptoethanol. The crystals reached a maximum size of 0.5 × 0.3 × 0.07 mm within 3 weeks.

### 2.4. *X-ray diffraction experiments*

A crystal was mounted in a thin-walled glass capillary. Precession photographs were taken with Ni-filtered Cu $K\alpha$ radiation from a Rigaku RU-200 rotating-anode. Intensity data were collected on an R-AXIS IIc imaging-plate area detector with monochromated Cu $K\alpha$ radiation from a Rigaku RU-300 rotating anode. Data were reduced using the R-AXIS IIc software (Sato *et al.*, 1992).

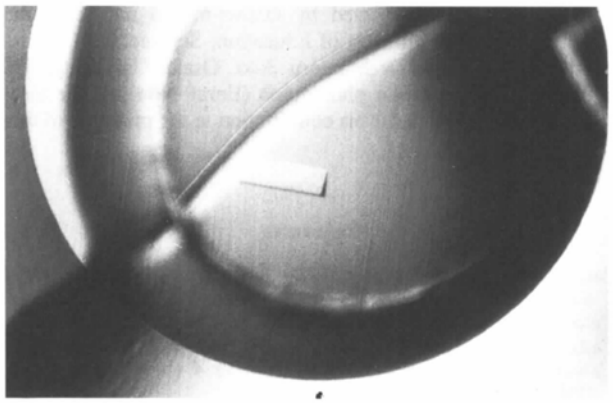
## 3. Results and discussion

To improve the quality of the crystals, we constructed the Cys-free asparagine synthetase by replacement of two Cys residues (Cys51 and Cys315) with Ala residues using site-directed mutagenesis. The specific activity of the mutant enzyme was 33% of the wild-type enzyme. The mutant enzyme formed good-quality crystals whereas the wild-type enzyme gave twinned plated or needled crystals (Figs. 1*a*,*b*). Addition of 22 m*M* asparagine was also necessary to obtain good-quality crystals.

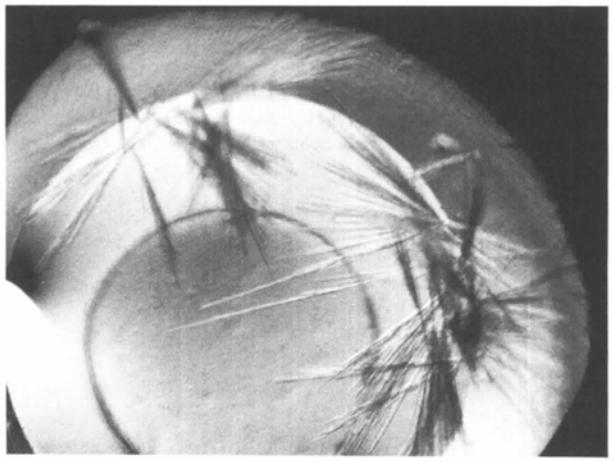
The unit-cell dimensions and the space group were determined from precession photographs (Fig. 2); the results are consistent with those from of the autoindexing routine of the R-AXIS IIc processing software. The crystals belong to the monoclinic space group $P2_1$, with $a$ = 52.9, $b$ = 126.2, $c$ = 52.8 Å, $\beta$ = 105.3°. Assuming one dimer in the asymmetric unit gives a solvent content of 47% and a $V_m$ of 2.3 Å$^3$ Da$^{-1}$. This specific value is well within the normal range of $V_m$ values for protein crystals (Matthews, 1968). Native data sets have been collected on an R-AXIS IIc imaging plate to a resolution of 2.7 Å with an $R_{\rm merge}$ of 8.4%. In the resolution shell from 2.75 to 2.7 Å, 60% of the reflection intensities are over 1$\sigma$.

Self-rotation functions were calculated for polar angles using *POLARRFN* of the *CCP*4 suit (Collaborative Computational Project, Number 4, 1994). The self-rotation function gave significant peaks only for the twofold rotation axis ($\kappa$ = 180°) oriented with the polar angles $\varphi$ = 90.5, $\psi$ = 166.1°. Using data from 10.0 to 3.5 Å, the peaks on the $\kappa$ = 180° section are 55% that of the origin peak. The peak indicates that a non-crystallographic twofold rotation axis relates the two subunits of the homodimer.

Now a samarium derivative has been obtained. A search for the other heavy-atom derivatives is in progress.



(*a*)



(*b*)

Fig. 1. Crystals of asparagine synthetase. (*a*) Cys-free mutant enzyme. (*b*) Wild-type enzyme.

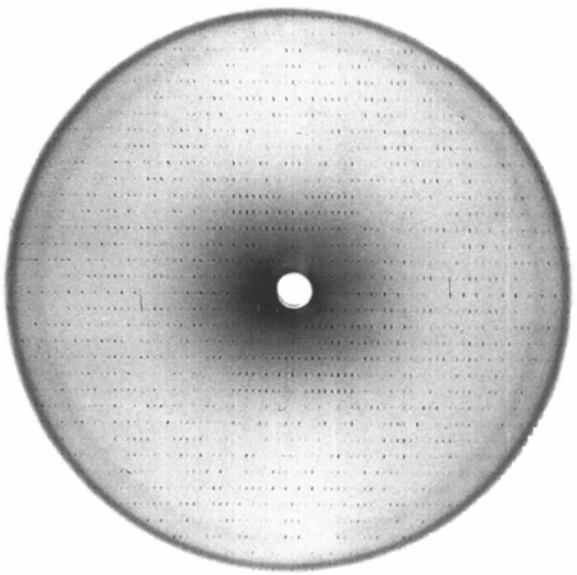


Fig. 2. Precession photograph of a Cys-free mutant crystal of asparagine synthetase, *hk*0 zone (precession angle: 15°, crystal-to-film distance: 100 mm, exposure time: 22 h).

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan. We thank Dr Mamoru Sato, Osaka University, for providing the precession photograph (Enraf–Nonius). We also thank Akio Sugiyama for his contribution in the mutation of the *asnA* gene.

### References

Andrulis, I. L., Chen, J. & Ray, P. N. (1987). *Mol. Cell Biol.* **7**, 2435–2443.

Cedar, H. & Schwartz, J. H. (1969*a*). *J. Biol. Chem.* **244**, 4112–4121.

Cedar, H. & Schwartz, J. H. (1969*b*). *J. Biol. Chem.* **244**, 4122–4127.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Cusack, S. (1995). *Nature Struct. Biol.* **2**, 824–831.

Cusack, S., Berthet-Colominas, C., Härtlein, M., Nassar, N. & Leberman, R. (1990). *Nature (London)*, **347**, 249–255.

Eriani, G., Deralue, M., Poch, O., Gangloff, J. & Moras, D. (1990). *Nature (London)*, **347**, 203–206.

Gantt, J. S. & Arfin, S. M. (1981). *J. Biol. Chem.* **256**, 7311–7315.

Gatti, D. L. & Tzagoloff, A. (1991). *J. Mol. Biol.* **218**, 557–568.

Hinchman, S. K., Henikoff, S. & Schuster, S. M. (1992). *J. Biol. Chem.* **267**, 144–149.

Hongo, S. & Sato, T. (1981). *Anal. Biochem.* **114**, 163–166.

Hongo, S. & Sato, T. (1985). *Arch. Biochem. Biophys.* **238**, 410–417.

Kunkel, T. A. (1985). *Proc. Natl Acad. Sci. USA*, **82**, 488–492.

Humbert, R. & Simoni, R. D. (1980). *J. Bacteriol.* **142**, 212–220.

Luehr, C. A. & Schuster, S. M. (1985). *Arch. Biochem. Biophys.* **237**, 335–346.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Mehlhaff, P. M., Luehr, C. A. & Schuster, S. M. (1985). *Biochemistry*, **24**, 1104–1110.

Meister, A. (1974). *The Enzymes*, Vol. 10, pp. 561–580. New York: Academic Press.

Nakamura, M., Yamada, M., Hirota, Y., Sugimoto, K., Oka, A. & Takanami, M. (1981). *Nucleic Acids Res.* **9**, 4669–4676.

Onesti, S., Miller, A. D. & Brick, P. (1995). *Structure*, **3**, 163–176.

Reitzer, L. J. & Magasanik, B. (1982). *J. Bacteriol.* **151**, 1299–1313.

Ruff, M., Krishnaswamy, S., Boeglin, M., Poterszman, A., Mitshler, A., Podjarny, A., Rees, B., Thierry, J. C. & Moras, D. (1991). *Science*, **252**, 1682–1689.

Sanger, F., Nicklen, S. & Coulson, A. R. (1977). *Proc. Natl Acad. Sci. USA*, **74**, 5463–5467.

Sato, M., Yamamoto, M., Imada, K., Katsube, Y., Tanaka, N. & Higashi, T. (1992). *J. Appl. Cryst.* **25**, 348–357.

Scofield, M. A., Lewis, W. S. & Schuster, S. M. (1990). *J. Biol. Chem.* **265**, 12895–12902.

Sugiyama, A., Kato, H., Nishioka, T. & Oda, J. (1992). *Biosci. Biotech. Biochem.* **56**, 376–379.