Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction studies of guanidinoacetate methyltransferase from rat liver

Junichi Komoto,[a] Yafei Huang,[a] Yongbo Hu,[a] Yoshimi Takata,[b] Kiyoshi Konishi,[b] Hirofumi Ogawa,[b] Tomoharu Gomi,[b] Motoji Fujioka[b] and Fusao Takusagawa[a]*

[a]Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045-0045, USA, and [b]Department of Biochemistry, Toyama Medical and Pharmaceutical University, Faculty of Medicine, Sugitani, Toyama 930-01, Japan

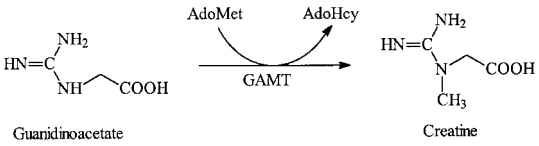
Correspondence e-mail: xraymain@ukans.edu

Received 27 April 1999
Accepted 27 July 1999

Guanidinoacetate methyltransferase is the enzyme which catalyzes the last step of creatine biosynthesis. The enzyme is found ubiquitously and in abundance in the livers of all vertebrates. Recombinant rat-liver guanidinoacetate methyltransferase has been crystallized with guanidinoacetate and *S*-adenosylhomocysteine. The crystals belong to the monoclinic space group $P2_1$, with unit-cell parameters a = 54.8, b = 162.5, c = 56.1 Å, β = 96.8 (1)° at 93 K, and typically diffract beyond 2.8 Å.

1. Introduction

Guanidinoacetate methyltransferase (*S*-adenosyl-L-methionine–guanidinoacetate *N*-methyltransferase, GAMT, E.C. 2.1.1.2), first found in pig liver by Cantoni & Vignos (1954), is the enzyme which catalyzes the last step of creatine biosynthesis. The enzyme is found ubiquitously and in abundance in the livers of all vertebrates.



In humans, the biosynthesis of creatine is reported to represent about 75% of the total utilization of methionine through *S*-adenosylmethionine (AdoMet; Mudd & Poole, 1975); GAMT is therefore believed to be the major enzyme involved in the metabolic conversion of AdoMet to *S*-adenosylhomocysteine (AdoHcy) in vertebrates. A hereditary disease with extrapyramidal motor disorder and extremely low concentrations of creatine in the brain, serum and urine has recently been described (Stöckler *et al.*, 1994) and shown to be a consequence of a deficiency of GAMT in the liver (Stöckler *et al.*, 1996, 1997).

GAMT was purified to homogeneity from the livers of pig and rat by Im *et al.* (1979) and Ogawa *et al.* (1983), respectively, and shown to be a monomeric protein with a relatively small molecular size (M_r = 26 kDa). The amino-acid sequences of the rat (Ogawa *et al.*, 1988) and human enzymes (Isbrandt & Figura, 1995) were deduced from the respective cDNA sequences (Ogawa *et al.*, 1988; Isbrandt & Figura, 1995), respectively. There is a 82.5% homology in the nucleotide sequence and a 86.9% homology in the deduced amino-acid sequence between the two enzymes. The rat-liver GAMT was produced recombinantly in large amounts in *Escherichia coli* (Ogawa *et al.*, 1988) and its structural and functional features have been studied by chemical modification, site-directed mutagenesis and limited proteolysis. These studies have revealed that Cys15, Cys90 and Cys219 occur spatially close together (Fujioka *et al.*, 1988; Takata *et al.*, 1991) and that the region around residues 19–24 is highly exposed to the solvent and is flexible (Takata & Fujioka, 1990; Fujioka *et al.*, 1991). The portion of the enzyme including these cysteine residues and the *N*-terminal region are apparently distant from the active site. On the other hand, ultraviolet irradiation of the enzyme in the presence of AdoMet resulted in covalent attachment of the compound to Tyr136 showing characteristics of affinity labeling (Takata & Fujioka, 1992). Thus, Tyr136 occurs at or near the active site. A subsequent site-directed mutagenesis study has indicated that Asp134 is critical for AdoMet binding and that Tyr136 has no direct role in the AdoMet binding (Takata *et al.*, 1994). Here, we describe the purification, crystallization and preliminary crystallographic characterization of GAMT from rat liver.

2. Materials and methods

2.1. Purification

Recombinant guanidinoacetate methyltransferase was produced in *E. coli* JM109 transformed with plasmid pUCGAT9-1 which contained the coding region of rat GAMT cDNA linked to the *lac* promoter (Ogawa *et al.*, 1988). The cells were grown in 2×YT medium containing 35 mg ml^{-1} ampicillin at 309 K. When the cell turbidity measured at 600 nm reached an absorbance of about 0.2, isopropyl-1-thio-β-D-galactopyranoside was added to a concentration of 1 m*M* and culture was continued for an additional 16 h.

Table 1
Data statistics.

Resolution range	Number of reflections	Completeness (%)	I/σ	R_{sym}
99.00–6.01	2437	98.0	18.1	0.053
6.01–4.77	2419	99.5	17.9	0.049
4.77–4.17	2386	98.6	17.8	0.049
4.17–3.79	2387	98.0	17.3	0.052
3.79–3.52	2385	98.8	16.8	0.056
3.52–3.31	2394	98.8	15.7	0.064
3.31–3.14	3413	99.4	14.5	0.073
3.14–3.01	2404	99.3	13.0	0.087
3.01–2.89	2393	99.3	11.7	0.110
2.89–2.79	1897	78.1	7.5	0.128
All reflections	23515	96.8	15.0	0.059

Cells harvested by centrifugation were suspended in 25 ml of 50 m*M* Tris–HCl pH 7.5 containing 2 m*M* EDTA and disrupted by treatment with egg-white lysozyme (1 mg ml^{-1}) for 30 min in ice. Following freeze-thawing and brief sonication, the cell debris was removed by centrifugation. The supernant was then treated with ammonium sulfate and the precipitate obtained between 40 and 55% saturation was dissolved in 1 ml of a buffer solution containing 10 m*M* Tris–HCl pH 7.5, 1 m*M* EDTA and 1 m*M* dithiothreitol. The solution was directly loaded onto a column of Sephacryl S200 (32 × 980 mm) equilibrated and eluted with the same buffer. The active fractions were combined and applied to a column of DEAE-cellulose (DE-52, 32 × 250 mm) which had been equilibrated with 10 m*M* Tris–HCl pH 7.5, 1 m*M* EDTA. The enzyme was eluted with a linear gradient between 60 ml each of solution *A* (10 m*M* Tris–HCl pH 7.5, 1 m*M* EDTA, 1 m*M* dithiothreitol) and solution *B* (70 m*M* potassium phosphate pH 7.2, 1 m*M* EDTA, 1 m*M* dithiothreitol). The enzyme was concentrated by ultrafiltration. The homogeneity of the enzyme was checked by SDS–PAGE. The recombinant enzyme lacks the N-terminal acyl group present in the liver enzyme, but shows the same kinetic and physical properties (Ogawa *et al.*, 1988).

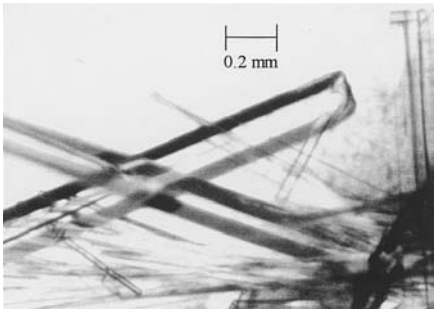


Figure 1
Crystals of guanidinoacetate methyltransferase with guanidinoacetate and *S*-adenosylhomocysteine.

2.2. Crystallization

Crystallization trials were performed at three different temperatures [incubator (299 K), room temperature (295 K), and cold room temperature (277 K)] by the hanging-drop vapor-diffusion method. Conditions for crystallization were searched initially by the PEG screen method. Protein solutions (20 mg ml^{-1}) with and without added 5 m*M* AdoHcy were utilized in the screening experiment. Having obtained needle micro-crystals of the enzyme from the solution containing 10% polyethylene glycol molecular weight 4000 (PEG 4000), 100 m*M* MES buffer (pH 6.5), the optimal conditions for growing crystals for X-ray diffraction study were searched by varying the pH, buffer concentration, precipitant and temperature (277–299 K). Crystals suitable for X-ray diffraction studies (∼0.70 × 0.15 × 0.15 mm) were grown in a solution containing 50 m*M* MES buffer (pH 6.5), 1 m*M* dithiothreitol, 5 m*M* AdoHcy, 20 m*M* guanidinoacetate, 8%(*w*/*v*) PEG 4000 with a protein concentration of 10 mg ml^{-1} in a 277 K cold room (Fig. 1).

2.3. X-ray diffraction experiment

The tetragonal prism-like crystals of GAMT diffracted well to 2.8 Å, but were very sensitive to radiation; it was only possible to collect a few frames from each crystal. Conditions for cryocooling the crystals were therefore sought in an attempt to circumvent this difficulty. A crystal (0.1 × 0.1 × 0.5 mm) in a hanging drop was scooped up in a nylon loop and dipped into a cryoprotectant solution containing 50 m*M* MES buffer (pH 6.5), 1 m*M* dithiothreitol, 5 m*M* AdoHcy and 20%(*v*/*v*) glycerol for 30 s before being flash-frozen in cold nitrogen gas (93 K) on a Rigaku R-AXIS IIc imaging-plate X-ray diffractometer with a rotating-anode X-ray generator as an X-ray source (Cu $K\alpha$ radiation generated at 50 kV and 100 mA). The diffraction data were measured to 2.8 Å resolution (Fig. 2). The data were processed with the program *DENZO* (Otwinowski & Minor, 1997). The space group and unit-cell parameters are $P2_1$ and a = 54.8, b = 162.5, c = 56.1 Å, β = 96.8°, respectively. From 72400 reflections recorded on the detector, 23515 independent reflections (96.8% complete) were obtained with R_{sym} (= $\sum |I - \langle I \rangle| / \sum |I|$) = 0.054. The data statistics are given in Table 1. The calculated unit-cell volume of 496961 Å^3 suggests that there are four monomeric GAMT molecules in the asymmetric unit, with a V_m of 2.39 Å^3 Da^{-1}, corresponding to a solvent content of 48% (Matthews, 1968). We have started screening possible heavy-atom derivatives. The structural determination of GAMT will be reported in the future.

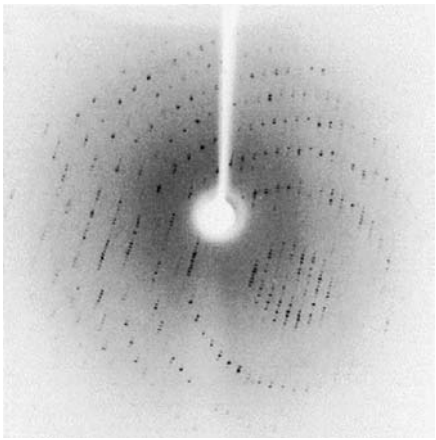


Figure 2
Diffraction pattern from a 1° oscillation image of the monoclinic crystal with 15 min exposure. The crystal-to-detector distance was 160 mm; the resolution at the edge of the image is 2.8 Å.

This research has been supported by the NIH grant GM37233.

References

Cantoni, G. L. & Vignos, P. J. Jr (1954). *J. Biol. Chem.* **209**, 647–659.
Fujioka, M., Konishi, K. & Takata, Y. (1988). *Biochemistry*, **27**, 7658–7664.
Fujioka, M., Takata, Y. & Gomi, T. (1991). *Arch. Biochem. Biophys.* **285**, 181–186.
Im, Y. S., Chiang, P. K. & Cantoni, G. L. (1979). *J. Biol. Chem.* **254**, 11047–11050.
Isbrandt, D. & von Figura, K. (1995). *Biochim. Biophys. Acta*, **1264**, 265–267.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mudd, H. S. & Poole, J. R. (1975). *Metabolism*, **24**, 721–735.
Ogawa, H., Date, T., Gomi, T., Konishi, K., Pitot, H. C., Cantoni, G. L., & Fujioka, M. (1988). *Proc. Natl Acad. Sci. USA*, **85**, 694–698.
Ogawa, H., Ishiguro, Y. & Fujioka, M. (1983). *Arch. Biochem. Biophys.* **226**, 265–275.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Stöckler, S., Holzbach, U., Hanefeld, F., Marquardt, I., Helms, G., Requart, M., Hänicke, W. & Frahm, J. (1994). *Pediatr. Res.* **36**, 409–413.
Stöckler, S., Isbrandt, D., Hanefeld, F., Schmidt, B. & von Figura, K. (1996). *Am. J. Hum. Genet.* **58**, 914–922.
Stöckler, S., Marescau, B., De Deyn, P. P., Trijbels, J. M. & Hanefeld, F. (1997). *Metabolism*, **46**, 1189–1193.
Takata, Y., Date, T. & Fujioka, M. (1991). *Biochem. J.* **277**, 399–4069.
Takata, Y. & Fujioka, M. (1990). *Int. J. Biochem.* **22**, 1333–1339.
Takata, Y. & Fujioka, M. (1992). *Biochemistry*, **31**, 4369–4374.
Takata, Y., Konishi, K., Gomi, T. & Fujioka, M. (1994). *J. Biol. Chem.* **269**, 5537–5542.