*Acta Cryst.* (1998). D**54**, 1030–1031

# Purification, crystallization and preliminary X-ray analysis of human recombinant cytosolic serine hydroxymethyltransferase

Suzanne B. Renwick,[a]† Jane V. Skelly,[b] Karen J. Chave,[c] Peter G. Sanders,[c] Keith Snell[a] and Ulrich Baumann[a]*‡ *at* [a]*Section of Structural Biology, Institute of Cancer Research, University of London, Cotswold Road, Sutton, Surrey SM2 5NG, England,* [b]*CRC Biomolecular Structure Unit, Institute of Cancer Research, University of London, Cotswold Road, Sutton, Surrey SM2 5NG, England, and* [c]*Molecular Microbiology Group, University of Surrey, Guildford, Surrey GU2 5XH, England. E-mail: ulrich.baumann@ibc.unibe.ch*

(*Received 1 December 1997; accepted 19 March 1998*)

**Abstract**

As an enzyme of the thymidylate synthase cycle, serine hydroxymethyltransferase (SHMT) has a key role in nucleotide biosynthesis. Elevated activities of SHMT have been correlated with the increased demand for nucleotide biosynthesis in tumors of human and rodent origin, making this enzyme a novel target for cancer chemotherapy. Here the purification and crystallization of recombinant human cytosolic SHMT are reported. Crystals belong to space group $P6_122$ or $P6422$ with cell parameters $a = b = 155.0$, $c = 235.5$ Å and diffract to at least 3.0 Å resolution.

## 1. Introduction

Serine hydroxymethyltransferase (SHMT) is one of the trio of enzymes which constitute the thymidylate synthase cycle for the production of the nucleotide precursor thymidylate. The enzyme catalyses the retro-aldol cleavage of serine to glycine, with the liberated hydroxymethyl group being transferred to tetrahydrofolate to produce 5,10-methylene tetrahydrofolate. Both products of this reaction are precursors for nucleotide biosynthesis.

The fundamental cellular importance of SHMT is highlighted in rapidly proliferating tissues where increased activity is required to satisfy the demands for nucleotide biosynthesis. Following mitogenic stimulation of human lymphocyte proliferation, elevated SHMT activity correlates with increased incorporation of radioactivity from [3-$^{14}$C] serine into DNA (Eichler *et al.*, 1981). Increased expression of SHMT has also been observed in a variety of tumors (Thorndike *et al.*, 1979; Bukin & Draudin-Krylenko, 1980), including sarcomas and carcinoma of human and rodent origin (Snell *et al.*, 1988). Thus, drug targeting of SHMT by anti-cancer agents is an attractive prospect.

SHMT is a pyridoxal 5′-phosphate-dependent enzyme and the primary structures of a large number of SHMTs from various species have been determined, including mammalian cytosolic and mitochondrial isoforms (Martini *et al.*, 1987, 1989; Garrow *et al.*, 1993). Sequence homology to other pyridoxal 5′-phosphate-dependent enzymes is low but a similarity to aspartate aminotransferase and related enzymes has been suggested (Alexander *et al.*, 1994). A wealth of enzymological and mutagenesis data have been collected, especially on the enzyme from *Escherichia coli* (Stover *et al.*, 1992; Schirch *et al.*, 1993). Currently, interpretation of these results is severely restricted by the lack of a three-dimensional structure. Crystals of the *E. coli* enzyme have been published (Stover *et al.*, 1993) but no structure has been reported yet.

## 2. Materials and methods

### 2.1. *Overexpression and purification of human recombinant cSHMT from E. coli*

Details of cloning will be published elsewhere. In brief, a cDNA encoding SHMT was isolated from a breast cancer cell line cDNA library (P. Byrne, personal communication). The SHMT cDNA was amplified by PCR and subcloned into pET-14b (Novagen) and used to transform BL21(DE3)pLysS. SHMT expression and purification was carried out according to Novagen's protocol. After resuspending the cell pellet in 10 ml binding buffer (20 m*M* Tris–HCl; 5 m*M* imidazole; 500 m*M* NaCl; 0.25 m*M* PMSF; 20 µg ml$^{-1}$ DNAase I; 10 µg ml$^{-1}$ RNAase A; 2 µg ml$^{-1}$ leupeptin; 3 µg ml$^{-1}$ aprotinin; pH 7.9) the cells were sonicated and the cell debris removed by centrifugation. The cleared lysate was loaded onto a His·Bind resin column (Novagen). After washing the column with Binding Buffer, SHMT was recovered by elution with imidazole. Following Novagen's protocol SHMT aggregated rapidly upon elution from the column. Protein precipitation was prevented by the addition of serine (final concentration 2 m*M*); pyridoxal 5′-phosphate (final concentration 2 m*M*) and dithiothreitol (final concentration 10 m*M*) to the eluted

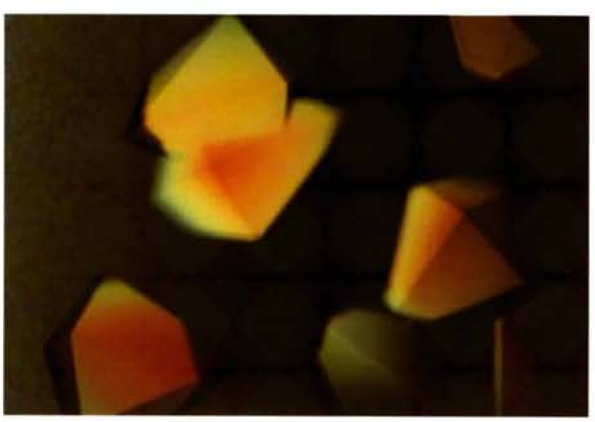

Fig. 1. Hexagonal bipyramidal crystals of recombinant human cytosolic SHMT.

† Present address: Celltech plc, 216 Bath Road, Slough, Berkshire SL1 4EN, England.

‡ Present address: Departement f. Chemie und Biochemie, Universität Bern, Freiestrasse 3. CH-3012 Bern, Switzerland.

sample, and by decreasing the concentration of imidazole in the elution buffer (1000 m*M* imidazole, 500 m*M* sodium chloride; 20 m*M* Tris–HCl, pH 7.9) from 1000 m*M* to 250 m*M*. The protein was concentrated using Amicon Centriplus-30 concentrators. The concentration step was also used to transfer the protein into 50 m*M* Tris–HCl pH 7.0, 2 m*M* serine, 2 m*M* pyridoxal 5′-phosphate and 10 m*M* dithiothreitol.

### 2.2. *Crystallization and data collection*

Screening for crystallization conditions was performed according to the sparse-matrix method (Jancarik & Kim, 1991), using commercially available buffers (Hampton Research, Laguna Hills, CA, USA) and the His-tagged SHMT. Hanging drops were prepared by mixing 5 µl of protein solution (22–27 mg ml$^{-1}$ SHMT) with 5 µl reservoir buffer over 700 µl of reservoir buffer. Crystals were flash cooled to 110 K for data collection. Glycerol was introduced by the serial transfer of crystals into crystallization buffer containing increasing concentrations [5–30%(*v*/*v*)] of cryoprotectant. Crystallographic data were recorded in-house using a Rigaku RU200 rotating-anode generator operating at 5.4 kW equipped with double-focusing mirrors, and an R-axis IIc detector. A native 3.0 Å data set was collected on one crystal in frames of 0.8° rotation and an exposure time of 20 min per frame. The crystal-to-detector distance was 250 mm and a swing angle of 9° was employed. Data were processed using the *HKL* suite (Otwinowski & Minor, 1997). 158 836 measurements were reduced to 28 884 unique reflections. The overall completeness is 85.7% (24.7% in the shell from 3.03 to 3.00 Å resolution) and the $R_{sym}$ based on intensities is 0.058 (0.247 for the outermost resolution shell). The average $\langle I\rangle/\langle\sigma I\rangle$ is 21.1 overall and 2.71 for the resolution shell 3.03–3.00 Å.

## 3. Results and discussion

Human cytosolic SHMT is a homotetramer, each monomer consisting of 483 amino acids with a molecular weight of 53 kDa. The recombinant His-tagged SHMT was overexpressed in *E. coli* and purified to homogeneity using metal-chelation chromatography. Using the protocol described above up to 15 mg of soluble, pure cytosolic SHMT was purified from 1 l of bacterial culture. This protein could be stored at 277 K for at least two months without loss of activity, as measured by a radioactivity assay using [3-$^{14}$C] serine as a substrate (Taylor & Weissbach, 1965). The purified protein was more than 98% pure as judged by Coomassie-blue staining of SDS–PAGE gels.

Yellow crystals of hexagonal bipyramidal shape grew in 1.0 *M* sodium acetate, 0.1 *M* imidazole pH 6.5 (Hampton Crystal Screen reagent 25) at 286 K after 1–2 d and grew to a typical size of 0.3 × 0.3 × 0.8 mm within one week. They belong to space group $P6_122$ or $P6422$ with cell dimensions $a = b = 155.0$, $c = 235.5$ Å and diffract to beyond 3.0 Å resolution. SHMT crystals were sensitive to X-ray exposure and survived for less than 10 min on a rotating-anode source. Consequently, crystals were flash cooled at 110 K for data collection, using glycerol as the cryoprotectant. The Matthews parameter (Matthews, 1985) $V_M$ was calculated as 7.5/*n* Å$^3$ Da$^{-1}$ (*n* is the number of monomers per asymmetric unit). Hence, $n = 2$ or $n = 3$ would yield a $V_M$ in the range usually observed with water-soluble proteins. Since human cytosolic SHMT is a tetramer in solution, only $n = 2$ would be consistent with the observed space-group symmetry. However, the susceptibility of the crystals to damage by X-rays and their modest diffraction limit are properties consistent with a loose crystal packing, containing a single protomer and a high solvent content. Self-rotation functions calculated using *X-PLOR* (Brünger, 1992) or *ALMN* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994), using different resolution ranges and integration radii, were invariably featureless and native low-resolution Patterson functions lacked any pronounced pseudo-origin peaks. Thus, we assume that the tetramer occupies a special position with 222 symmetry and the asymmetric unit contains only one monomer and about 85% solvent ($V_M$ = 7.5 Å$^3$ Da$^{-1}$). Matthews parameters above 4.0 Å$^3$ Da$^{-1}$ are rare for water-soluble proteins but do occur. The search for heavy atom derivatives is now under way.

We gratefully acknowledge financial support from the Institute of Cancer Research and the Cancer Research Campaign. KC was funded by the University of Surrey. We are grateful to Professor Stephen Neidle (CRC Biomolecular Structure Unit, Institute of Cancer Research) for providing the X-ray and computing facilities.

## References

Alexander, F. W., Sandmeier, E., Mehta, P. K. & Christen, P. (1994). *Eur. J. Biochem.* **219**, 953–960.
Brünger, A.T. (1992). *X-PLOR, A System for X-ray Crystallography and NMR*. Yale University Press, New Haven, USA.
Bukin, Y. V. & Draudin-Krylenko, V. A. (1980). *Vest. Akad. Med. Nauk. SSSR*, **6**, 67–69.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* **D50**, 760–763.
Eichler, H. G., Hubbard, R. & Snell, K. (1981). *Biosci. Rep.* **1**, 101–106.
Garrow, T. A., Brenner, A. A., Whitehead, V. M., Chen, X. N., Duncan, R. G., Korenberg, J. R. & Shane, B. (1993). *J. Biol. Chem.* **268**, 11910–11916.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Martini, F., Angelaccio, S., Pascarella, S., Barra, D., Bossa, F. & Schirch, V. (1987). *J. Biol. Chem.* **262**, 5499–5509.
Martini, F., Maras, B., Tanci, P., Angelaccio, S., Pascarella, S., Barra, D., Bossa, F. & Schirch, V. (1989). *J. Biol. Chem.* **264**, 8509–8519.
Matthews, B. W. (1985). *Methods Enzymol.* **114**, 176–187.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Schirch, D., Delle Fratte, S., Iurescia, S., Angelaccio, S., Contestabile, R., Bossa, F. & Schirch, V. (1993). *J. Biol. Chem.* **268**, 23132–23138.
Snell, K., Natsumeda, Y., Eble, J. N., Glover, J. L. & Weber, G. (1988). *Br. J. Cancer.* **57**, 87–90.
Stover, P., Kruschwitz, H., Schirch, V. & Wright, H. T. (1993). *J. Mol. Biol.* **230**, 1094–1096.
Stover, P., Zamora, M., Shostak, K., Gautambasak, M. & Schirch, V (1992). *J. Biol. Chem.* **267**, 17679–17687.
Taylor, R. T. & Weissbach, H. (1965). *Anal. Biochem.* **13**, 80–84.
Thorndike, J., Pelliniemi, T. T. & Beck, W. S. (1979). *Cancer Res.* **39**, 3435–3440.