

Crystallization and preliminary crystallographic characterization of recombinant L-methionine- α -deamino- γ -mercaptomethane lyase (methioninase)

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L-Methionine- α -deamino- γ -mercaptomethane lyase (rMETase) is involved in the α,γ -elimination of methionine to α -ketobutyrate, methanethiol and ammonia. The reaction catalyzed by rMETase reduces the methionine concentration of methionine-dependent tumor cells, arresting their growth. Towards the goal of developing rMETase into an effective antitumor therapeutic and also to understand the catalytic mechanism of this enzyme, rMETase from *Pseudomonas putida* has been expressed, purified and crystallized. The crystals belong to space group $P2_12_12$ and diffract X-rays to at least 2.68 Å resolution at 100 K using synchrotron radiation. The unit cell has parameters $a = 152.8$, $b = 154.6$, $c = 80.8$ Å and contains four molecules in the asymmetric unit.

Received 8 June 2000

Accepted 26 September 2000

1. Introduction

The chemotherapy of solid tumors, with a few exceptions, has had only limited efficacy (Devita *et al.*, 1993). Thus, the majority of solid cancers are generally not responsive to current chemotherapy regimens. For the most part, the existing drugs used for solid-tumor chemotherapy are not tumor selective and are therefore active against both tumor and normal cells, which gives the drugs limited efficacy and significant toxicity. Therefore, it is of critical importance to identify targets and agents that are tumor selective.

Asparagine dependence is one such target for the enzyme asparaginase. However, the asparagine-dependence target may be limited only to hematological tumors (Devita *et al.*, 1993). On the other hand, studies have shown that the amino acid methionine may be a tumor-specific target. Observations have indicated that a number of malignant cell lines have an absolute requirement for methionine, as they fail to grow on homocysteine (Tan *et al.*, 1996). Recent studies have demonstrated that *in situ* patient tumors are also frequently methionine dependent (Tan *et al.*, 1996). Normal cells and tissues, unlike methionine-dependent tumors, were found to use homocysteine in place of methionine for proliferation and are therefore methionine independent (Tan *et al.*, 1996). *In vitro* and *in vivo* studies have suggested that targeting the methionine dependence of tumors may exert tumor-selective efficacy *via* a tumor-specific cell-cycle block (Hoffman & Jacobson, 1980; Guo *et al.*, 1993). Under conditions of a limiting methionine source, methionine-dependent tumor cells

arrest in the late-S/G₂ phase in the cell cycle (Guo *et al.*, 1993).

Recently, a number of investigators have attempted to exploit the methionine dependence of tumors for therapeutic effects *in vivo*. Breillout *et al.* (1987, 1990) observed that a methionine-depleted diet lowered the metastatic potential of methionine-dependent tumors without significant effects on local tumor growth in rats. Goseki *et al.* (1995) observed that a methionine-free total parental nutrition (TPN) mixture for rats bearing the Yashida sarcoma slowed tumor growth and extended the survival of the rats, especially with the use of doxorubicin. With the use of a methioninase, Kreis & Hession (1973) demonstrated an attenuation of growth of W-256 rat carcinosarcoma growing in rats. This clinical trial demonstrated that methionine depletion has clinical activity. However, dietary methionine starvation is insufficient in completely depleting the serum methionine and therefore does not completely arrest tumor growth.

A methionine-cleaving enzyme would lower methionine levels more than methionine starvation and thereby could have more therapeutic efficacy. For this purpose, Kreis & Hession (1973) attempted to purify a METase from *Clostridium sporogenes*. The enzyme reduced the growth of the Walker 256 carcinoma in rats more than a methionine-free diet. Subsequently, Ito *et al.* (1976) purified a METase from *P. putida*. This enzyme was composed of four identical subunits of molecular weight 43 kDa and catalyzed the α,γ -elimination of methionine to α -ketobutyrate, methanethiol and ammonia in the

A cross-rotation function was calculated in the resolution range 20.0–5.0 Å with a sampling step of 2.5° and an integration radius of 35 Å using the program *AMoRe* (Navaza, 1994). The cross-rotation functions calculated using the tetramer as the search model resulted in four peaks of equal height and equal correlation coefficients (CC). These four peaks could be grouped into two sets. The two peaks in the first set had values of $\alpha = 82.3$, $\beta = 78.0$, $\gamma = 103.1^\circ$ and $\alpha = 82.0$, $\beta = 78.2$, $\gamma = 283.8^\circ$; the two peaks in the second set had values of $\alpha = 98.3$, $\beta = 78.1$, $\gamma = 77.0^\circ$, and $\alpha = 97.7$, $\beta = 78.2$ and $\gamma = 257.0^\circ$. The four best solutions with equal CC were used in translation-function searches in the same resolution range. All the four rotation functions resulted in the same translation function with comparable CC and *R* factors.

3. Results and discussion

Crystals of *P. putida* rMETase belong to orthorhombic space group $P2_12_12$ and

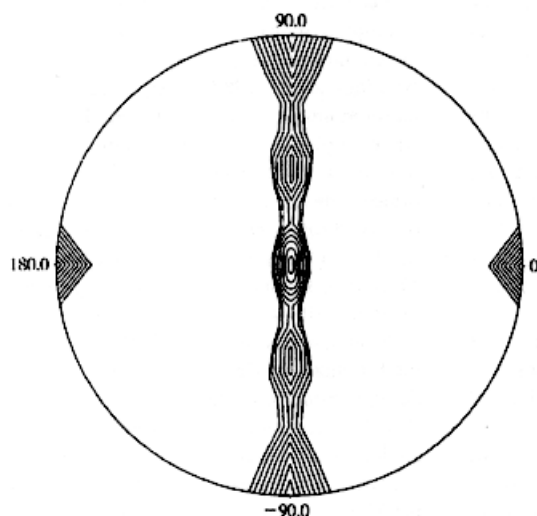


Figure 2
The $\kappa = 180^\circ$ section of the self-rotation function of recombinant methioninase. The integration radius is 25.0 Å and was calculated using the data in the resolution range 30.0–3.0 Å.

diffract to at least 2.68 Å resolution. The unit-cell parameters and data-collection statistics for the native data set are given in Table 1. Based on the unit-cell volume, it is assumed that the asymmetric unit contains one tetramer (172 kDa). Self-rotation calculations revealed two strong peaks in the $\kappa = 180^\circ$ section in addition to the peaks arising from the 222 point-group symmetry (Fig. 2). The two peaks correspond to rotations of $\omega = 48.0$, $\varphi = 90$, $\kappa = 180^\circ$, and $\omega = 47.6$, $\varphi = -90$ and $\kappa = 180^\circ$ and are consistent with the cross-rotation function results (§2). The top four solutions of the cross-rotation function can be grouped in two sets of two, each set having the same α and β values but differing by 180° in the γ value. Consequently, the cross- and self-rotation function values together suggest that four monomers of a tetramer in the asymmetric unit are packed as two independent dimers, with each monomer in the dimer being related by a twofold non-crystallographic axis. This arrangement of the subunits of the rMETase tetramer in the asymmetric unit is similar to that observed in the crystal structure of *E. coli* cystathionine β -lyase and is defined as the $\alpha 2$ dimer (Clausen *et al.*, 1996). Based on this model, the CC was 39.2% and the *R* factor was 51.3%. Rigid-body refinement improved the CC to 45.5% and lowered the *R* factor to 49.9%. Complete structure determination and refinement of rMETase is in progress.

We would like to thank Dr C. D. Stout for his support and encouragement. The staff at Stanford Synchrotron Research Laboratory (SSRL) is gratefully acknowledged for their assistance during data collection.

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