Acta Cryst. (1995). D**51**, 1092–1093

Preliminary X-ray crystallographic study of methyltetrahydrofolate:corrinoid/iron sulfur protein methyltransferase from *Clostridium thermoaceticum*.

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(*Received* 12 *December* 1994; *accepted* 20 *April* 1995)

Abstract

Methyltetrahydrofolate:corrinoid/iron sulfur protein methyltransferase from *Clostridium thermoaceticum* has been crystallized in two polymorphic forms and characterized by X-ray diffraction measurements. Form I displayed orthorhombic symmetry with $a = 63.9$, $b = 53.8$, $c = 164.0$ Å. Form II also displayed orthorhombic symmetry with $a = 63.5$, $b = 87.1$, $c = 117.9$ Å. Crystals of form I diffract to approximately 3 Å resolution; those of form II diffract to approximately 2.7 Å.

Introduction

Clostridium thermoaceticum is an anaerobic thermophilic bacterium that performs CO_2 fixation by the reductive acetyl-CoA pathway (Ragsdale, 1991). In this pathway, a CH_3-H_4folate:corrinoid/iron sulfur protein methyltransferase (MeTr) transfers the N^5-methyl group of (6*S*)-methyltetrahydrofolate (CH_3-H_4folate) to the cobalt center of a corrinoid/iron sulfur protein (C/FeSP) (see Fig. 1).

MeTr was purified to homogeneity earlier (Drake *et al.*, 1981) and recently cloned, sequenced, and over-expressed in *Escherichia coli* (Roberts, Zhao, Doukov & Ragsdale, 1994). An additional step was included that consisted of incubating the cell extract at 343 K for 10 min. This significantly improved the micropurity of the protein and allowed us to obtain better quality single crystals. MeTr occurs as a homodimer with a subunit molecular weight of 28 641 Da (Roberts *et al.*, 1994). The enzyme contains no metals at levels above 0.1 g atm mol^{-1} protein as tested by plasma emission spectroscopy and lacks chromophoric prosthetic groups.

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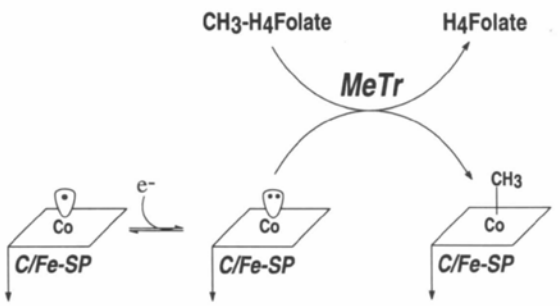

Fig. 1. The reaction of MeTr.

MeTr is functionally related to a cobamide-dependent methyltransferase from methanogenic bacteria and to methionine synthase. The cobamide-dependent methyltransferase from methanogens differ from MeTr in the utilization of 5-methyltetrahydromethanopterin or 5-methyltetrahydrosarcinopterin as a one-carbon source instead of CH_3-H_4folate (Fischer & Thauer, 1989). Cobamide-dependent methionine synthase (E.C. 2.1.1.13, 5-methyl tetrahydrofolate homocysteine methyltransferase) from *E. coli* is a single-chain multi-domain enzyme ($M_r = 136\,000$ Da). Methionine synthase transfers the methyl group of CH_3-H_4folate to the bound Cob(I)alamin to form methyl-Cob(III)alamin. Subsequently, the methyl group is transferred to homocysteine to form methionine. Methionine synthase contains separate domains for binding CH_3-H_4folate, cobamide, and S-adenosyl methionine (Drummond, Huang, Blumenthal & Matthews, 1993). The cobalamin-binding domain has recently been crystallized and its structure determined (Luschinsky, Drummond, Matthews & Ludwig, 1992, Drennan, Huang, Drummond, Matthews & Ludwig, 1994). A region in the *C. thermoaceticum* MeTr sequence shares significant homology with residues 350–650 of the cobalamin-dependent methionine synthase suggesting that this constitutes the CH_3-H_4folate binding domain (Roberts *et al.*, 1994). The residues in the adjacent region (650–850) are involved in binding cobalamin (Drennan, Drummond, Matthews & Ludwig, 1994). With determination of the crystal structure of MeTr, it will be possible to better understand this class of methyl transferases at a structural level.

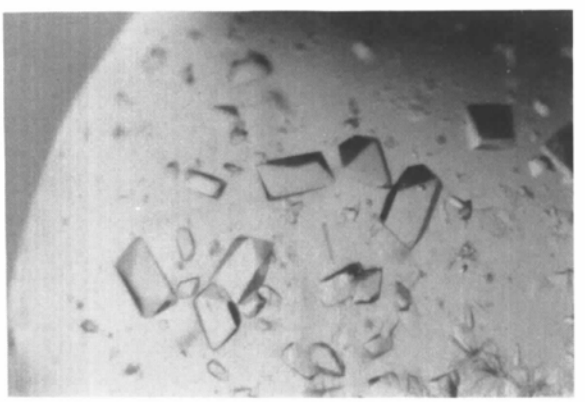

Fig. 2. Typical crystals (with dimensions up to about 0.5 mm) of form II of CH_3-H_4folate methyltransferase from *Clostridium thermoaceticum*.

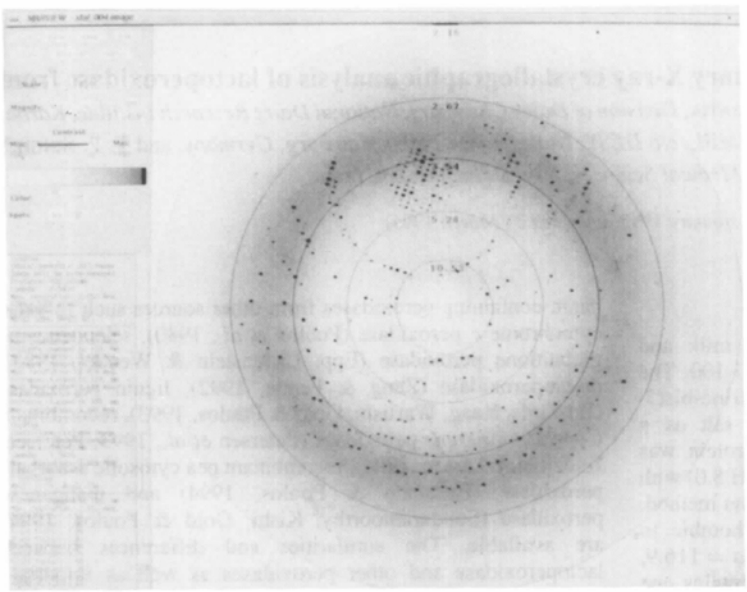

Fig. 3. A typical 1° oscillation image for a crystal of form II of CH_3-H_4folate methyltransferase from *Clostridium thermoaceticum*. The image was recorded on a MAR Research imaging plate area detector on a Siemens rotating anode generator (Cu$K\alpha$ monochromatized radiation) operating at 50 kV and 100 mA. The resolution is indicated by concentric circles on the image.

Experimental

Single crystals of MeTr were grown either by the sitting or hanging-drop techniques (McPherson, 1982). Crystals of the enzyme isolated from *C. thermoaceticum* (form I) were grown by the sitting-drop technique using 10 µl of a MeTr solution at a concentration of 2.5 mg ml^{-1}, and 10 µl of the well solution containing 0.35 M $CaCl_2$, 0.1 *M* Hepes (pH 7.5) and 20% PEG 4000. Crystals of form II were grown using a solution of 18 mg ml^{-1} of the enzyme purified from *E. coli* mixed with an equal volume (5 µl) of a precipitant solution containing 11.6% polyethylene glycol monomethyl ether 5000, 0.0775 *M* HEPES buffer (pH = 7.5), 0.0775 *M* $CaCl_2$, and a trace (0.1%) of NaN_3.

Results

Crystals of form I were characterized by precession photographs taken at room temperature and found to display space-group symmetry $P2_12_12_1$ with $a = 63.9$, $b = 53.8$, $c = 164.0$ Å. They diffracted initially to 3 Å resolution but deteriorated after a few hours to 6 Å resolution. Assuming a molecular weight for the monomer of 28 600 Da and eight monomers per unit cell (two per asymmetric unit), the volume per unit molecular weight (V_m) is 2.46 Å^3 Da^{-1}. Crystals of form II, Fig. 2, were also characterized at room temperature, but with a MAR Research imaging-plate area detector on a Siemens rotating anode X-ray generator. Based on the data available, the space group is most probably $P2_12_12_1$ with $a = 63.5$, $b = 87.1$, $c = 117.9$ Å. Space groups $P222$, P_122 and $P2_12_12$ cannot yet be ruled out. Fresh crystals diffracted to 2.7 Å resolution, Fig. 3. Under the same assumptions applied to form I, V_m for form II is 2.84 Å^3 Da^{-1}.

In conclusion, good quality crystals of CH_3-H_4folate:-corrinoid/iron sulfur protein methyltransferase from *C. thermoaceticum* have been grown and characterized by X-ray diffraction measurements. The eventual structure determination has the potential to be the first crystal structure reported for a CH_3-H_4folate utilizing enzyme.

This research was supported in part by NIH grant GM39451 to SWR, NSF grant CHE9214428 to JJS, and NSF award No. OSR-9255225.

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