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# Crystal structure of heterotetrameric sarcosine oxidase from *Corynebacterium* sp. U-96 ☆

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**Abstract**

Sarcosine oxidase from *Corynebacterium* sp. U-96 is a heterotetrameric enzyme. Here we report the crystal structures of the enzyme in complex with dimethylglycine and folinic acid. The α subunit is composed of two domains, contains $NAD^+$, and binds folinic acid. The β subunit contains dimethylglycine, FAD, and FMN, and these flavins are approximately 10 Å apart. The γ subunit is in contact with two domains of α subunit and has possibly a folate-binding structure. The δ subunit contains a single atom of zinc and has a $Cys_3His$ zinc finger structure. Based on the structures determined and on the previous works, the structure–function relationship on the heterotetrameric sarcosine oxidase is discussed.


*Keywords:* Crystal structure; Flavoenzyme; Heterotetramer; Sarcosine oxidase; Cofactor binding; FAD; FMN; Folinic acid; $NAD^+$; Zinc finger

The concentration of serum creatinine is widely used as an indicator of renal function [1]. Sarcosine oxidase (SO) [sarcosine: oxygen oxidoreductase (demethylating), EC 1.5.3.1] has been used in the enzymatic determination of creatinine in the clinical laboratory. SO has been isolated from various bacterial strains [1]. It was first purified from *Corynebacterium* sp. U-96 and shown to be a heterotetrameric enzyme ($M_r$ 174,000) [2]. The enzyme is composed of four nonidentical subunits (α, $M_r$ 110,000; β, $M_r$ 44,000; γ, $M_r$ 21,000; δ, $M_r$ 10,000) [2]. Since then, heterotetrameric SO has been purified from various sources: *Corynebacterium* sp. P-1 [3], *Arthrobacter ureafaciens* [4], and *Arthrobacter* sp. 1-IN [5]. We have been studying SO from *Corynebacterium* sp. U-96 (SO-U96), and have characterized it [1,6–9]. To understand the structure–function relationship of SO-U96 more in detail, we aimed to determine the three-dimensional structure of the enzyme. This paper describes the crystal structure of SO-U96. Based on the structure and on the previous works by us and others, the structure–function relationship on the heterotetrameric sarcosine oxidase is discussed.

## Materials and methods

*Crystallization*. Recombinant SO-U96 (recSO-U96) was expressed and purified as previously described [6], except that the chromatographic procedures were performed by ÄKTA prime system (Amersham Biosciences), using HiTrap DEAE FF, HisTrap HP, HiTrap Phenyl FF, Resources Q, and HiLoad 16/60 Superdex 200 prep grade columns (Amersham Biosciences). The recSO-U96 had the same enzymatic properties as the native one [6]. The homogeneity of the purified SO-U96 was confirmed by SDS–PAGE. The purified recSO-U96 in 10 mM Tris–HCl (pH 8.0) was concentrated to

☆ *Abbreviations:* SO, sarcosine oxidase; SO-U96, SO from *Corynebacterium* sp. U-96; SO-P1, SO from *Corynebacterium* sp. P-1; MSO, monomeric sarcosine oxidase; rec, recombinant; DMGO, dimethylglycine oxidase; THF, tetrahydrofolate; FON, folinic acid (5-formyltetrahydrofolate); 5,10-$CH_2$-THF, 5,10-methylenetetrahydrofolate; IAM, iodoacetamide.

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approximately 5 mg/ml in an Amicon Ultra (Millipore) at 277 K. Crystallization was performed by vapor diffusion method. Preliminary screening of crystallization conditions was performed using Hampton Research Crystal Screen kits and Emerald Biostructure Screen kits at 293 K. Flower-like crystals were obtained by ammonium sulfate as a precipitant. Refinement of the crystallization conditions to 0.1 M Tris–HCl (pH 8.5), 1.9 M ammonium sulfate, and 10 mM $CuSO_4$ improved the quality and size of the crystals. The crystals grew to approximate dimensions of $0.2 \times 0.2 \times 0.3$ mm within a few weeks. The isomorphous heavy-atom derivative was prepared at 293 K by soaking the native crystal in crystallization buffers containing 0.1 mM $HgSO_4$ for 1 day. The crystal of folinic acid (FON) complex was prepared by soaking the native crystal in the crystallization mother liquor containing 10 mM FON for 30 min at 293 K.

*Data collection, structure solution, and refinement*. Diffraction data for $HgSO_4$ derivative data sets and native data set were collected on NW12 in PF-AR using an ADSC Quantum 210 CCD detector, and FON complex data set was collected on BL5 in PF using an ADSC Quantum 315 CCD detector. A crystal of the protein was frozen using a cryoprotectant solution containing 30% (v/v) glycerol in the crystallization mother liquor. The data were processed using the MOSFLM [10] and scaled using the SCALA [11]. The crystal structure was solved using mercury derivative crystal and MAD method. The experimental phase was calculated to a resolution of 2.9 Å with SOLVE [12] and density modification with RESOLVE [13]. The structure factor replacement for the native data and phase extension to 2.15 Å resolution were carried out by DM [14]. Initial model building was carried out automatically by ARP/wARP [15], and additional model building was manually performed with O [16]. Refinement of the model was carried out using CNS [17]. Ten percent of the data set was selected for free *R* factor calculations. Water molecules were assigned automatically by ARP/wARP. The initial structure of FON complex was derived from the native model. The structure was refined with CNS, and manual modifications were made with O. A summary of the statistics for structural determination is given in Table 1.

*Coordinate*. The refined coordinates of the two structures have been deposited in Protein Date Bank (Accession Nos.: 1X31 for folinic acid-free, 1VRQ for folinic acid complex).

## Results and discussion

### *Overall description*

The three-dimensional structure of recSO-U96 is shown in Fig. 1A. Overall dimension of the molecule is approximately 65 Å × 85 Å × 120 Å. The missing residues (α 964; β 403, 404; γ 1–5; δ 91–98) have no electron density. The initiator Met has been removed in the α and β subunits of the native enzyme [20], but not in the δ subunit [20,21]. As for γ subunit, the N-terminal sequence (MANDT) has been removed [20,21]. The α subunit is composed of two large domains, the N-terminal half (nicotine domain, residue 1–565) and the C-terminal half (folate domain, residue 576–963). These two domains are linked with a 10 residue chain. The nicotine domain contains 1 mol $NAD^+$, and the folate domain binds 1 mol folinic acid (FON) to a probable site of 5,10-methylenetetrahydrofolate (5,10-$CH_2$-THF) synthesis. The β subunit contains flavin cofactors, 1 mol of noncovalent FAD and 1 mol of covalent FMN, and 1 mol of dimethylglycine. The flavin cofactors are approximately 10 Å apart. The γ subunit is along the linker between the nicotine and folate domains of the α subunit (Fig. 1A). Interestingly, the γ subunit and the part of folate domain are related by a noncrystallo-

Table 1
Data collection, refinement, and model statistics

| | Native | Folinic acid | Hg-peak | Hg-edge | Hg-remote |
|---|---|---|---|---|---|
| *Data collection* | | | | | |
| Beamline | PF-AR (NW12) | PF (BL5) | | PF-AR (NW12) | |
| Wavelength (Å) | 1.00817 | 1.00000 | 1.00868 | 1.00934 | 1.02000 |
| Space group | $P6_522$ | $P6_522$ | | $P6_522$ | |
| Unit cell (Å) | $a = 199.112$, $c = 197.034$ | $a = 199.401$, $c = 197.205$ | | $a = 198.065$, $c = 197.308$ | |
| Resolution range (Å)[a] | 70.1–2.15 (2.27–2.15) | 99.5–2.20 (2.32–2.20) | 88.74–2.85 (3.00–2.85) | 88.74–2.85 (3.00–2.85) | 88.74–2.85 (3.00–2.85) |
| Total reflections | 1,276,782 | 1,677,601 | 1,147,445 | 1,150,636 | 1,153,212 |
| Unique reflections | 124,207 | 116,783 | 53,641 | 53,807 | 53,934 |
| Redundancy[a] | 10.3 (7.9) | 14.4 (14.5) | 21.4 (20.9) | 21.4 (20.9) | 21.4 (20.9) |
| Completeness[a] (%) | 99.8 (98.3) | 100.0 (100.0) | 100.0 (100.0) | 100.0 (100.0) | 100.0 (100.0) |
| $R_{merge}$[a,b] (%) | 9.2 (31.9) | 8.5 (31.2) | 8.9 (31.3) | 9.1 (34.1) | 9.4 (37.4) |
| *Refinement* | | | | | |
| Resolution range (Å) | 70.1–2.15 | 99.5–2.20 | | | |
| $R_{work}$ (%) | 18.8 | 19.2 | | | |
| $R_{free}$ (%) | 23.2 | 22.4 | | | |
| *Model statistics* | | | | | |
| R.m.s.ds | | | | | |
| Bond length (Å) | 0.791 | 0.786 | | | |
| Bond angles (deg) | 1.7 | 1.5 | | | |
| Average *B* factor | 24.6 | 24.1 | | | |

[a] Values in parentheses correspond to the reflections observed in the highest resolution shell.
[b] $R_{merge} = \sum_{hkl}\sum_i | I_{hkl,i} - \langle I_{hkl}\rangle | \sum_{hkl}\sum_i I_{hkl,i}$, where $I$ is the observed intensity and $\langle I\rangle$ is the averaged intensity for multiple measurements.