5A, NW12A /2004G372

Crystal Structure of Heterotetrameric Sarcosine Oxidase

Koh IDA[1*], Tomotaka MORIGUCHI[2] and Haruo SUZUKI[1,2]
[1]Department of Biosciences, School of Science, and [2]Division of Biosciences, Graduate School of Fundamental Life Science, Kitasato University, 1-15-1 Kitasato, Sagamihara, Kanagawa 228-8555, Japan

Introduction

The concentration of serum creatinine is widely used as an indicator of renal function. Sarcosine oxidase (SO) has been used in the enzymatic determination of creatinine in the clinical laboratory. SO has been isolated from various bacterial strains. It was first purified from *Corynebacterium* sp. U-96 (SO-U96) and shown to be a heterotetrameric enzyme. SO-U96 is a complex, bifunctional enzyme that catalyzes both sarcosine oxidation and the synthesis of 5,10-methylenetetrahydrofolate. SO-U96 contains four different subunits (α, β, γ, δ). The isolated enzyme contains three cofactors (noncovalently bound FAD, NAD^+ and covalently bound FMN). We determined the three dimensional structure of heterometric SO from *Corynebacterium* sp. U-96 [1]. In this report, we show the structure concisely.

Experiments

SO-U96 was expressed from BL21(DE3) with pET system. The purification was performed using Ni affinity chromatography and five several columns. The purifed SO was concentrated to approximate 5 mg/ml. Crystallization was performed by vapour diffusion method. The crystallization condition is 0.1 M Tris-HCl (pH 8.5), 1.9 M ammonium sulphate and 10 mM $CuSO_4$. The crystals grew to approximate dimensions of 0.2 x 0.2 x 0.3 mm within a few weeks.

Diffraction data for $HgSO_4$ derivative data sets and native data set were collected on NW12A using an ADSC Quantum 210 CCD detector. The $HgSO_4$ derivative crystal was prepared by soaking in crystallization buffers containing 0.1 mM $HgSO_4$. The mercuric ion inhibited the sarcosine oxidation activity. The crystal structure was solved using Hg-MAD method. The experimental phase was calculated to a resolution of 2.9 Å with *SOLVE* and density modification with *RESOLVE*. The structure factor replacement for the native data and phase extension to 2.15 Å resolution were carried out *DM*.

Folinic acid (FON) is inhibitor of the synthesis by SO-U-96 of 5,10-methylenetetrahydrofolate. We carried out soaking FON to the crystal. The complex data was collected on BL5A using an ADSC Quantum 315 CCD detector. The complex crystal diffracted beyond 2.20 Å resolution. The initial structure of FON complex was derived from native model. The refined structure model showed a clear *Fo–Fc* map of FON.

Result and Discussions

The structural model of SO-U96 is shown in Fig. 1. The α subunit is composed of two large domains, N-terminal half (nicotine domain) and the C-terminal half (folate domain). The nicotine domain contains NAD^+ and the folate domain binds FON to a probable synthesis site of 5,10-methylenetetrahydrofolate. Theβ subunit contains FAD, FMN and dimethylglycine (DMG). The DMG bound to FAD, but the DMG is not a substrate of SO. The hydrogen flows from sarcosine to oxygen; sarcocine → FAD → FMN → oxygen. The flavin cofactors are approximately 10 Å apart. The orientation of the flavins is very similar to L-proline dehydrogenase from *Pyrococcus horikoshii* [2]. Probably, they belong to a new family of electron transfer flavoprotein. The δ subunit contains a single atom of zinc and has unexpected structure, Cys_3His zinc finger structure. The enzyme has a large spheroid-like cavity with approximately 10,000 $Å^3$. The internal cavity enables to reduce formation of toxic formaldehyde. Dimethylglycine oxidase [3] has the similar cavity.

The investigations of hydrogen transfer mechanism and the role of the large cavity are in progress.

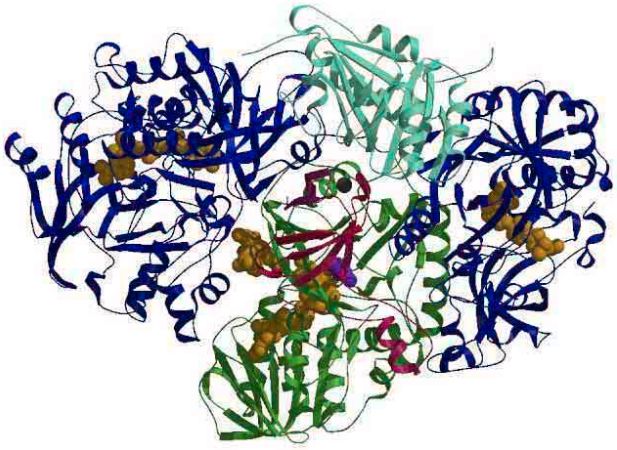

Fig. 1 Structure model of sarcosine oxidase

References

[1] Ida K., Moriguchi T. and Suzuki H. Biochem. Biophys. Res. Commun. 333(2), 359-366 (2005)
[2] Tsuge H. *et al.*, J. Biol. Chem. 280(35), 31045-31049 (2005)
[3] Leys D., *et al.*, EMBO J. 22(16), 4038-4048 (2003)
*idakoh@sci.kitasato-u.ac.jp