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X-ray Crystallographic analysis of heterotetrameric sarcosine oxidase

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Introduction

The concentration of serum creatinine is widely used as an indicator of renal function. 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. It was first purified from *Corynebacterium* sp. U-96 and shown to be a heterotetrameric enzyme (Mr 174,000). The enzyme is composed of four nonidentical subunits (α, Mr 110,000; β, Mr 44,000; γ, Mr 21,000; δ, Mr 10,000). To understand the structure-function relationship of SO more in detail, we aimed to determine the three dimensional structure of the enzyme.

Experiments and Result

Sarcosine oxidase was expressed and purified as previously described [1], except that the chromatographic procedures were performed by ÄKTA prime system (Amersham Bisciences), using HiTrap DEAE FF, HisTrap HP, HiTrap Phenyl FF, Resources Q and HiLoad 16/60 Superdex 200 prep grade columns (Amersham Biosciences). The purifed SO in 10 mM Tris-HCl (pH 8.0) was concentrated to approx. 5 mg/ml. Crystallization was performed by vapour diffusion method. 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 (Figure 1).

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. 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 (CCP4 suite) and scaled using the SCALA (CCP4 suite). The data processing statistic are given Table 1. After measurement, the crystal lost yellow colour. The crystals have two problems that the cell dimensions are too large and the crystal take large radiation damage. These causes overlap and non-isomorphism. To obtain high quality data, we used large detector or fine slice method and minimum X-ray exposed time. Structure determination by the multiple-wavelength anomalous diffraction (MAD) method is in progress.

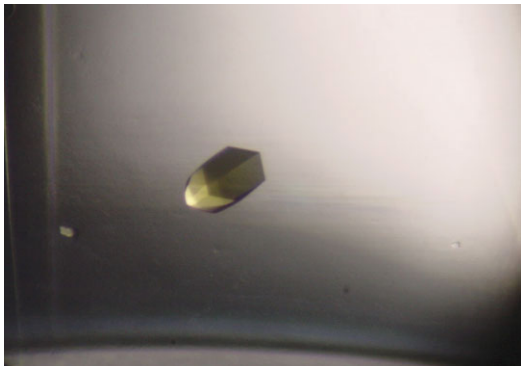

Figure 1. Crystal of sarcosine oxidase

Table 1. Data processing statistics

Data set	Native	Peak	Edge	Remote
S. G.	$P6_522$			
Cell dimensions	a=199.1 c=197.2		a=199.4 c=197.3	
Resolution	2.15		2.85	
Wavelength		1.00868	1.00934	1.02000
Oscillation	0.3		0.5	
Observ. ref.	1276782	1147445	1150636	1153212
Unique ref.	124207	53641	53807	53934
Comp.	99.8	100	100	100
R_{merge}	9.2	8.9	9.1	9.4
I/σ	5.1	7.0	7.0	6.6
Multiplicity	10.3	21.4	21.4	21.4

References

[1] H. Suzuki *et al*., Biosci. Biotechnol. Biochem. 69 (5), 952-956 (2005).

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