Note

Crystallization and Characterization of Sarcosine Oxidase from *Alcaligenes denitrificans* subsp. *denitrificans*

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We have isolated three microorganisms that form sarcosine oxidase (EC 1.5.3.1) as the sole enzyme for sarcosine oxidation when cultivated with creatinine. The report provided the first evidence for the involvement of sarcosine oxidase in the degradation of creatinine.¹⁾ Here, we describe the crystallization and characterization of sarcosine oxidase from *Alcaligenes denitrificans* subsp. *denitrificans* J9. Sarcosine oxidase from this strain is different from other enzymes that oxidize sarcosine in that it is composed of two different subunits.

The enzyme was purified as described previously.¹⁾ Crystals of the enzyme were prepared by the addition of powdered ammonium sulfate to about 40% saturation with gentle shaking to the purified enzyme in 0.02 M Tris-HCl buffer, pH 7.5, containing 0.1 mM dithiothreitol. After for one day storage at 4°C, long bar-like crystals were formed (Fig. 1).

The purified enzyme was studied by electrophoresis. With 5% polyacrylamide gels,²⁾ the enzyme migrated as a single band. With 10% polyacrylamide gels and SDS,³⁾ two protein bands were produced; their molecular weight were 100,000 (α) and 55,000 (β) as estimated from parallel runs of standard proteins (Fig. 2). The molecular weight of the enzyme is 190,000,¹⁾ so we think that the subunit structure of the enzyme was $\alpha\beta$ or $\alpha\beta_2$.

The K_m value of the enzyme for sarcosine was 4.2 mM, and the enzyme was specific for this substrate. *N*-Methyl-L-alanine was also oxidized by the enzyme at about one-third of the rate of sarcosine. Dimethylglycine and betaine were not oxidized at all. The enzyme did not oxidize sarcosine with phenazine methosulfate as an electron carrier. The absorption spectrum of the enzyme had maxima at 365 and 452 nm in the visible region, both of which were completely bleached upon addition of excess sarcosine, suggesting that the enzyme is a flavoprotein, like other sarcosine-oxidizing enzymes.^{4~9)}

The optimum pH and optimum temperature for sar-

FIG. 1. Crystals of the Sarcosine Oxidase from *A. denitrificans* subsp. *denitrificans*.

FIG. 2. SDS-Polyacrylamide Gel Electrophoresis of the Purified Sarcosine Oxidase from *A. denitrificans* subsp. *denitrificans*.

Upper, the standards: (from left) phosphorylase b ($M_r=94,000$), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (29,000), trypsin inhibitor (20,000), and lactoalbumin (14,000). Lower, the enzyme (10 μ g).

cosine oxidation were 8 and 37°C, respectively. After 10 min of incubation in 0.05 M potassium phosphate buffer, pH 7.5, the enzyme was stable at 30°C. The enzyme was stable when incubated at 30°C for 10 min in the pH range 7 to 9.

Sarcosine-oxidizing enzymes of different organisms vary greatly in their molecular structure. The molecular weight of *A. denitrificans* subsp. *denitrificans* sarcosine oxidase ($M_r=190,000$) was much higher than the enzyme from *Cylindrocarpus didymus* M-1 (45,000) and also higher than that of sarcosine dehydrogenase (105,000) or dimethylglycine dehydrogenase (90,000) from rat liver mitochondria.^{1,4,10)} *Corynebacterium* sp. U-96 sarcosine oxidase and *Pseudomonas putida* sarcosine dehydrogenase are close to each other in the molecular weight of their native forms, although their subunit structures are different; the former is a homotetramer and the latter is composed of four nonidentical subunits.^{5,6)} In this respect, *A. denitrificans* subsp. *denitrificans* sarcosine oxidase seems not to resemble any known sarcosine-oxidizing enzyme. *P. putida* sarcosine dehydrogenase⁷⁾ had a substrate spectrum similar to that of *A. denitrificans* subsp. *denitrificans* sarcosine oxidase, but the K_m values for sarcosine were different, 29 and 4.2 mM, respectively. The K_m values of *C.*

didymum sarcosine oxidase and rat liver sarcosine dehydrogenase were 1.8 and 0.5 mM, respectively. However, these two enzymes do not oxidize *N*-methyl-L-alanine at all,^{4,7)} whereas *A. denitrificans* subsp. *denitrificans* sarcosine oxidase does. In its substrate specificity and *K_m* value, the enzyme resembles *Corynebacterium* sarcosine oxidase most. Their *K_m* values for sarcosine were 4.2 and 2.9 mM, respectively, and both of the enzymes oxidize *N*-methyl-L-alanine at comparable rates. However, *A. denitrificans* subsp. *denitrificans* sarcosine oxidase did not oxidize sarcosine with phenazine methosulfate as an electron carrier, whereas *Corynebacterium* sarcosine oxidase does.⁵⁾

From the above results, we concluded that *A. denitrificans* subsp. *denitrificans* sarcosine oxidase was comparable only with *Corynebacterium* sarcosine oxidase in various physicochemical properties. However, these enzymes have different subunit structures; the former is composed of two nonidentical subunits whereas the latter has four. Only two protein bands were detected during the SDS-polyacrylamide gel electrophoresis of 50 µg of the enzyme from *A. denitrificans* subsp. *denitrificans* J9.

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