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Crystallization of a Photosensitive Nitrile Hydratase from *Rhodococcus* sp. N-771

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A photosensitive nitrile hydratase from *Rhodococcus* sp. N-771 has been crystallized in two different crystal forms in its inactive form. One crystal form belongs to an orthorhombic space group $P2_12_12$ with unit cell dimensions of $a = 117{\cdot}4$ Å, $b = 145{\cdot}7$ Å and $c = 52{\cdot}1$ Å, and the other form belongs to a hexagonal space group $P6_322$ with unit cell dimensions of $a = 110{\cdot}2$ Å and $c = 412{\cdot}1$ Å.

Keywords: crystallization; nitrile hydratase; *Rhodococcus*; photosensitive; crystallographic parameter

Nitrile hydratase (NHase§) hydrates various nitrile compounds to the corresponding amide groups. The NHase enzymes from *Rhodococcus* sp. N-771 and sp. N-774 show an intriguing property towards light, and have both active and inactive forms. We have already reported that the NHase can be inactivated in the dark *in vivo* depending on the conditions of both dissolved oxygen concentration and temperature, and that the inactivated NHase can be reactivated by light (Nagamune *et al.*, 1990*a*). In addition, the inactive enzyme purified from the inactivated cells could also be photoactivated *in vitro* by near-ultra-violet light irradiation, but the active enzyme could not be inactivated by dark and aerobic incubation (Nagamune *et al.*, 1990*b*). Upon photo-irradiation, drastic changes of NHase spectra are observed in absorption, circular dichroism, electron spin resonance and Mössbauer measurements, and these changes are most probably due to the electronic changes of the iron center composed of prosthetic non-heme iron and PQQ or a PQQ-like compound (unpublished results).

The NHase gene of *Rhodococcus* sp. N-774 has been cloned, and the amino acid sequence of the enzyme deduced from the nucleotide sequence of the cloned gene indicated that the NHase consists of two heterogeneous subunits, α (207 amino acid residues, relative molecular mass $M_r = 22{,}918$) and β (212 amino acid residues, relative molecular mass $M_r = 23{,}428$; Ikehata *et al.*, 1989). Recently, we also cloned the NHase gene from *Rhodococcus* sp. N-771 and clarified that the nucleotide sequence of the gene was identical with that from *Rhodococcus* sp. N-774 (unpublished results).

The NHase enzymes have also been found in several other microorganisms; *Arthrobacter* (Asano *et al.*, 1980), *Brevibacterium* (Bui *et al.*, 1982), *Pseudomonas* (Asano *et al.*, 1982), *Corynebacterium* (Amarant *et al.*, 1989; Tani *et al.*, 1989), but the photoactivation phenomenon has not been observed in these enzymes. The enzyme from *Brevibacterium* R312 contains non-heme iron and PQQ as prosthetic groups, both of which seem to play an essential role for catalytic function (Nagasawa & Yamada, 1987; Sugiura *et al.*, 1988). Since PQQ has so far been found only in oxidoreductases such as methylamine dehydrogenase, alcohol dehydrogenase, glucose dehydrogenase and amine oxidase, its role in combination with non-heme iron for the

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§ Abbreviations used: NHase, nitrile hydratase; PQQ, pyrroloquinoline quinone; PEG, polyethylene glycol; V_m, volume per unit protein molecular mass.

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hydration reaction is an interesting subject in enzymology.

With the purpose of acquiring some insight into the structural features of the NHase, which may explain its properties of catalytic reaction and photoactivation processes, we have initiated a study which attempts to elucidate its tertiary structure. The present work describes the crystallization and the crystallographic parameters of the photosensitive NHase from *Rhodococcus* sp. N-771 in its inactivated form.

The inactivated NHase from *Rhodococcus* sp. N-771 was purified using the methods described by Nagamune *et al.* (1990*b*), and the enzyme was further purified for crystallization by two-column chromatography. For the first column (Phenyl-Sepharose CL-4B, Pharmacia), the enzyme solution containing 15% (w/v) ammonium sulfate was loaded onto the column equilibrated with buffer (20 mM-phosphate, 10 mM-*n*-butyric acid, 15% ammonium sulfate (pH 7·5)) and eluted with a linear gradient of 15% to 0% ammonium sulfate. The eluted enzyme was loaded onto a second column (Bio-Gel HTP, Biorad) and eluted with a linear gradient of 5 mM to 50 mM-phosphate buffer (pH 6·8). Crystallization trials were carried out at 5°C and 20°C within the pH range from 5 to 8 using the hanging-drop vapor diffusion method with various conditions of NHase concentration (10 to 20 mg/ml) and of reagents (buffers, cations of chloride salt and precipitants) and their concentrations in the drop. The concentration of the precipitant in the reservoir solution was twice that in the drop. All procedures of protein purification and crystallization were performed in the dark to prevent the photoactivation of inactive NHase.

Using ammonium sulfate and PEG 8000 as the precipitants, two different crystals of the NHase gradually grew at 5°C over a period of two months. One crystallized at an ammonium sulfate concentration of 20%, in 20 to 60 mM-Hepes buffer (pH 6 to 7), 10 mM-*n*-butyric acid, 10 to 15 mM-zinc chloride, and has a diamond shape (size 0·7 mm × 0·5 mm × 0·1 mm). The other crystallized at a concentration of 5% (w/v) PEG 8000, in 20 to 60 mM-cacodylate buffer (pH 6 to 6·5), 10 mM-*n*-butyric acid, 5 to 15 mM-calcium chloride and has a hexagonal shape (size 0·7 mm × 0·7 mm × 0·2 mm). No crystals grew at 20°C, most probably due to the instability of the NHase at this temperature (Nagamune *et al.*, 1990*b*).

Crystallographic parameters were determined by X-ray precession photography using Ni-filtered X-rays from a Rigaku rotating anode X-ray generator (60 mA, 50 kV, 4 h exposure). The hexagonal-shaped crystals belong to the hexagonal space group $P6_322$ with unit cell dimensions of $a = 110{\cdot}2$ Å and $c = 412{\cdot}1$ Å (1 Å = 0·1 nm), and diffract up to 2·8 Å on photographs. The V_m value is calculated to be 2·6 Å^3/Da assuming three molecules of NHase in the asymmetric unit. The diamond-shaped crystals belong to the orthorhombic space group $P2_12_12$ with dimensions $a = 117{\cdot}4$ Å, $b = 145{\cdot}7$ Å and $c = 52{\cdot}1$ Å. The V_m value is calculated to be 2·4 Å^3/Da for two molecules of NHase per asymmetric unit. These V_m values are comparable to the values of 1·68 to 3·53 Å^3/Da usually found for other proteins (Matthews, 1968). The diamond-shaped crystals diffract to beyond 2·5 Å resolution and are suitable for high-resolution data collection.

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