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Preliminary X-ray Crystallographic Study of Amicyanin From *Thiobacillus versutus*

Two forms of single crystals have been obtained of amicyanin from *Thiobacillus versutus*, a blue copper protein that serves as an electron acceptor for methylamine dehydrogenase. The crystals of one form belong to the monoclinic space group $P2_1$ and have unit cell parameters: $a=54{\cdot}0$ Å, $b=54{\cdot}9$ Å, $c=37{\cdot}3$ Å and $\beta=107{\cdot}9°$ (1 Å = 0·1 nm). There are two molecules in the asymmetric unit. The crystals diffract to about 2 Å resolution. The crystals of the second form are orthorhombic, space group $P2_12_12$ or $P2_12_12_1$ and the unit cell parameters are: $a=81{\cdot}6$ Å, $b=70{\cdot}6$ Å and $c=38{\cdot}7$ Å. There are again two molecules in the asymmetric unit. The crystals diffract to 2·9 Å resolution.

Methylotrophic bacteria can use, as a sole carbon and energy source, carbon compounds like CH_3OH or CH_3NH_2 (Anthony, 1982). The periplasmic oxidoreductase methylamine dehydrogenase, which is an inducible enzyme, catalyses the oxidation of CH_3NH_2 to HCHO and NH_4^+ (Eady & Large, 1968). The enzyme is a quinoprotein and carries as prosthetic group pyrrolo-quinoline quinone (De Beer *et al.*, 1980). It has been proved (Tobari & Harada, 1981) that a blue copper protein named amicyanin mediates *in vivo* the electron transfer between the methylamine dehydrogenase and a *c*-type cytochrome which follows in the electron transfer chain. Although the optical and electron paramagnetic resonance spectra of amicyanins studied so far are very similar to the blue copper proteins (Husain *et al.*, 1986), the properties of the amicyanins have been considered as sufficiently distinct from those of the plastocyanins and azurins to grant them the status of a different subclass in a recently published classification of type-I copper proteins (Adman, 1985).

At present the best characterized copper proteins are the type-I, so-called blue copper proteins. Crystal structures are known to date of the *Pseudomonas aeruginosa* and *Pseudomonas denitrificans* azurins (Adman *et al.*, 1978; Norris *et al.*, 1983), the poplar plastocyanin (Guss & Freeman, 1983) and the pseudoazurin from *Alcaligenes faecalis* S-6 (Petratos *et al.*, 1987).

The three-dimensional structure of methylamine dehydrogenase from the facultative methylotroph *Thiobacillus versutus* is being pursued (Vellieux *et al.*, 1986). In an effort of our laboratory to elucidate the mechanism of electron transfer between proteins we have begun a crystallographic investigation of amicyanin from the same bacterial species first isolated and characterized by van Houwelingen *et al.* (1985), followed by van Beeumen & Canters (personal communication).

Among other crystallisation attempts 5 µl of a 0·6% (w/v) protein solution in water were mixed with 5 µl of 150 mM-CH_3COONa buffer (pH 4·2), 5 µl of 0·5% (w/v) β-octylglucoside and 5 µl of 30% (w/v) polyethelene glycol (M_r = 8000) in a well of a soft tissue culture cluster and allowed to equilibrate at room temperature *via* the vapour phase with 0·5 ml of 30% polyethelene glycol (M_r=8000) solution containing 0·5 M-NaCl (Davies & Segal, 1971). Single prismatic crystals appeared about five weeks later with average dimensions 0·3 mm × 0·2 mm × 0·2 mm. They belong to the monoclinic system, space group $P2_1$ with unit cell parameters $a = 54{\cdot}0$ Å, $b = 54{\cdot}9$ Å, $c = 37{\cdot}3$ Å (1 Å = 0·1 nm) and $\beta = 107{\cdot}9°$. These yield a unit cell volume of 105,200 Å^3. On the assumption of one dimer (2 × 11,000) per asymmetric unit one obtains $V_M=2{\cdot}4$ Å^3/dalton (Matthews, 1968), which implies a 50% (v/v) solvent content in the crystals. They diffract X-rays at the DESY synchrotron source to 2 Å resolution. Complete native data have been collected to 2·6 Å.

In the absence of β-octylglucoside from the protein solution long thin needles (2 mm × 0·1 mm × 0·05 mm) appeared a few weeks later. They belong to the orthorhombic space group $P2_12_12$ or $P2_12_12_1$ with unit cell dimensions $a=81{\cdot}6$ Å, $b=70{\cdot}6$ Å and $c=38{\cdot}7$ Å. These yield a unit cell volume of 223,000 Å^3. On the assumption of one dimer (2 × 11,000) per asymmetric unit one obtains $V_M=2{\cdot}5$ Å^3/dalton, which implies a 52% (v/v) solvent content in the crystals. This crystalline form of amicyanin diffracts to 2·9 Å resolution, therefore it is not suitable for high resolution X-ray work.

For the solution of the three-dimensional structure of the protein we are trying the molecular replacement method using as a model the β-barrel of plastocyanin. Knowledge of the structure of amicyanin will provide evidence for the mechanism of electron transport and will contribute to the understanding of evolution of the blue copper proteins as has been done for the cytochromes (Matthews, 1985).

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