SHORT COMMUNICATIONS

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Crystallization and preliminary crystallographic data for the azurin mutant Ala 114 from *Pseudomonas aeruginosa*.

By LI-CHU TSAI,* VRATISLAV LANGER and LENNART SJÖLIN, *Department of Inorganic Chemistry, Chalmers University of Technology and The University of Göteborg, S-412 96 Göteborg, Sweden*, and TORBJÖRN PASCHER, *Department of Biochemistry and Biophysics, Chalmers University of Technology and The University of Göteborg, S-412 96 Göteborg, Sweden*

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Abstract

The site-specific mutant alanine 114 of the blue copper protein azurin from *Pseudomonas aeruginosa* in *Escherichia coli* has been crystallized from PEG 4000 in a new crystal form compared to the wild type utilizing the hanging-drop procedure. The crystals are blue well-formed prisms. Monoclinic, $P2_1$, $a = 51{\cdot}03\,(5)$, $b = 83{\cdot}36\,(5)$, $c = 66{\cdot}30\,(6)$ Å and $\beta = 111{\cdot}0\,(1)°$. 14 875 reflections up to 2·7 Å have been collected using a modified Syntex $P2_1$ automated four-circle diffractometer.

1. Introduction

Pseudomonas aeruginosa azurin belongs to a group of small copper-containing proteins which are found in various bacterial species (Fee, 1975; Lappin, 1981). Azurins are electron-transfer proteins and it is believed that azurin receives the electron from the cytochrome c_{551} protein before the electron is subsequently passed to the cytochrome oxidase/nitrite reductase system.

Several amino-acid residues such as the residues near His 35, the residues in the vicinity of His 83, the surface residue Gln 91, and the hydrophobic residues which are located close to His 117 are considered to be important (Farver, Blatt & Pecht, 1982; Van de Kamp, Silvestrini, Brunori, Beeumen, Hali & Canters, 1990) for the azurin's particular function. Recently, the structure of the *P. aeruginosa* azurin mutants Gln 35 and Leu 35 were reported by Nar, Messerschmidt, Huber, Van de Kamp & Canters (1991). In order to establish the possible role of the hydrophobic region in electron transfer, Phe 114 a conserved residue located near His 117 has been suggested as an interesting and important residue for the mechanism. Therefore, structural knowledge obtained for the wild-type azurin and similar knowledge deduced from the structures of selected azurin mutants will probably be very important for the understanding of, for example, the electron-transport mechanism.

Crystallization of *P. aeruginosa* azurin was reported by Adman, Stenkamp, Sieker & Jensen (1978). Norris, Anderson, Baker & Rumball (1979) and Baker (1988) obtained *Alcaligenes denitrificans* azurin crystals. The tertiary structure of azurin from *P. denitrificans* (known as *Alcaligenes sp.* NCIB 11015) was determined by Korszun (1987). Nar, Messerschmidt, Huber, Van de Kamp & Canters (1991) crystallized two *P. aeruginosa* azurin mutants, namely Gln 35 and Leu 35.

In this paper we present a new crystal form of the azurin mutant Ala 114, and in addition, some preliminary crystallographic data.

2. Materials and methods

The azurin mutant Ala 114 (Pascher, Bergström, Malmström, Vänngård & Lundberg, 1989) used in this work was supplied by the Department of Biochemistry and Biophysics, University of Göteborg.

Crystals of the azurin mutant Ala 114 were grown using the hanging-drop method. Crystallization droplets of 20 μl initial volume were placed on coverslips suspended over the reservoir. The crystallization droplets consisted of 10 μl of protein solution (15 mg ml^{-1}) and 10 μl of reservoir solution.

The reservoir solution was prepared as follows: A few drops of 1 *M* CH_3COOH were added to 6·0 ml 1·75 *M* $CaCl_2.2H_2O$ and 6·5 ml 1·82 *M* CH_3COONa solution to achieve a buffer mixture at pH 6·0. The mixture was then made up to 25 ml with PEG 4000 (1 g ml^{-1}). The petri dishes were kept at six different temperatures: in a refrigerator at 6°C, in temperature-controlled rooms at temperatures of 15, 21 and 24°C, and in heat chambers at temperatures of 28 and 37°C.

3. Results and discussion

Single crystals were obtained using the method described above and small crystal seeds could be observed within 2–3 days. The complete formation of crystals was accomplished in 18–20 days. The largest crystals were found in the dishes kept at 24°C, where each droplet contained 1–2 prismatically formed crystals of roughly 0·8 × 0·8 × 0·2 mm. For the prismatic crystals obtained at 6 and 15°C mass crystallization always occurred and hundreds of small crystals aggregated in each droplet. No crystallization occurred in the petri dishes stored at 28 and 37°C.

* Author for correspondence.

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Table 1. *Data statistics showing the number of collected and observed reflections based on different σ levels and at various resolution intervals*

Crystal number	Shell number	Resolution (Å)	Number of reflections collected	Number (%) of observed reflections $I \geq 3\sigma_I$	$I \geq 1.96\sigma_I$	$I \geq \sigma_I$
1	1	20·5–5·0	2315	1725 (74·5)	1864 (80·5)	1932 (83·5)
1	2	5·0–3·5	4651	2272 (48·8)	2651 (57·0)	2977 (64·0)
2	3	3·5–3·0	4133	1818 (44·0)	2010 (48·6)	2265 (54·8)
2	4	3·0–2·7	3776	1930 (51·1)	1989 (52·7)	2169 (57·4)
	Total	20·5–2·7	14 875	7745 (52·1)	8514 (57·2)	9343 (62·8)

Table 2. *Data-collection parameters*

Detector aperture (mm)	3·0 horizontal, 3·0 vertical
Crystal-to-detector distance (cm)	25
Scan type	ω
Scan rate (° min^{-1})	1·0 (2 rescans)
Scan width (°)	0·80 + 0·30tanθ
Software	*TEXSAN* (Molecular Structure Corporation, 1985)

Two crystals grown at 24°C (Fig. 1), of approximate size 0·6 × 0·6 × 0·15 mm, were mounted in two capillaries each of 1 mm diameter. Diffraction data for the Ala 114 azurin mutant were collected at room temperature using a modified Syntex $P2_1$ diffractometer. From 20 reflections, in the range $10{\cdot}72 \leq 2\theta \leq 16{\cdot}61°$ using Cu $K\alpha$ graphite-monochromated radiation, the cell constants were determined and refined to be $a = 51{\cdot}03$ (5), $b = 83{\cdot}36$ (5), $c = 66{\cdot}30$ (6) Å and $\beta = 111{\cdot}0$ (1)° and the space group was considered to be $P2_1$ based on the systematic reflection extinctions (Fig. 2). 14 875 reflections were then collected in four shells from two crystals according to the data given in Tables 1 and 2. The data extend to 2·7 Å resolution. No absorption correction was applied, but a decomposition correction was carried out.

The decomposition allowed during data collection was 25% in intensity. The data were scaled together with the help of three standard reflections.

Assuming there are four azurin molecules per asymmetric unit, each of them with a molecular weight of 13 925 daltons, the calculated value of V_m (Matthews, 1968) is 2·36 Å^3 dalton^{-1}.

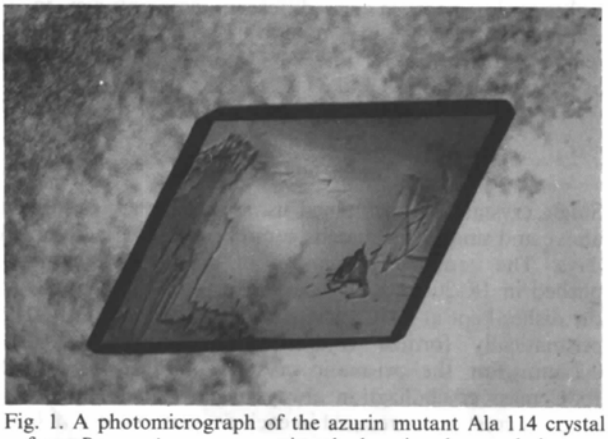
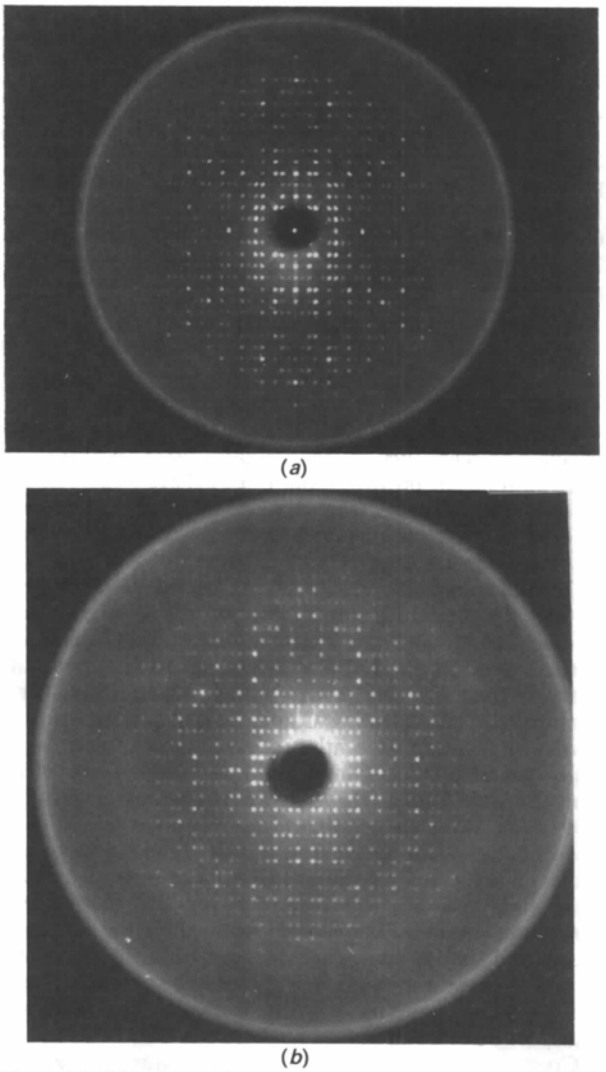


Fig. 1. A photomicrograph of the azurin mutant Ala 114 crystal from *P. aeruginosa* grown using the hanging-drop technique.

One conclusion drawn from our experiment is that the concentration of precipitating agent is seemingly an important parameter. Clearly, no crystals could be obtained from a 0·5 *M* buffer while the best crystals formed in the buffer at a concentration of around 0·25 *M*. When the buffer concentration was lowered to 0·05 *M* only small crystals grew in the hanging drop, and in some cases the protein precipitated.

It is also very clear that for the azurin system the crystallization process is very dependent on temperature and the best crystals were formed at 24°C. We also analyzed the pH dependence of the crystallization process. The optimal pH condition for crystal growth was found to be pH 6·0 although crystals grew in the pH range 5·0–7·0.

Structure interpretation of the Ala 114 mutant is in progress using rotation–translation function calculations.



(*a*)

(*b*)

Fig. 2. (*a*) 0*kl* and (*b*) 2*kl* precession photographs obtained from a crystal of the azurin mutant Ala 114. The crystal-to-film distance was 75·0 mm and the precession angle 17·5°. The Cu $K\alpha$ radiation was generated by rotating anode at 40 kV and 100 mA.

Biophysical data such as redox potentials and their temperature dependence are currently being measured at the Department of Biochemistry and Biophysics, University of Göteborg, and these data will then be viewed in the light of the structural changes in the mutants.

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Preliminary crystallographic study of peanut peroxidase.

By NENAD BAN, *Department of Biochemistry, University of California at Riverside, Riverside, California* 92521, *USA*, ROBERT B. VAN HUYSTEE, *The Department of Plant Science, University of Western Ontario, London, Ontario N6A 4B7, Canada*, JOHN DAY, AARON GREENWOOD and STEVE LARSON, *Department of Biochemistry, University of California at Riverside, Riverside, California* 92521, *USA*, ROBERT ESNAULT, *Institute des Sciences Vegetales, CNRS*, 91198 *Gif-sur-Yvette CEDEX, France*, and ALEXANDER MCPHERSON,* *Department of Biochemistry, University of California at Riverside, Riverside, California* 92521, *USA*

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Abstract

The cationic isozyme of peroxidase isolated from suspension cultures of peanut cells is a heme-containing and calcium-dependent glycoprotein having four covalently attached oligosaccharide chains. Attempts were made to crystallize the glycoprotein for X-ray diffraction analysis, and these have met with some success. Crystals have now been grown that are suitable for a full three-dimensional structural analysis. The crystals are thin plates and we have shown them to be of the orthorhombic space group $P2_12_12_1$ with $a = 48{\cdot}1$, $b = 97{\cdot}2$, $c = 146{\cdot}2$ Å. The crystals diffract to beyond 2·8 Å resolution, appear to be stable to lengthy X-ray exposure, and contain two molecules of 40 000 daltons each in the asymmetric unit.

Introduction

Most higher plants produce a variety of isozymic forms of the enzyme peroxidase (E.C. 1.11.1.7) which has been used as a convenient marker in genetic, physiological and pathological studies (Greppin, Penel & Gaspar, 1986; van Huystee, 1987). In all cases, the enzyme is a glycoprotein that contains a heme prosthetic group responsible for its activity. The pattern of expression in plants is influenced by environmental stimuli, is developmentally regulated, and is tissue specific (Cassab & Varner, 1988). Although the function of peroxidases in plants is still uncertain, it has been implicated in polysaccharide cross-linkages with extensin monomers, indoleacetic acid oxidation, liquification, wound healing, phenol oxidation, defense against pathogens and regulation of cell elongation (Greppin, Penel & Gaspar, 1986; Cassab & Varner, 1988).

Peroxidases are synthesized by cultured plant cells which then secrete the cationic isozyme into the medium. It provides, thus, a straightforward means for its purification (van Huystee, 1987; Stephan & van Huystee, 1981). Peanut cell peroxidases have been shown to consist predominantly of two cationic and one anionic species (Stephan & van Huystee, 1981). The major cationic isozyme represents 75% of the medium's peroxidase activity. The cDNAs for both cationic forms of peanut peroxidase have now been cloned and sequenced (Buffard, Breda, van Huystee, Asemota, Pierre, Ha & Esnault, 1990), and the four oligosaccharides covalently bound to the protein from the major cationic isozyme have been extensively studied (Hu & van Huystee, 1989; van Huystee, Hu & Sesto, 1990).

The major cationic isozyme of peanut cell peroxidase has a total molecular weight of 40 000 daltons. It consists of a single polypeptide chain 307 residues in length of 32 954 daltons molecular weight. This protein component is covalently attached to four polysaccharide chains of total weight 8500 daltons comprising 21% of the total glycoprotein molecular weight. The isoelectric point of the protein is 8·9. The enzyme contains a single heme group that is essential for its activity and gives it a red color and absorption maximum in the Soret region at 405 nm. The

* Address all correspondence to this author.

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