Acta Cryst. (1994). D**50**, 37–39

Crystallization and Preliminary Crystallographic Data for the Azurin Mutant End-121 from *Pseudomonas aeruginosa*

BY RICHARD W. STRANGE AND LORETTA MURPHY

Molecular Biophysics Group, SERC Daresbury Laboratory, Warrington, Cheshire WA4 4AD, England

B. GÖRAN KARLSSON

Department of Biochemistry and Biophysics, Chalmers University of Technology and The University of Göteborg, S-412 96 Göteborg, Sweden

PETER F. LINDLEY

Division of Structural Biology, SERC Daresbury Laboratory, Warrington, Cheshire WA4 4AD, England

LENNART G. LUNDBERG AND BENGT REINHAMMAR

Department of Biochemistry and Biophysics, Chalmers University of Technology and The University of Göteborg, S-412 96 Göteborg, Sweden

AND S. SAMAR HASNAIN

Molecular Biophysics Group, SERC Daresbury Laboratory, Warrington, Cheshire WA4 4AD, England

(*Received* 11 *June* 1993; *accepted* 9 *August* 1993)

Abstract

Pseudomonas aeruginosa azurin has been crystallized from a mutant where residues from Met121 to Lys128 have been deleted from the protein. The crystals form pale-blue well formed prisms in the orthorhombic space group $P2_12_12_1$, with cell dimensions $a = 60.79$ (5), $b = 123.47$ (5), $c = 187.77$ (5) Å. The crystals diffract to 3.0 Å and there are eight molecules in the asymmetric unit.

Introduction

Pseudomonas aeruginosa azurin is an electron-transfer protein belonging to a small class of bacterial type 1 copper proteins (Ryden, 1984; Adman, 1985). The crystal structure of *P. aeruginosa* has been reported by Adman, Stenkamp, Sieker & Jensen (1978) and Adman & Jensen (1981). In recent years site-directed mutagenesis has provided an important means of studying the structure–function relationships in the type 1 proteins. Nar, Messerschmidt, Huber, van de Kamp & Canters (1991*a*) have obtained the high-resolution crystal structure of the native form of *P. aeruginosa* azurin expressed in *Escherichia coli*. Several residues believed to be important in the electron-transfer mechanism of azurin have been mutated and the crystal structures of two of them, Gln35His and Leu35His, have been determined (Nar, Messerschmidt, Huber, van de Kamp & Canters, 1991*b*). Preliminary crystallographic results on an Ala114Phe mutant have also been reported (Tsai, Langer, Sjölin & Pascher, 1992). The methionine 121 residue, which provides one of the copper ligands in the native protein and has been implicated in tuning the redox potential of the blue copper site, has been replaced by glutamine and has been successfully crystallized (Karlsson, Tsai, Nar, Langer & Sjölin, 1993).

In this paper we present preliminary crystallographic data on a mutant of *P. aeruginosa* where residues from Met121 to Lys128 have been deleted. Electron-spin resonance, X-ray absorption, optical and redox-potential measurements for this mutant (End-121) have previously been reported (Murphy *et al.*, 1993). Deletion of these eight residues does not prohibit the redox function of the protein. The redox potential (205 mV) is close to that of the type 1 copper protein stellacyanin from *Rhus vernicefera*, which lacks the methionine ligand (Reinhammar, 1970). The structure of the End-121 mutant may shed light on the structure of stellacyanin, which has so far proved difficult to crystallize (H. Freeman, personal communication).

Materials and methods

The azurin mutant End-121 was prepared by methods previously described (Karlsson, Pascher,

Nordling, Arvidsson & Lunderg, 1989; Karlsson, Nordling, Pascher, Tsai, Sjolin & Lundberg, 1991). Crystals were grown using the hanging-drop method. Crystallization droplets consisting of 10 μl protein solution (13 mg ml^{-1}) and 10 μl reservoir were used. The reservoir solution consisted of 50% saturated ammonium sulfate solution, 0.6 *M* $CdCl_2$, buffered with 0.1 *M* sodium acetate pH 5.7. The crystallization trays were kept at 277, 291 and 297 K.

Results and discussion

Single crystals were obtained at 291 and 297 K but not at 277 K. Crystals formed very slowly and small seeds appeared only after 2–3 weeks. Crystal growth ceased after 4–6 weeks. The largest crystals obtained were about 0.3 × 0.3 × 0.1 mm, Fig. 1.

Crystals were mounted in 1 mm diameter capillary tubes. Diffraction data were taken at station 7.2 at the Synchrotron Radiation Source, Daresbury Laboratory, and recorded photographically using an Arndt–Wonacott oscillation camera (Arndt & Wonacott, 1977) at a wavelength of 1.488 Å and a film-to-crystal distance of 100.1 mm. The crystals were cooled to 275 K during data collection. Diffraction extended to 3 Å, Fig. 2, and the crystals proved relatively unstable even at low temperature. They ceased to diffract after 2–3 h exposure to X-rays, only 30° of a data set has been collected so far. A complete data set will be obtained from freshly grown crystals in the near future.

The photographic films were digitized using a Joyce–Loebl Scandig 3 microdensitometer using a raster step size of 50 μm and an optical density range 0–2 units. Preliminary cell parameters were measured directly from film and the values obtained were used in the *REFIX* program (Kabsch, 1988) to auto-index the oscillation film and determine the single-crystal orientation matrix. The film data were subsequently processed using the *MOSFLM* suite of programs based on the Cambridge program *MOSCO* (Nyborg & Wonacott, 1977). The cell dimensions were refined to be $a = 60.79\ (5)$, $b = 123.47\ (5)$, $c = 187.77\ (5)$ Å and the space group was considered to be $P2_12_12_1$ based upon observed systematic reflection absences.

Assuming that about 50% of the volume of the unit cell is occupied by water, the estimated number of molecules in the each of the four asymmetric units is eight. The packing of the molecules in the unit cell of native azurin comprises four molecules in the asymmetric unit which form a dimer of dimers (Adman & Jensen, 1981; Nar, Messerschmidt, Huber, van de Kamp & Canters, 1991*a*). There are several points of interaction between adjacent mol-

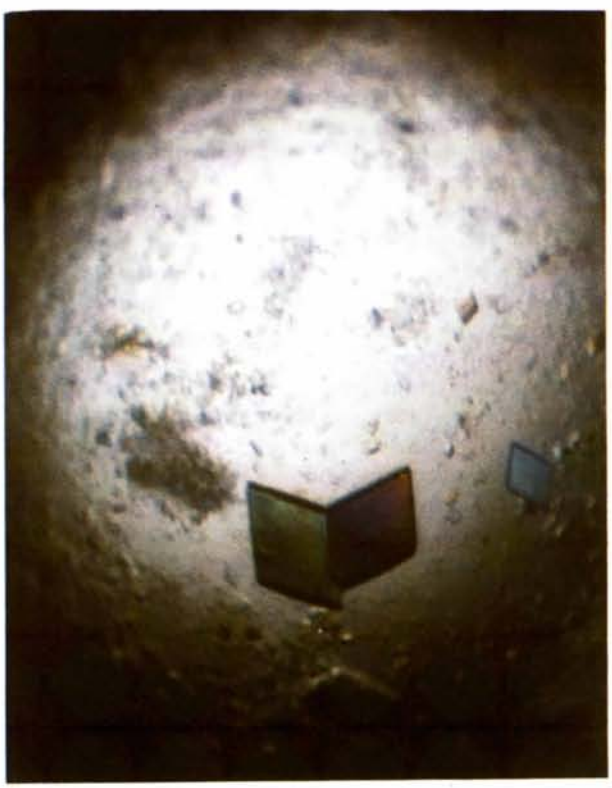

Fig. 1. A photomicrograph (×35) of End-121 mutant azurin crystals from *P. aeruginosa*.

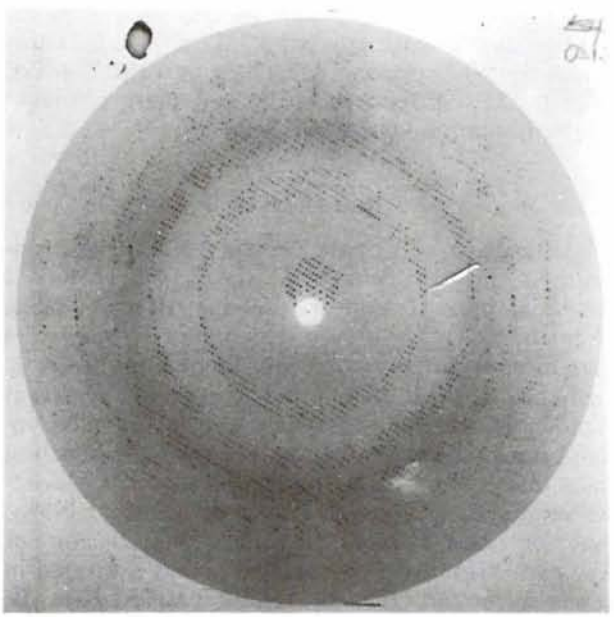

Fig. 2. A 3° oscillation photograph obtained from a crystal of the azurin mutant End-121 using station 7.2 at the SRS, SERC Daresbury Laboratory. The crystal-to-film distance was 100.1 mm and X-ray wavelength 1.488 Å.

ecules of the two dimers which occur *via* the residuals deleted in the End-121 mutant. Their deletion may thus lead to a change in the arrangement of azurin molecules in the mutant.

We would like to thank the UK SERC and Swedish NFR for their financial support.

References

ADMAN, E. T. (1985). *Topics in Molecular and Structural Biology: Metalloproteins*, edited by P. HARRISON, pp. 1–46. London: MacMillan.

ADMAN, E. T. & JENSEN, L. H. (1981). *Isr. J. Chem.* **21**, 8–12.

ADMAN, E. T., STENKAMP, R. E., SIEKER, L. C. & JENSEN, L. H. (1978). *J. Mol. Biol.* **123**, 35–47.

ARNDT, U. W. & WONACOTT, A. J. (1977). Editors. *The Rotation Method in Crystallography*. Amsterdam: North-Holland.

KABSCH, W. (1988). *J. Appl. Cryst.* **21**, 67–71.

KARLSSON, B. G., NORDLING, M., PASCHER, T., TSAI, L.-C., SJOLIN, L. & LUNDBERG, L. G. (1991). *Protein Eng.* **4**(3), 343–349.

KARLSSON, B. G., PASCHER, T., NORDLING, M., ARVIDSSON, R. H. A. & LUNDBERG, L. G. (1989). *FEBS Lett.* **246**(1/2), 211–217.

KARLSSON, B. G., TSAI, L.-C., NAR, H., LANGER, V. & SJÖLIN, L. (1993). *J. Biol. Chem.* Submitted.

MURPHY, L. M., STRANGE, R. W., KARLSSON, G., LUNDBERG, L., PASCHER, T., REINHAMMAR, B. & HASNAIN, S. S. (1993). *Biochemistry*, **32**, 1965–1975.

NAR, H., MESSERSCHMIDT, A., HUBER, R., VAN DE KAMP, M. & CANTERS, G. W. (1991*a*). *J. Mol. Biol.* **221**, 765–772.

NAR, H., MESSERSCHMIDT, A., HUBER, R., VAN DE KAMP, M. & CANTERS, G. W. (1991*b*). *J. Mol. Biol.* **218**, 427–447.

NYBORG, J. & WONACOTT, A. J. (1977). *The Rotation Method in Crystallography*, edited by U. W. ARNDT & A. J. WONACOTT. Amsterdam: North-Holland.

REINHAMMAR, B. (1970). *Biochim. Biophys. Acta*, **205**, 35–47.

RYDEN, L. (1984). *Copper Proteins and Copper Enzymes*, edited by R. LONTIE, p. 157. Boca Raton: CRC Press.

TSAI, L.-C., LANGER, V., SJÖLIN, L. & PASCHER, T. (1992). *Acta Cryst.* **B48**, 107–109.