Acta Cryst. (1997). **D53**, 129–130

Crystallization and preliminary X-ray studies of plastocyanin from *Silene* expressed in *E. coli*

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(*Received 29 May 1996; accepted 29 August 1996*)

Abstract

Plastocyanin from *Silene* (SilPc) expressed in *E. coli* has been crystallized in a form suitable for X-ray diffraction analysis by a macroseeding method using ammonium sulfate as a precipitant in acetate buffer (pH = 5.5). These crystals belong to the trigonal space group $P3_121$ or $P3_221$ with lattice parameters $a = b = 76.6$, $c = 65.5$ Å, indicating an asymmetric unit containing two plastocyanin molecules. The crystals diffracted to at least 2.0 Å.

1. Introduction

Plastocyanins are type I copper proteins with a single polypeptide chain (10.5 kDa). They function as a mobile electron carrier between the cytochrome *b*6–cytochrome *f* complex (Cyt. *b*6–*f*) and photosystem I (PS-I) complex within the electron-transport chain of oxygen-evolving photosynthetic organisms. They are found in plants, algae and cyanobacteria (Haehnel, 1984; Sykes, 1990; Redinbo, Yeates & Merchant, 1994). Characteristic properties include an intense blue color, a narrow hyperfine splitting in the copper ESR spectrum, and an unusually high reduction potential.

Plastocyanins from higher plants and green algae are acidic proteins, whereas those from cyanobacteria can be either basic or acidic. Differences between the kinetics of reaction with PS-I and Cyt. *f* have been reported for the plastocyanins of plants, algae and cyanobacteria (Farver, Shahak & Pecht, 1982; Adam & Malkin, 1989; Haehnel *et al.*, 1994). In order to understand changes of the electron-transfer mechanisms during the evolution from cyanobacteria to plants, it is important to know the structures of plastocyanins from various sources. So far the crystal structures of only a few plastocyanins have been reported. The structure of plastocyanin from poplar was described at 2.7 Å resolution in 1983 (Guss & Freeman, 1983) and at 1.33 Å resolution in 1992 (Guss, Bartunik & Freeman, 1992). The other two structurally characterized plastocyanins are derived from green algae *Enteromorpha prolifera* (Collyer, Guss, Sugimura, Yoshizaki & Freeman, 1990) and *Chlamydomonas reinhardtii* (Redinbo *et al.*, 1993). No information is available for plastocyanins from cyanobacteria.

It has been suggested that plastocyanins are unique proteins among the type I copper proteins in having two different electron-transfer sites (Sykes, 1990, 1991), namely a hydrophobic site adjacent to the copper, and an acidic patch on a more remote region of the surface of the molecule. The precise specificities of the two sites, the binding of electron-transfer partners, and the intramolecular electron transfer to and from the Cu atom, are not yet fully understood.

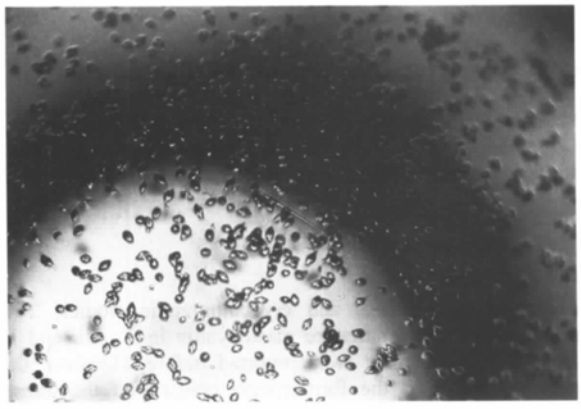
With this background, we have started a program to develop a superior expression systems for plastocyanin and to carry out site-directed mutagenesis and crystallographic studies. Two expression systems for mature plastocyanin in *E. coli* and in transgenic tobacco plant were reported before. However, the expression vector in the former case consists of the transit peptide sequence of azurin as well as the mature sequence of plastocyanin, and the yield of mutant plastocyanin in the latter case is quite low (Nordling, Olausson & Lundberg, 1990; Nordling, Sigfridsson, Young, Lundberg & Hansson, 1991; He, Modi, Bendall & Gray, 1991). An expression system capable of yielding large amounts of plastocyanin precursor from *Silene* has now been established (Hibino, Boer, Weisbeek & Takabe, 1991; Hibino, Lee & Takabe, 1994) and several mutants have also been made (Lee, Hibino, Takabe, Weisbeek & Takabe, 1995). Since the yields are very high, it is possible to explore further the relationship between the function and the structures. Here we report the crystallization and preliminary X-ray studies of SilPc expressed in *E. coli*.

2. Methods and results

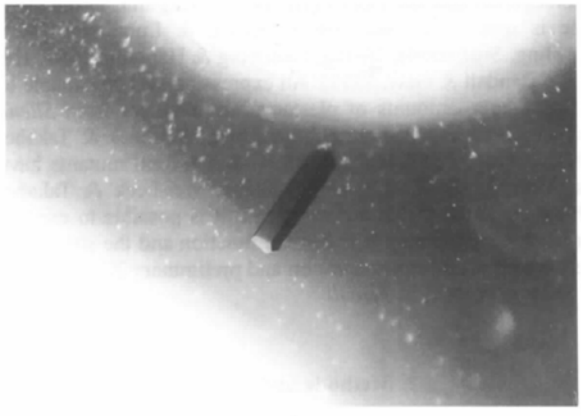
All reagents were of chemical grade and were used without further purification. The expression of SilPc in *E. coli*, its isolation, and purification were carried out by the previously reported method (Hibino *et al.*, 1991). The spectra and kinetic properties of the purified SilPc are indistinguishable from those of purified spinach plastocyanin. The protein as a solution (about 15 mg ml^{-1}) was stored in potassium phosphate buffer (0.1 *M*, pH 6.0) at 277 K. Crystallization experiments were performed using the hanging-drop vapor-diffusion method at 293 K.

A 6 µl droplet of SilPc solution (7.5 mg ml^{-1}) containing sodium acetate buffer (0.1 *M*, pH 5.5) and 26.5% saturated ammonium sulfate was equilibrated against 500 ml reservoir solution containing sodium acetate buffer (0.1 *M*, pH 5.5) and 53% saturated ammonium sulfate. Rhombic crystals with maximum dimensions of 0.01 × 0.01 × 0.01 mm appeared in the droplet after one week (Fig. 1*a*). However, these crystals were too small to be used for X-ray experiments and modification of the crystallization conditions did not give a better result. High-quality crystals were then chosen as seeds for macroseeding using the same crystallization conditions. One week later SilPc crystals with maximum dimensions of 0.2 × 0.2 × 1.0 mm were obtained (Fig. 1*b*).

The crystals of SilPc were sealed in glass capillaries with a small amount of mother liquor as usual. X-ray diffraction data were collected on an R-AXIS IIc image-plate system (Rigaku) using Cu $K\alpha$ radiation, $\lambda = 1.5418$ Å with a crystal-to-detector distance of 97.6 mm. Image-plate data were processed using the R-AXIS software. The crystals diffracted to at least 2.0 Å resolution. There was no evidence of radiation damage. The space group is trigonal, $P3_121$ or $P3_221$, with lattice parameters



(*a*)



(*b*)

Fig. 1. (*a*) A crystal of the SilPc with maximum dimensions of $0.01 \times 0.01 \times 0.01$ mm appeared in the droplet of ammonium sulfate solution after one week. (*b*) A crystal of the SilPc with maximum dimensions of $0.2 \times 0.2 \times 1.0$ mm obtained by macroseeding method.

$a = b = 76.6$, $c = 65.5$ Å. These dimensions correspond to an asymmetric unit of two SilPc molecules. The calculated V_m is 2.64 Å^3 Da^{-1}, which is close to the average for proteins (Matthews, 1968). The structure analysis is in progress.

Ms Chunmin Li thanks Yoneyama Rotary Scholarship Foundation in Japan for offering a studentship.

References

Adam, Z. & Malkin, R. (1989). *Biochim. Biophys. Acta*, **975**, 158–165.
Collyer, C. A., Guss, J. M., Sugimura, Y., Yoshizaki, F. & Freeman, H. C. (1990). *J. Mol. Biol.* **211**, 617–632.
Farver, O., Shahak, Y. & Pecht, I. (1982). *Biochemistry*, **21**, 1885–1890.
Guss, J. M., Bartunik, H. D. & Freeman, H. C. (1992). *Acta Cryst.* B**48**, 790–811.
Guss, J. M. & Freeman, H. C. (1983). *J. Mol. Biol.* **169**, 521–563.
Haehnel, M. (1984). *Annu. Rev. Plant Physiol.* **35**, 659–693.
Haehnel, W., Jansen, T., Gause, K., Klosgen, R. B., Stahl, B., Michl, D., Huvermann, B., Karas, M. & Herrmann, R. G. (1994). *EMBO J.* **13**, 1028–1038.
He, S., Modi, S., Bendall, D. S. & Gray, J. C. (1991). *EMBO J.* **10**, 4011–4016.
Hibino, T., Boer, A. D. D., Weisbeek, P. J. & Takabe, T. (1991). *Biochim. Biophys. Acta*, **1058**, 107–112.
Hibino, T., Lee, B. H. & Takabe, T. (1994). *J. Biochem.* **116**, 111–117
Lee, B. H., Hibino, T., Takabe, T., Weisbeek, P. J. & Takabe, T. (1995). *Biochem.* **117**, 1209–1217.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Nordling, M., Olausson, T. & Lundberg, L. G. (1990). *FEBS Lett.* **276**, 98–102.
Nordling, M., Sigfridsson, K., Young, S., Lundberg, L. G. & Hansson, O. (1991). *FEBS Lett.* **291**, 327–330.
Redinbo, M. R., Cascio, D., Choukair, M. K., Rice, D., Merchant, S. & Yeates, T. O. (1993). *Biochemistry*, **32**, 10560–10567.
Redinbo, M. R., Yeates, T. O. & Merchant, S. (1994). *J. Bioenerget. Biomemb.* **26**, 49–66.
Sykes, A. G. (1990). *Struct. Bonding*, **75**, 175–224.
Sykes, A. G. (1991). *Adv. Inorg. Chem.* **36**, 377–408.