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Crystallization and preliminary crystallographic analysis of the high-potential iron–sulfur protein from *Thermochromatium tepidum*

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The high-potential iron–sulfur protein (HiPIP) is an electron carrier between the photosynthetic reaction centre and the cytochrome bc_1 complex in the electron-transfer chain of photosynthesis. The purified HiPIP from *Thermochromatium tepidum* (formerly *Chromatium tepidum*) was crystallized in a solution of 1.4 *M* ammonium sulfate and 0.1 *M* sodium citrate pH 3.5. The crystals diffract X-rays beyond 1.4 Å resolution and belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 47.12 (6), b = 59.59 (10), c = 23.62 (3) Å. The structure was preliminarily solved by the molecular-replacement method. The crystal structure of HiPIP from *T. tepidum* showed that the proteins exist as monomers, although HiPIPs from several other species can form dimers.

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1. Introduction

The high-potential iron–sulfur protein (HiPIP) is a soluble electron carrier containing a single [4Fe–4S] cluster. HiPIPs show the 2+/3+ redox transition and their redox potentials are positive and relatively high compared with other ferredoxins with the 1+/2+ redox transition (De Klerk & Kamen, 1966; Dus *et al.*, 1967). HiPIPs exist abundantly in the periplasmic space in many photosynthetic bacteria. They transfer electrons from the cytochrome bc_1 complex to the photosynthetic reaction centre (RC) in photosynthesis reactions, which results in the reduction of photo-oxidized special-pair bacteriochlorophyll. In this electron transfer, HiPIPs interact with the peripheral regions of the RC, namely the *c*-type cytochrome subunit, which possesses four haems acting as electron acceptors. Several kinetic studies have proposed the possible docking site of electron carriers and the electron-transfer pathway in the cytochrome subunit of the RC (Hochkoeppler *et al.*, 1996; Knaff *et al.*, 1991; Meyer *et al.*, 1993). Site-directed mutagenesis has indicated that HiPIPs possibly bind near haem 1, the most distal haem from the special pair (Osyczka *et al.*, 1998, 1999), although haem 1 seems unfavourable for accepting electrons from the viewpoint of its redox potential (Dracheva *et al.*, 1988).

We have already crystallized RC from *T. tepidum* (formerly *C. tepidum*), whose physiological electron donor is HiPIP (Katayama *et al.*, 1994; Kobayashi & Nozawa, 1993), and its structure determination is almost complete (Nogi *et al.*, in preparation). We have therefore purified and crystallized the HiPIP from *T. tepidum* so as to analyze the molecular recognition on the basis of structures originating from the same species.

Crystal structures of HiPIPs have been determined from several bacteria (Breiter *et al.*, 1991; Freer *et al.*, 1975; Kerfeld *et al.*, 1998; Parisini *et al.*, 1999; Rayment *et al.*, 1992). Most of these HiPIPs were crystallized in the dimeric state and the dimerizations of these HiPIPs are mediated by the hydrophobic surface adjacent to the [4Fe–4S] cluster. Spectroscopic study has also suggested that HiPIPs can form dimers in solution in the same manner (Bertini *et al.*, 1993; Couture *et al.*, 1999). In order to determine how these proteins behave as electron carriers, it is important to determine whether and how HiPIPs dimerize physiologically, as different molecular surfaces must interact with the RC in monomeric and dimeric HiPIPs during electron-transfer reactions.

The crystal packing and intermolecular contacts of HiPIP from *T. tepidum* are therefore also of interest. HiPIP from *T. tepidum* has 83 amino acids and its measured molecular weight is 9136 Da (Moulis *et al.*, 1993). As mentioned above, one [4Fe–4S] cluster is linked covalently to the polypeptides by four cysteine side chains and its redox midpoint potential is +323 mV (Moulis *et al.*, 1993). Here, we report the crystallization conditions and the preliminary structure determination of HiPIP from *T. tepidum*, which affords information regarding the protein's monomeric and dimeric architectures.

2. Materials and methods

T. tepidum was grown according to the published procedure (Madigan, 1984;

Table 1
Crystal data and data-collection statistics.

X-ray source	BL-6A, Photon Factory, KEK
Wavelength (Å)	1.0
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	
a	47.12 (6)†
b	59.59 (10)†
c	23.62 (3)†
Resolution (Å)	20.0–1.4 (1.43–1.40)‡
No. of observations	55667
No. of unique reflections	11477
Multiplicity	4.9
$\langle I/\sigma(I)\rangle$	20.1 (3.1)‡
Completeness (%)	81.4 (54.5)‡
$R_{\rm merge}$§ (%)	7.1 (32.1)‡

† Estimated standard deviation in parentheses. ‡ Values for the highest resolution shell in parentheses. § $R_{\rm merge} = \sum_{hkl}|I - \langle I\rangle|/\sum_{hkl}I$.

Madigan, 1986). A crude lysate was obtained by suspending cells in 5 m*M* Tris–HCl pH 8.0 (buffer *A*) and disrupting the cells by sonication. After particulate material had been removed by ultracentrifugation at 150 000*g* for 90 min, the supernatant was subjected to anion-exchange chromatography on DEAE–Toyopearl 650S (TOSOH). HiPIP was eluted from the column with buffer *A* containing 10 m*M* NaCl. Column fractions containing HiPIP were then concentrated and applied to a Superdex-200 gel-filtration column (Amersham Pharmacia Biotech) equilibrated with 50 m*M* NaCl in buffer *A* and then eluted with the same buffer. The purity of HiPIP was confirmed by absorption spectra and the presence of a single band corresponding to a molecular weight of 9136 Da on sodium dodecyl sulfate–polyacrylamide gels. The purified protein was concentrated to 20 mg ml^{-1} using a Centricon-3 concentrator (Amicon).

Dynamic light scattering (DLS), which allows the analysis of the state of HiPIPs in solution (Ferre-D'Amare & Burley, 1994; Mikol *et al.*, 1990; Zulauf & D'Arcy, 1992), was measured with a DynaPro-801 (Protein Solutions) and the data were analyzed with *DYNAMICS* version 3.00 software. 2 mg ml^{-1} of HiPIP solutions were analyzed under several conditions and each measurement included 10–15 samplings.

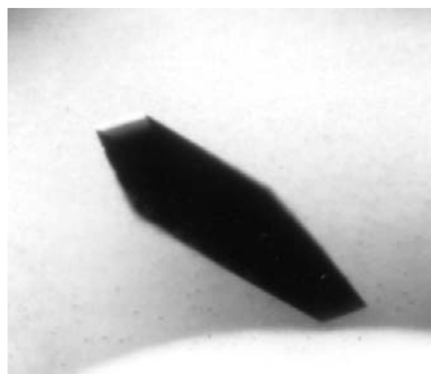

Figure 1
Crystal of HiPIP from *T. tepidum* (approximate dimensions 0.2 × 0.1 × 0.4 mm).

Intensity data used for the structure determination were collected at room temperature using synchrotron radiation at the BL-6A beamline of the Photon Factory, KEK, Tsukuba, Japan. X-rays were monochromated to 1.00 Å using an Si(111) monochromator system. A screenless Weissenberg camera was used with a 0.1 mm aperture collimator and a cylindrical cassette with a 286.5 mm radius (Sakabe *et al.*, 1995). The diffraction intensities were recorded on 200 × 400 mm imaging plates (Fuji Photo Film Co. Ltd) and read on a Fuji BAS2000 scanner (Miyahara *et al.*, 1986). The intensity data were processed with the program *DENZO* and merged with the program *SCALEPACK* (Otwinowski & Minor, 1997).

The molecular replacement was carried out using *AMoRe* (Navaza, 1994) from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). The molecular model was built by manual fitting to the electron-density map using the program *O* (Jones *et al.*, 1991) followed by structure refinement using the program *X-PLOR* (Brünger, 1992; Brünger *et al.*, 1990).

3. Results and discussion

Plate-like crystals (Fig. 1) were obtained under the following conditions: 4 µl of 20 mg ml^{-1} protein solution containing 0.7 *M* ammonium sulfate and 0.05 *M* sodium citrate pH 3.5 was equilibrated at 297 K against a reservoir solution consisting of 1.4 *M* ammonium sulfate and 0.1 *M* sodium citrate pH 3.5. The symmetry and systematic absences of the reflections indicated that the crystals belong to the orthorhombic space group $P2_12_12_1$. The unit-cell parameters are a = 47.12 (6), b = 59.59 (10), c = 23.62 (3) Å. The statistics for the intensity data are shown in Table 1.

The structure was solved by the molecular-replacement method using the crystal structure of HiPIP of *Allochromatium vinosum* (formerly *C. vinosum*; PDB code 1hip; Freer *et al.*, 1975) as a search model. The rotation and translation searches were calculated using the reflections in the 6.0–3.0 Å resolution range and each of them gave a clear solution. No unfavourable molecular contacts were observed in the crystal packing. After rigid-body refinement, the structural model including water molecules and a [4Fe–4S] cluster was refined using the simulated-annealing protocol combined with *B*-factor refinement, which gave a crystallographic *R* factor of 22.4% and a free *R* factor of 31.0% for the reflections in the resolution range 8.0–2.0 Å. Further refinement at higher resolution is now under way.

Based on the crystals containing only one molecule per asymmetric unit and the space group $P2_12_12_1$ having no twofold axes, HiPIPs of *T. tepidum* in the crystal are thought to exist as monomers. Moreover, there is no crystallographic molecular interaction mediated by hydrophobic surfaces as is found in the dimerization of HiPIPs from other species. On the other hand, the DLS measurement indicated that the HiPIP solution was polydisperse, which implies that HiPIP monomers easily associate and dissociate even under conditions that are close to the crystallization conditions. It was reported in the DLS analysis of HiPIP from *Marichromatium purpuratum* (formerly *C. purpuratum*) that the estimated average molecular weight corresponded to the dimeric state; the solution was also polydisperse (Kerfeld *et al.*, 1998). Crystallographic studies of HiPIPs from other species have shown that HiPIPs can form dimers, but the two monomers in the dimeric structure are not exactly related by the twofold axes; the dimeric interfaces must be significantly deviated and flexible in each dimeric structure. These findings suggest that HiPIPs do not form a rigid dimer in which dimer–monomer exchange may easily occur in solution. This behaviour of HiPIPs may affect the possible electron-transfer partners of HiPIP; electrons are possibly transferred not only from the reduced HiPIP to the oxidized RC but also to the oxidized HiPIP.

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References

Bertini, I., Gaudemer, A., Luchinat, C. & Piccioli, M. (1993). *Biochemistry*, **32**, 12887–12893.

Breiter, D. R., Meyer, T. E., Rayment, I. & Holden, H. M. (1991). *J. Biol. Chem.* **266**, 18660–18667.

Brünger, A. T. (1992). *X-PLOR. Version* 3.1. *A System for X-ray Crystallography and NMR.* Yale University, New Haven, Connecticut, USA.

Brünger, A. T., Krukowski, A. & Erickson, J. W. (1990). *Acta Cryst.* A**46**, 585–593.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Couture, M. M., Auger, M., Rosell, F., Mauk, A. G., Boubour, E., Lennox, R. B. & Eltis, L. D. (1999). *Biochim. Biophys. Acta*, **1433**, 159–169.

De Klerk, H. & Kamen, M. D. (1966). *Biochim. Biophys. Acta*, **112**, 175–178.

Dracheva, S. M., Drachev, L. A., Konstantinov, A. A., Semenov, A., Skulachev, V. P., Arutjunjan, A. M., Shuvalov, V. A. & Zaberezhnaya, S. M. (1988). *Eur. J. Biochem.* **171**, 253–264.

Dus, K., De Klerk, H., Sletten, K. & Bartsch, R. G. (1967). *Biochim. Biophys. Acta*, **140**, 291–311.

Ferre-D'Amare, A. R. & Burley, S. K. (1994). *Structure*, **2**, 357–359.

Freer, S. T., Alden, R. A., Carter, C. W. Jr & Kraut, J. (1975). *J. Biol. Chem.* **250**, 46–54.

Hochkoeppler, A., Zannoni, D., Ciurli, S., Meyer, T. E., Cusanovich, M. A. & Tollin, G. (1996). *Proc. Natl Acad. Sci. USA*, **93**, 6998–7002.

Jones, T. A., Zou, J. Y., Cowan, S. W. & Kjeldgaard, M. (1991). *Acta Cryst.* A**47**, 110–119.

Katayama, N., Kobayashi, M., Mottojima, F., Inaka, K., Nozawa, T. & Miki, K. (1994). *FEBS Lett.* **348**, 158–160.

Kerfeld, C. A., Salmeen, A. E. & Yeates, T. O. (1998). *Biochemistry*, **37**, 13911–13917.

Knaff, D. B., Willie, A., Long, J. E., Kriauciunas, A., Durham, B. & Millett, F. (1991). *Biochemistry*, **30**, 1303–1310.

Kobayashi, M. & Nozawa, T. (1993). *Bull. Chem. Soc. Jpn*, **66**, 3834–3836.

Madigan, M. (1984). *Science*, **225**, 313–315.

Madigan, M. (1986). *Int. J. Syst. Bacteriol.* **36**, 222–227.

Meyer, T. E., Bartsch, R. G., Cusanovich, M. A. & Tollin, G. (1993). *Biochemistry*, **32**, 4719–4726.

Mikol, V., Hirsch, E. & Giege, R. (1990). *J. Mol. Biol.* **213**, 187–195.

Miyahara, J., Takahashi, K., Amemiya, Y., Kamiya, N. & Satow, Y. (1986). *Nucl. Instrum. Methods A*, **246**, 572–578.

Moulis, J. M., Scherrer, N., Gagnon, J., Forest, E., Petillot, Y. & Garcia, D. (1993). *Arch. Biochem. Biophys.* **305**, 186–192.

Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.

Osyczka, A., Nagashima, K. V., Shimada, K. & Matsuura, K. (1999). *Biochemistry*, **38**, 2861–2865.

Osyczka, A., Nagashima, K. V., Sogabe, S., Miki, K., Yoshida, M., Shimada, K. & Matsuura, K. (1998). *Biochemistry*, **37**, 11732–11744.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Parisini, E., Capozzi, F., Lubini, P., Lamzin, V., Luchinat, C. & Sheldrick, G. M. (1999). *Acta Cryst.* D**55**, 1773–1784.

Rayment, I., Wesenberg, G., Meyer, T. E., Cusanovich, M. A. & Holden, H. M. (1992). *J. Mol. Biol.* **228**, 672–686.

Sakabe, N., Ikemizu, S., Sakabe, K., Higashi, T., Nakagawa, A., Watanabe, N., Adachi, S. & Sasaki, K. (1995). *Rev. Sci. Instrum.* **66**, 1276–1281.

Zulauf, M. & D'Arcy, A. (1992). *J. Cryst. Growth*, **122**, 102–106.