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# Isolation and Partial Characterization of Two Ferredoxins from the Photosynthetic Bacterium *Heliobacillus mobilis*

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Two ferredoxin (Fd) fractions, namely, Fd-A and Fd-B were isolated from *Heliobacillus mobilis* cells, and purified by ammonium sulfate fractionation, DEAE, gel-permeation and Phenyl-Superose column chromatographies under anaerobic conditions. Their absorption spectra were typical of 2[4Fe-4S] cluster type Fds with peaks at about 385 and 280 nm and a shoulder at about 305 nm. Their *N*-terminal amino acid sequences were determined, which showed that both of them contain a [4Fe-4S] cluster binding motif. Fd-B was sensitive to oxygen, and its $A_{385}$ value decreased by about 50% in 2 h at 4℃ under aerobic conditions. In contrast, $A_{385}$ of Fd-A was essentially unchanged up to 24 h under the same conditions.

**Key words :** heliobacteria, ferredoxin, iron sulfur cluster, electron transport

## INTRODUCTION

Heliobacteria are relatively recently found anoxygenic phototrophic prokaryotes [1] and have bacteriochlorophyll (BChl) *g* as the major photosynthetic pigment whose chemical structure is rather similar to chlorophyll (Chl) *a* than to BChl *a* [2] The reaction center (RC) of heliobacteria is considered to be similar to that of green sulfur bacteria and PSI of higher plants and cyanobacteria in that they contain very low-potential Fe-S clusters as the secondary electron acceptors. The electron transfer pathway around the RC in heliobacteria remains uncertain. In order to study the electron transfer pathway in heliobacteria from RC to external acceptors, we have purified two ferredoxin (Fd) fractions from the heliobacterium *Heliobacillus mobilis*. Fd of the green sulfur bacterium *Chlorobium thiosulfatophilum* was reported to be extremely sensitive to oxygen [3, 4] and it had been generally assumed that Fds from green sulfur bacteria are sensitive to oxygen. However, recent studies indicated that Fds from the moderately thermophilic green sulfur bacterium *Chlorobium tepidum* are not extremely sensitive to oxygen [5]. We studied oxygen sensitivity of Fds from *Heliobacillus mobilis*.

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## MATERIALS AND METHODS

Cells of *H. mobilis* (ATCC 43427) were grown anaerobically in 500 ml glass bottles for 18 h at 37°C, and harvested by centrifugation under anaerobic conditions. Fds were prepared essentially as described in Seo et al. [5] except that all the operations were performed under anaerobic conditions. The cells were suspended in a buffer containing 50 mM Tris-HCl (pH 7.8), 5 mM sodium ascorbate, 0.5 mM sodium dithionite, 1 mM phenyl-methanesulfonyl fluoride, 1 mM *p*-aminobenzamidine-HCl, 1 mM 6-amino-*n*-caproic acid, and 5 units/ml DNase, and disrupted by a French pressure cell. After removing unbroken cells by centrifugation at 20,000 x *g* for 20 min, the dark green supernatant was further centrifuged at 160,000 x *g* for 60 min. Ammonium sulfate was added to the supernatant to 40% saturation, and the solution was gently stirred for 2 h at 4°C. The mixture was centrifuged at 20,000 x *g* for 20 min, and ammonium sulfate was added to the supernatant to 80% saturation. After gently stirring for overnight, a light brown precipitate was collected by centrifugation at 20,000 x *g* for 20 min and the precipitate was suspended in a buffer containing 50 mM Tris-HCl (pH 7.8) and dialyzed against the same buffer. The sample was diluted twofold with the buffer and then applied to a DEAE-cellulose column that had been equilibrated with 50 mM Tris-HCl (pH 7.8), 0.1 mM EDTA, 100 mM NaCl. After washing the column with a buffer containing 50 mM Tris-HCl (pH 7.8), 0.1 mM EDTA, and 200 mM NaCl, the Fds were eluted with a buffer containing 50 mM Tris-HCl (pH 7.8), 0.1 mM EDTA, 500 mM NaCl. The brown colored fractions were collected and applied to a Sephadex G-50 column equilibrated with 50 mM Tris-HCl (pH 7.8), 300 mM NaCl and eluted with the same buffer. The combined Fd-rich fractions were mixed with an equal volume of saturated ammonium sulfate solution in 50 mM Tris-HCl buffer (pH 7.8), and applied to a Phenyl Superose 10/10 equilibrated with 50 mM Tris-HCl buffer (pH 7.8) containing 2 M ammonium sulfate at room temperature. The column was washed with two column volumes of the medium for equilibration, and the Fds were eluted as two major peaks (385nm) with a 60 ml inverse linear concentration gradient of ammonium sulfate of 2 to 0.8 M in 50mM Tris-HCl (pH 7.8). Each peak fraction was applied to an Mono Q 10/10 equilibrated with 50 mM Tris-HCl (pH 7.8) containing 100 mM NaCl, and Fds were eluted with a 20 ml linear gradient of NaCl from 100 mM to 600 mM in the same Tris-HCl buffer. N-terminal sequences were determined with a protein sequencer (Procise 491, Perkin-Elmer). Absorption spectra were measured with UV-2500PC (SIMADZU).

## RESULTS AND DISCUSSION

When Fds monitored at $A_{385}$ were eluted from a Phenyl Superose column with a decreasing concentration gradient of ammonium sulfate, they eluted in two discernible peaks (A and B, in the order of elution from the column). These Fds were purified to apparent homogeneity, and their absorption spectra are shown in Fig. 1. Their absorption spectra were typical of 2[4Fe-4S] cluster type Fds with peaks at 385 and 280 nm and a shoulder at about 305 nm. In the presence of Fd-$NADP^+$ reductase from spinach and purified reaction center complex from the green sulfur bacterium *C. tepidum*, both Fd-A and Fd-B were active in photoreduction of $NADP^+$ (data not shown). These results suggest that both Fds may serve as electron acceptors from RC in *H. mobilis*, although purification of photoactive RC from heliobacteria has not yet been reported.

The absorption spectra of these Fds were unchanged for at least 8 h at 4°C in anaerobic conditions. When air was bubbled to Fd-containing solutions, $A_{385}$ of Fd-A were essentially unchanged for 20 h at 4°C, while, $A_{385}$ of Fd-B were decreased to about a half in 2 h at 4°C (Fig. 1). Fd of the green sulfur bacterium *C. thiosulfatophilum* was

reported to be extremely sensitive to oxygen [3, 4]. The latter authors [4] reported that $A_{385}/A_{280}$ ratio of the purified Fd dropped from 0.71 to 0.60 when stored for 5 h at 4°C in air. More recently, Seo et al. [5] reported that at least three kinds of 2[4Fe-4S] are present in the green sulfur bacterium *C. tepidum* and that these Fds are stable against oxygen. With heliobacterial Fds, although Fd-A is very stable against oxygen, Fd-B is very sensitive to oxygen, suggesting a possibility that the physiological functions of Fd-A and Fd-B in heliobacteria differ.

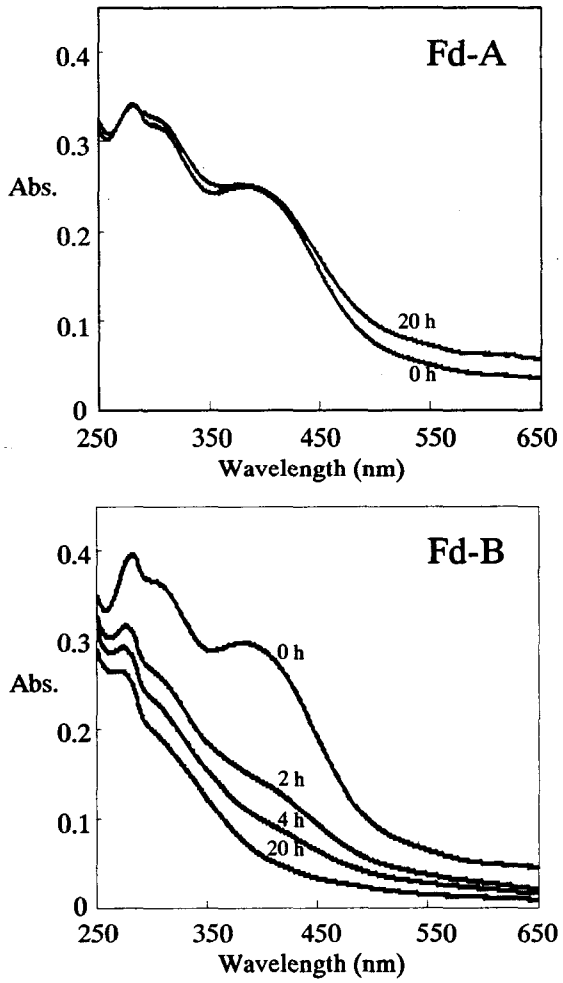


**Figure 1. Changes of absorption spectra of ferredoxins exposed to air**

Purified Fds were incubated at 4°C under aerobic conditions for indicated time and their absorption spectra were measured.

Table 1 shows N-terminal sequences of the Fds. Both Fds have a motif for binding iron-sulfur cluster, CxxCxxCxxxC. The determination of whole amino acid sequences of these Fds is underway (Hatano et al. in preparation).

**Table 1. N-terminal amino acid sequence of ferredoxins**

| |
|---|
| **Fd-A :** |
| A/M YKISDA■VN■GS■VDA■PVGAIE |
| **Fd-B :** |
| MY/GN KIDASQ■TG■GA■VSG■YTNA |

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