Note

Purification and Characterization of Ferredoxin from \textit{Hydrogenobacter thermophilus} Strain TK-6

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Ferredoxin was purified from cells of \textit{Hydrogenobacter thermophilus} strain TK-6. Purification was performed aerobically by the addition of octyl-β-glucoside to the buffers. The purified ferredoxin had a molecular mass of 13,000 and contained a [4Fe-4S] cluster. The protein had a long stretch at the N-terminal region; however, the sequence was not similar to the sequences of ferredoxins with a long stretch from \textit{Archaeabacteria}.

Key words: ferredoxin; hydrogen bacterium; reductive TCA cycle

\textit{Hydrogenobacter thermophilus} strain TK-6 (IAM 12695) is an aerobic, thermophilic, obligately autotrophic, hydrogen-oxidizing bacterium isolated in our laboratory. Pitulle \textit{et al.} reported that the species belongs to \textit{Aquificales}, one of the earliest branching orders of \textit{Bacteria}. The strain fixes carbon dioxide via the reductive TCA cycle, unlike other aerobic organisms. The reductant used by strain TK-6 for the pyruvate synthase and 2-oxoglutarate synthase reaction has not been identified, though it should be to prove that the reductive TCA cycle is operating. In other, anaerobic bacteria, ferredoxin is the usual reductant, except that F$_{420}$, a deazaflavin compound, is the reductant in \textit{Methanobacterium thermophilum}. Our earlier attempt to measure both enzyme activities by using ferredoxins from \textit{Clostridium pasteurianum} or a \textit{Chlorella} sp. failed. In this report, we describe the purification and some properties of ferredoxin from \textit{H. thermophilus} strain TK-6.

Strain TK-6 was grown and harvested as described previously. A cell suspension (1 g wet cells/4 ml of 50 mM phosphate buffer, pH 7.0) was prepared and stored at -80°C if not used immediately. A cell extract was prepared by sonication of 50 ml of the cell suspension at 140 W and 4°C for 10 min. Ultracentrifugation of the fluid at 30,000 x g and 4°C for 1 h yielded a supernatant that was used as the cell extract. A 45-ml portion of this extract was condensed by ultrafiltration with a Centri-cell apparatus (molecular weight cut-off, 10,000; Polysciences, Inc., PA, U.S.A.) to about 5 ml. All of the buffers used during purification were degassed so that protein would not be oxidized, although the manipulation itself was not anaerobic. The condensed solution was put on a column of Q-Sepharose Fast Flow (φ1.5 x 13 cm, Pharmacia) equilibrated with 10 mM Tris·HCl (pH 8.0) containing 1 mM MgCl$_2$ and 0.02% octyl-β-glucoside (the basal buffer). The column was washed with 100 ml of the basal buffer, and elution was done stepwise, with 90 ml of the buffer containing 0.3 M NaCl first, 90 ml of the buffer containing 0.5 M NaCl next, and 100 ml

![Molecular Mass Estimation by Gel Filtration](image)

Fig. 1. Purity and Molecular Mass of the Ferredoxin.
Purified ferredoxin was analyzed by gel filtration (Superdex 75, left) and PAGE (15%, right). On the left, the molecular mass is plotted versus retention time. The flow rate was 0.6 ml/min. The molecular weight standards for gel filtration were from Bio-rad (ovalbumin, M.W. 44,000; myoglobin, 17,000; vitamin B$_{12}$, 1350). The right shows the entire gel, with the dye front at the bottom. Electrophoresis was done at a constant electric current of 15 mA. Coomassie Brilliant Blue was used for staining.

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Abbreviation: TCA, tricarboxylic acid.
of the buffer containing 1.0 M NaCl last. The flow rate was 3.0 ml/min. Fractions were collected every 3 min. The fractions containing ferredoxin, detected by an $A_{410}/A_{280}$ ratio of more than 0.05, were eluted at 0.5 M NaCl. The pooled fractions (18 ml) were condensed with a Centricle-cell apparatus to about 0.5 ml. Next, with a Pharmacia HPLC system, the condensed solution was put on a column of DEAE-5PW (ø7.5 mm x 7.5 cm, Tosoh) equilibrated with the basal buffer. The flow rate was 0.5 ml/min. The column was washed with 20 ml of the buffer, and then sequential linear gradient elution was done, first with 0 to 0.15 M NaCl in the buffer (30 ml), and then with 0.15 to 0.5 M NaCl in the buffer (15 ml). The fractions containing ferredoxin, detected by an $A_{410}/A_{280}$ ratio of more than 0.15, were eluted when the NaCl concentration was about 0.14 M. The pooled fractions (1 ml) were condensed with a Centricle Zalt apparatus (Sartorius) to about 0.5 ml. The solution was put on a column of Superdex 75 (ø1 x 30 cm, Pharmacia) equilibrated with the basal buffer containing 0.1 M NaCl. The flow rate was 0.6 ml/min. The protein was eluted as a single peak at the molecular mass of 13,000 (Fig. 1). The fraction obtained gave a single band by PAGE (Fig. 1) and SDS-PAGE. By the scheme described above, about 1 mg of purified ferredoxin was obtained from 270 mg of total protein in the cell extract.

The iron in the protein was assayed by the $\alpha$-phenanthroline method and the labile sulfur in the protein was assayed in terms of methylene blue formation. The protein was estimated to have one $[Fe4S4]$ cluster. An EPR spectrum of the protein was taken on a JES-RE 2X ESR spectrometer (Fig. 2). The spectrum confirmed the presence of the $[Fe4S4]$ cluster in the protein.

A UV-visible spectrum of the protein had a typical spectrum for bacterial ferredoxin, with an absorption maximum at 410 nm and a shoulder at 320 nm. When dithionite was added to the protein solution, the absorbance around 410 nm decreased, so the purified ferredoxin was in oxidized form (Fig. 3).

The N-terminal amino acids of the protein were sequenced by a method reported previously. The sequence was MKDVKIYEKLGELKDYLEKNYATNPYDYEFLRXPYDXGF. The sequence had a long stretch at the N-terminal, unlike other eubacteria that have been investigated. Ferredoxins from Thermoplasma acidophilum, Sulfurovum, and Halobacterium also have a long stretch at the N-terminal region. However, those sequences and that from $H. thermophilus$ were less than 20% identical. Recently, we found evidence that the purified ferredoxin acts as a reductant in the 2-oxoglutarate synthase reaction of the strain (submitted). Ferredoxins from the archaeabacteria mentioned above also are involved in 2-oxoglutarate metabolism, although the direction of metabolism is toward oxidation. Differences in the physiological roles of the ferredoxins would be why the ferredoxin from $H. thermophilus$ has an unusual sequence at the N-terminal region.

When purification was done with buffers without octyl-$\beta$-glucoside, ferredoxin was not obtained, so octyl-$\beta$-glucoside, a nonionic detergent, stabilized the ferredoxin. The Fe-S cluster of the ferredoxin is probably stabilized by the detergent. Triton X-100 stabilized 2-oxoglutarate synthase activity when we purified the enzyme, which also has an Fe-S cluster (submitted).

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**References**