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The Reductive Tricarboxylic Acid Cycle of Carbon Dioxide Fixation in *Chlorobium tepidum*

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Carbon dioxide (CO_2) may serve as the sole carbon source for the bulk of living organisms found on earth. These organisms include aerobic and anaerobic bacteria and archaea as well as eukaryotic algae and plants. Several metabolic pathways and schemes enable organisms to reduce and assimilate CO_2 to organic matter. Currently, there are four known pathways by which autotrophic microorganism use CO_2 as their sole source of carbon. In order of their discovery, these pathways are the Calvin-Benson-Bassham reductive pentose phosphate pathway, the reductive tricarboxylic acid cycle, the Wood-Lungdahl acetyl-CoA pathway, and the hydroxypropionate cycle (1). Among these, the reductive tricarboxylic acid (RTCA) cycle is an important carbon dioxide fixation pathway and serves as the major means by which carbon is assimilated by diverse prokaryotes, including archaea and several types of bacteria. Despite the importance of this process in nature, many key aspects of the biochemistry and molecular control of this process are unknown.

During the course of investigating the enzymology of the RTCA cycle from *Chlorobium tepidum*, several iron-sulfur cluster proteins were isolated, some of which were associated with pyruvate oxidation, CO_2 fixation, and light-dependent energy generation. *C. tepidum* is a moderately thermophilic, anoxygenic green sulfur photosynthetic bacterium capable of obtaining all its cell carbon through the RTCA cycle (2). Pyruvate synthase (PS)/pyruvate ferredoxin oxidoreductase (PFOR) and α-ketoglutarate synthase (KGS)/α-ketoglutarate ferredoxin oxidoreductase (KGOR) are the key enzymes of the RTCA pathway. PS/PFOR, and KGS/KGOR, which use acetyl-CoA or succinyl-CoA as the CO_2 acceptor, respectively, are classically thought to catalyze PFOR and KGOR reactions, in which pyruvate or α-ketoglutarate is

oxidized to acetyl-CoA or succinyl-CoA and CO_2. PS/PFOR and KGS/KGOR were shown to catalyze the physiologically important CO_2 fixation reaction using photochemically reduced ferredoxin (Fd) as electron donor. The physiological important CO_2 fixation (PS/PFOR and KGS/KGOR) reactions have only been demonstrated for the purified enzyme from *Hydrogenobacter thermophilus* (3, 4). PS/PFOR, and KGS/KGOR are different from pyruvate dehydrogenase, and α-ketoglutarate dehydrogenase in subunit composition, electron donor, and catalytic properties.

While stuying the RTCA cycle enzymes from photoautotrophically grown *C. tepidum*, we purified PS/ PFOR, KGS/KGOR, as well as the electron carriers, rubredoxin (2) and two different ferredoxins, Fd I and Fd II (5). PS/PFOR was shown to possess a homodimeric structure of 126.2 kDa subunits. KGS/KGOR was a heterodimer of comprised of two polypeptide chains, a large subunit of 67.3 kDa and a small subunit of 37.7 kDa (the manuscripts in draft form). The enzymes required thiamine diphosphate as cofactor, as well as Mg^{2+} for maximal activity. Spectroscopic studies, including electron paramagnetic resonance and UV-visible spectral determinations clearly indicated that both enzymes of PS/PFOR and KGS/KGOR possess [4Fe-4S] clusters. Fd I and Fd II were purified to homogeneity under anaerobic conditions as these proteins were deemed potential electron donors or electron acceptors for this bifunctional PS/PFOR enzyme from *C. tepidum*. Several spectroscopic studies were performed including UV-visible, Resonance Raman, Circular Dichroism, and Electron Paramagnetic Resonance spectra, along with the reduction potential determined by Cyclic voltammetry. These results indicated that *C. tepidum* Fd I and Fd II had unique properties characteristic of both clostridial- and chromatium-type Fds. This was supported by deduced amino acid sequences, a deduced folding structure, and various physiological properties. From the amino acid sequences, *C. tepidum* Fd I and Fd II appear to be novel type Fds, which may originate from the 2[4Fe-4S] cluster of the clostridial and chromatium type Fds after a gene duplication event. From the amino acid sequence homology, modeled structures of *C. tepidum* Fd I and Fd II exhibit core structures very similar to Fds of the clostridial class, and also both *C. tepidum* Fd I and Fd II display a similar extended loop structure of the chromatium class around the second [4Fe-4S]

cluster. From these studies, it was proposed that Rd functions as the electron acceptor for the pyruvate oxidation reaction (2), while the two different Fds appear to be the preferred electron donors for the pyruvate synthetic reaction of PS/PFOR (5).

This research focuses on the physiological and biological properties of the two Fds and the catalytic function of purified *C. tepidum* PFOR/PS and KGS/KGOR. In addition, the hydrogen-oxidizing or evolutional enzyme hydrogenase has led us to propose the mechanistic aspects of hydrogen metabolism and its electron transport system in the autotrophic bacteria.

Reference

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