Rubredoxin from the Green Sulfur Bacterium Chlorobium tepidum Functions as an Electron Acceptor for Pyruvate Ferredoxin Oxidoreductase*

(Received for publication, May 24, 1999, and in revised form, July 30, 1999)

Ki-Seok Yoon† §, Russ Hille§, Craig Hemmann§, and F. Robert Tabita‡¶

From the †Department of Microbiology and the Plant Biotechnology Center and the §Department of Medical Biochemistry, The Ohio State University, Columbus, Ohio 43210-1292

Rubredoxin (Rd) from the moderately thermophilic green sulfur bacterium Chlorobium tepidum was found to function as an electron acceptor for pyruvate ferredoxin oxidoreductase (PFOR). This enzyme, which catalyzes the conversion of pyruvate to acetyl-CoA and CO₂, exhibited an absolute dependence upon the presence of Rd. However, Rd was incapable of participating in the pyruvate synthase or CO₂ fixation reaction of C. tepidum PFOR, for which two different reduced ferredoxins are employed as electron donors. These results suggest a specific functional role for Rd in pyruvate oxidation and provide the initial indication that the two important physiological reactions catalyzed by PFOR/pyruvate synthase are dependent on different electron carriers in the cell. The UV-visible spectrum of oxidized Rd, with a monomer molecular weight of 6500, gave a molar absorption coefficient at 492 nm of 6.89 mm⁻¹ cm⁻¹ with an A₂₈₀/A₂₉₀ ratio of 0.343 and contained one iron atom/molecule. Further spectroscopic studies indicated that the CD spectrum of oxidized C. tepidum Rd exhibited a unique absorption maximum at 385 nm and a shoulder at 420 nm. The EPR spectrum of oxidized Rd also exhibited unusual anisotropic resonances at g = 9.675 and g = 4.322, which is composed of a narrow central feature with broader shoulders to high and low field. The midpoint reduction potential of C. tepidum Rd was determined to be −87 mV, which is the most electronegative value reported for Rd from any source.

Chlorobium tepidum is a moderately thermophilic, anoxygenic green sulfur photosynthetic bacterium (1) capable of obtaining cell carbon through the action of two reduced ferredoxins (Fd)⁻¹-linked CO₂ fixation reactions, much like other specialized prokaryotes. The enzymes that catalyze these reactions, pyruvate synthase (PS) and α-ketoglutarate synthase, are key components of the reductive tricarboxylic acid pathway of CO₂ fixation (2–7). PS (Equation 1) is classically thought to also catalyze a pyruvate ferredoxin/flavodoxin oxidoreductase (PFOR) reaction, in which pyruvate is oxidized to acetyl-CoA and CO₂, essentially the reverse of the PS reaction (Equation 2).

\[
\text{acetyl-CoA} + \text{CO}_2 + \text{Fd}_{\text{red}} \rightarrow \text{pyruvate} + \text{coenzyme A} + \text{Fd}_{\text{ox}} \quad \text{(Eq. 1)}
\]

\[
\text{pyruvate} + \text{coenzyme A} + \text{Fd}_{\text{red}} \rightarrow \text{acetyl-CoA} + \text{CO}_2 + \text{Fd}_{\text{ox}} \quad \text{(Eq. 2)}
\]

In the PFOR reaction, coenzyme A and thiamine diphosphate (ThDP) are necessary coenzymes, with oxidized Fd or flavodoxin previously shown to be necessary electron acceptors (Equation 2) (8–13). PFOR is thus distinct from the well characterized pyruvate dehydrogenase multienzyme complex, which catalyzes coenzyme A- and NAD-dependent oxidation of pyruvate (Equation 3) (14, 15). PFOR also differs from pyruvate decarboxylase, which catalyzes the ThDP-dependent conversion of pyruvate to acetaldehyde and CO₂ (Equation 4) (16, 17).

\[
\text{pyruvate} + \text{coenzyme A} + \text{NAD}^+ \rightarrow \text{acetyl-CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+ \quad \text{(Eq. 3)}
\]

\[
\text{pyruvate} \rightarrow \text{acetaldehyde} + \text{CO}_2 \quad \text{(Eq. 4)}
\]

To date, the physiologically important CO₂ fixation (or PS) reaction has only been demonstrated for one purified PFOR/PS enzyme, the protein from Hydrogenobacter thermophilus (7, 12); however, the reaction was initially discovered in another green sulfur bacterium, C. limicola forma thiosulfatophilum (3, 18). The PS carboxylation reaction must overcome a large negative potential energy barrier, which is facilitated by the use of Fd, one of the most electronegative electron carriers known in cellular metabolism (19, 20). Thus, PS catalyzes a carboxylase reaction, which distinguishes it from pyruvate dehydrogenase multienzyme complex, pyruvate decarboxylase, and perhaps even some sources of PFOR.

During the course of studying the carboxylation enzymes of the reductive tricarboxylic acid cycle of C. tepidum, a Rd was isolated and purified to homogeneity from photoautotrophically grown cells. Rd is a small nonheme iron protein and is an important electron transfer component of many bacteria, especially anaerobic organisms. Rd contains a single iron atom coordinated to four cysteine residues in a tetrahedral arrangement (21) with the redox-active site located at the surface of the protein, indicating a directed outer sphere electron transfer mechanism. There are many specific examples where Rd has been shown to participate in a variety of metabolic reactions. For example, Rd is proposed to be the primary electron carrier for the acetogenic CO dehydrogenase of several anaerobes (22) and is an intermediary electron carrier in the NAD(P)H-dependent nitrate reduction system in Clostridium perfringens (23). Similarly, in Pseudomonas oleovorans, a soluble Rd reduc-
tase and a membrane-bound \(\omega\)-hydroxylase are coupled to the hydroxylation of aliphatic hydrocarbons, functionalized hydrocarbons, and various aromatic compounds (24, 25). In Azotobacter vinelandii (26) and in Rhizobium leguminosarum (27), the genes coding for proteins with sequences very similar to Rd have recently been recognized as part of hydrogenase gene clusters, which may indicate the involvement of Rd in hydrogen oxidation or reduction (28). The Rd of Desulfovibrio gigas has also shown to function as a redox coupling protein between NADH oxidoreductase and Rd oxidoreductase, allowing this organism to increase ATP production via the degradation of internal polyglucose in the presence of oxygen (21, 29). Despite these disparate studies, the actual physiological function of Rd is still unclear, especially in organisms that may obtain much of their organic carbon from CO\(_2\) assimilation, such as green photosynthetic bacteria (30–33). Certainly, from the foregoing, Rd might have very important catabolic and also anabolic functions in different organisms. In this study, it is demonstrated that Rd from C. tepidum exhibits unique characteristics; most interestingly, it serves as a high potential electron acceptor for C. tepidum PFOR, resulting in the conversion of pyruvate to acetyl-CoA + CO\(_2\). However, Rd has thus far not been shown to participate in the PS reaction of this protein, suggesting a potential specific physiological role in pyruvate oxidation.

**EXPERIMENTAL PROCEDURES**

**Bacterium and Growth Conditions—** C. tepidum strain TLS was grown photoautotrophically in 20-liter Carboy bottles according to a modification of a method described previously (34). The cells were harvested anaerobically, using continuous centrifugation at 35,000 \(\times g\) at 4 °C under a flow of nitrogen gas. The harvested cells were washed twice with an anaerobic 50 mM phosphate buffer, pH 7.0, containing 50 mM \(\beta\)-mercaptoethanol. The cells were then resuspended in the same buffer (4 g of wet weight/1 ml of buffer), after which the cell suspension was disrupted by two passes through a French pressure cell at 10,000 psi under a flow of argon gas. Cell debris and unbroken cells were removed by centrifugation (20,000 \(\times g\), 10 min); the supernatant fraction was then centrifuged at 150,000 \(\times g\) for 1.5 h. The supernatant from this step was used for the purification of all soluble proteins, including Rd, two Fds, and PFOR. The high speed precipitate fraction was used for the isolation of membrane-bound reaction center particles.

**Purification of Rd—** All purification procedures were carried out at room temperature under anaerobic conditions using a Coy anaerobic chamber using the Biological Autoprogam System from Bio-Rad. The supernatant following ultracentrifugation was loaded onto a DEAE-Sepharose fast flow column (2.5 \(\times\) 15 cm), pre-equilibrated with 20 mM Tris-HCl, pH 8.0, 1 mM dithiothreitol, 1 mM sodium dithionite, 2 mM MgCl\(_2\), and 0.1 mM ThDP. The column was washed with 100 ml of the same buffer containing 0.1 M NaCl at a flow rate of 7 ml/min. A stepwise gradient method was used that consisted of 600 ml of buffer containing a gradient of 0.1–0.3 M NaCl, 200 ml of 0.3–0.5 M NaCl, and 100 ml of 0.5–2.0 M NaCl. PFOR, Rd, and Fds were eluted around 0.2, 0.3, and 0.4 M NaCl, respectively, from this first column. The Rd fraction, eluting at approximately 0.32 to 0.34 M NaCl, was diluted 2-fold with nonsalt buffer and then applied onto a Q-Sepharose high performance column (1.6 \(\times\) 10 cm) pre-equilibrated with wash buffer. After sample application, the column was washed with wash buffer and then eluted isocratically using buffer containing 0.25 M NaCl, followed by a gradient of 0.25–0.4 M NaCl at a flow rate of 3 ml/min. The total gradient volume was 180 ml. The Rd-containing fractions from this column were then directly loaded onto a hydroxyapatite column (1.0 \(\times\) 15 cm) pre-equilibrated with 1 mM potassium phosphate, pH 6.8, containing 1 mM dithiothreitol. At this stage, the Rd-containing fraction was concentrated using Ultrafree-15, PBC 5,000 NMWL (Millipore). Then the Rd was loaded onto a Superose-12 column (1.6 \(\times\) 50 cm) pre-equilibrated with 20 mM Tris-HCl, pH 8.0, containing 0.2 M NaCl and eluted at a flow rate of 1.0 ml/min. PFOR and two different Fds were also purified to homogeneity via several column chromatographic steps under anaerobic conditions.\(^2\)

**EPR, CD, and UV-visible Spectra of Rd—** EPR spectra were obtained with a Bruker instruments, Inc. ER 300 spectrometer equipped with ER 053M NMR gaussmeter and a Hewlett-Packard 5352B microwave frequency counter. Double integration of the EPR spectra was performed with the ESP 300 Software package from Bruker Instruments. Air-oxidized Rd was injected into quartz EPR tubes and frozen by plunging the tubes into liquid nitrogen. Samples of oxidized Rd were stored under anaerobic conditions with either an enzymatic protocol using the activity of PFOR or by chemical means after the addition of sodium dithionite. The reduced liquid samples were collected directly into EPR tubes and sealed with a rubber stopper; the samples were then frozen at specific times after mixing in a dry ice/acetone bath by plunging the tube into liquid nitrogen. EPR spectra were measured at 4.7–20 K using 10 mW of microwave power under liquid helium. CD spectra of oxidized or reduced Rd were measured at room temperature on an AVIV 632DS Spectropolarimeter. CD data were collected at 1-mm intervals using averaging times of 1 s/measurement, depending on the signal to noise ratio. UV-visible spectra were measured with a Beckman DU-8/70 spectrophotometer.

**General Properties of C. tepidum Rd—** The midpoint reduction potential value of the C. tepidum Rd was determined by cyclic voltammetry at a glassy carbon electrode at 25 °C (35). The scan rate was 20 mVs over the potential range of 0 to –600 mV (versus the Ag/AgCl electrode). All values were referenced to the standard hydrogen electrode. The N-terminal amino acid sequence and amino acid analyses of Rd were determined using standard procedures. The purity of Rd was established by SDS-PAGE using a discontinuous Tris-Tricine buffer system (36); samples were heated at 75 °C for 1 h. The apparent molecular weight of Rd was estimated by calibration with a commercial kit of small molecular weight markers from Sigma. Protein concentrations were routinely estimated by established procedures (37). The iron content of the protein was determined by the o-phenanthroline procedure (38). The molecular weight of Rd was determined by gel filtration using a Superose-12 HR (10/30) column calibrated with standard proteins (39). The molecular weight of Rd was estimated by gel filtration using a Superose-12 HR (10/30) column calibrated with standard proteins (39). The molecular weight of Rd was estimated by gel filtration using a Superose-12 HR (10/30) column calibrated with standard proteins (39). The molecular weight of Rd was estimated by gel filtration using a Superose-12 HR (10/30) column calibrated with standard proteins (39). The molecular weight of Rd was estimated by gel filtration using a Superose-12 HR (10/30) column calibrated with standard proteins (39). The molecular weight of Rd was estimated by gel filtration using a Superose-12 HR (10/30) column calibrated with standard proteins (39).

**RESULTS**

**General Properties of C. tepidum Rd—** After purification, the Rd preparation was oxidized and pink-red in color; it eluted from DEAE-Sepharose columns at salt concentrations between 0.32 and 0.34 M NaCl or about 20–25 mS/cm conductivity. This column effectively separated other important soluble proteins, including PFOR, two Fds, and others. Furthermore, the hy-
FIG. 1. A, UV-visible absorption spectra, including inset, of C. tepidum Rd in 50 mM Tris-HCl, pH 8.0. The solid line refers to air oxidized protein, and the dotted line was obtained after reduction in the presence of sodium dithionite. B, SDS-PAGE of purified Rd from C. tepidum. Lane 1, standard marker proteins (Mr = 17–25 kDa); lane 2, Rd treated with 2% SDS and heated at 75 °C for 1 h in the presence of β-mercaptoethanol. C, SDS-PAGE of purified C. tepidum PFOR. Lane 1, standard marker protein (Mr = 36–205 kDa); lane 2, purified PFOR.

droxyapatite column was crucial to separate rubredoxin from another rubredoxin-like molecule that was found to compromise the spectral and other molecular properties obtained. Rd was reduced and blanched in a reaction mixture containing PFOR; similar results were obtained after incubation with sodium dithionite. When reoxidized in air, the pink-red color returned after a few minutes. The UV-visible absorption spectra of oxidized Rd showed several peaks at 250, 370, 492, 570, and 774 nm (Fig. 1A); at 492 nm, the absorption coefficient was 6.89 mmól−1 cm−1, calculated using a molecular weight of 6,500. C. tepidum Rd differed from other bacterial Rds by the presence of at least two absorption bands in the 300–400 nm region, a property that may be unique to only green photosynthetic bacteria (32, 33). The A397/A690 ratio was 0.343. A molecular weight of 6,500 ± 1,000 was obtained from gel filtration experiments in the presence of 1.0 M NaCl. In the presence of 0.1 M NaCl, the molecular weight increased to 13,000 ± 1,000, indicating that the protein exists as a monomer at high ionic strength. The amino acid composition of C. tepidum Rd also yielded an approximate molecular weight of 6,500 (data not shown), which is typical of other Rds (30, 31, 43–45). Electrophoresis of the purified Rd gave rise to a single protein band (Fig. 1B), which migrated with the dye front after SDS-PAGE using a discontinuous Tris-Tricine buffer system (36). This behavior is typical of acidic proteins such as Rd or Fd even in the presence of 20% acrylamide (46). PFOR was shown to have a homodimeric structure comprised of two 125 ± 1 kDa subunits, as determined by SDS-PAGE (Fig. 1C), with a native molecular weight of 250,000 ± 10,000, as determined by gel filtration. The N-terminal amino acid sequence of the C. tepi-
dum Rd was determined to be MQKWCVPGY-DPAD-, which indicated high similarity with other previously isolated Rds of anaerobic bacteria (30, 31, 43, 44). The midpoint reduction potential value of C. tepidum Rd, −87 mV (Fig. 2), is more negative than other sources of Rd (31, 33, 45, 47–49), perhaps indicating that the C. tepidum Rd has more negatively charged amino acids at neutral pH. The reduction potential of Rd from the hyperthermophilic archaeon Pyrococcus furiosus is highly temperature-dependent, changing from about 0 mV at 25 °C to about −160 mV at 90 °C at pH 8.0 (47). Thus, the in vivo reduction potential of Rd from C. tepidum, with an optimum growth temperature of around 48 °C, must be even more negative than the value determined in vitro (−87 mV at 25 °C).

EPR and CD Spectra of C. tepidum Rd—The near UV-visible CD spectra of oxidized C. tepidum Rd closely resembled spectra obtained previously (31, 45, 50), with the exception of the absorption maximum at 385 nm and the shoulder at 420 nm (Fig. 3A). The optical absorption and CD spectra of reduced and oxidized Rd at wavelengths from 300 to 600 nm arise from optically active charge-transfer transitions in the iron-sulfur chromophore, which are presumably sensitive to changes in conformation. The CD features above 320 nm were lost when the protein was reduced (Fig. 3A). The far UV-CD spectrum of C. tepidum Rd showed a weak negative band near 225 nm, a strong positive transition between 200 and 215 nm, and also a strong negative band between 185 and 190 nm (Fig. 3B), which usually signifies an antiparallel β-sheet environment. This property is very similar to Rd from P. furiosus (50).

Reduced Rd was produced when air-oxidized Rd was added to a complete PFOR assay system under anaerobic conditions; this occurred in the complete absence of artificial electron acceptors such as methyl viologen. Under these conditions, PFOR apparently uses oxidized Rd as an electron acceptor. This was manifested by a change from the pink-red color observed upon air oxidation and the appearance of a strong EPR signal (Fig. 4A). Reduced Rd was not EPR-active, as expected (Fig. 4B). Oxidized Rd from C. tepidum exhibited a principal resonance at g = −4.3, which is composed of a narrow sharp component, at g = 4.322 with broad shoulders to high and low field; in addition, a smaller feature at g = 9.675 was observed. The EPR features at g = −4.3 and g = −9.7 were temperature-dependent, increasing in amplitude with decreasing temperature below 16.0 K (Fig. 5, A and B). These results differ from other Rds where the g = −4.3 region decreases with temperature (31, 45, 51). The relative change in intensity of the temperature-dependent EPR signal
at the $g = \sim 9.7$ region was over 2.5-fold more affected than the $g = \sim 4.3$ region under 10 K (Fig. 5C). Thus, it is conceivable that future studies below the 4.7 K limit of the presently available instrumentation might indicate that the $g = \sim 9.7$ region may be increased while the $g = \sim 4.3$ region may be reduced.

C. tepidum PFOR-dependent Reduction of C. tepidum Rd—As a result of the EPR results, which indicated that PFOR activity caused reduction of oxidized Rd, additional experiments were undertaken to characterize the specificity of this interaction. The catalytic activity of PFOR was coupled to the time-dependent and pyruvate/coenzyme A-dependent change of the absorption spectra of Rd (Fig. 6A). Rd was not reduced if pyruvate, coenzyme A, or PFOR were omitted from the reaction mixture, suggesting that full PFOR activity was necessary to reduce oxidized Rd. During this reaction, the amounts of acetyl-CoA produced, as well as the amount of pyruvate consumed, were also determined (Fig. 6B). The rate of acetyl-CoA formation from pyruvate depended on the amounts of added Rd supplied. Moreover, the specificity of Rd was suggested by substituting either of two different Fd molecules isolated from this organism for an equivalent amount of Rd (approximately 90 $\mu$m for each electron donor), resulting in 30- and 64-fold less activity, respectively (results not shown). In this catabolic reaction, the iron-sulfur clusters of PFOR release electrons to Rd, resulting in the formation of acetyl-CoA (52, 53). In the presence of excess oxidized Rd, this catalytic reaction was continuous. However, after the iron-sulfur clusters of PFOR are reduced in the absence of Rd or some other electron acceptor, this catalytic reaction eventually stops and acetyl-CoA can no longer be generated. As expected, no acetyl-CoA was produced if pyruvate, coenzyme A, or PFOR were omitted (Table I). If Rd was omitted or if it was heated at 100 °C for 1 h, virtually all pyruvate-dependent acetyl-CoA formation was lost. Rd did not blanch, however, when boiled at 100 °C over 1 h. Only after dithionite reduction.

FIG. 3. Circular dichroism spectra of isolated C. tepidum Rd (in 50 mM potassium phosphate buffer, pH 7.2) at 25 °C in the near UV-visible (A) and far UV (B). The solid line refers to air oxidized protein, and the dotted line indicates the spectrum obtained after dithionite reduction.

over 1.5–2 h at 100 °C did the pink-red color of Rd disappear, as determined by the visible absorption spectrum at 492 nm (data not shown). Thus, Rd from this moderate thermophile possesses high thermostability. Because it is known that P. furiosus PFOR catalyzes the conversion of pyruvate to acetaldehyde (53) via a coenzyme A-dependent pyruvate decarboxylase-like reaction (Equation 4), it is conceivable that the approximate 2-fold greater amount of pyruvate consumed compared with acetyl-CoA formed might be due to ancillary reactions of this type. However, we were unable to detect a pyruvate decarboxylation reaction catalyzed by C. tepidum PFOR; this and other unique properties of C. tepidum PFOR/PS will be described at a later date.2

Finally, Rd was found to function specifically in the PFOR reaction of the bifunctional C. tepidum PFOR/PS protein and not the PS reaction. A CO$_2$ fixation assay for PS activity was optimized for this enzyme, based on a previously employed method for measuring the reductive carboxylation of acetyl-CoA (2, 3). It was apparent that two different reduced Fd proteins isolated from this organism2 were much more effective than Rd in supporting PS activity (Table II). It was also apparent that there was some specificity in the Fd that best supported PS activity.

DISCUSSION

During the course of investigating the enzymology of reductive tricarboxylic acid cycle enzymes of C. tepidum, several low molecular weight electron transfer proteins were isolated, some of which were associated with CO$_2$ fixation and light-dependent energy generation. Rd was of particular interest because it was shown to be required as an electron acceptor for PFOR activity. Further work indicated that this Rd was highly stable because little change in the absorption spectrum at 492 nm was
noted even after exposure to boiling temperatures for 1 h or less. Because virtually all pyruvate-dependent acetyl-CoA formation was lost under the same conditions, it would appear that this catalytic function might serve as a convenient measure of thermal stability independent of any bleaching of its redox center. The major EPR signals resembled those obtained for other Rds. The signal at $g \approx 4.322$ was much more prominent than the signal at $g \approx 9.675$; in addition, the sharp EPR signal at $g \approx 4.322$ was different from other Rds, which usually exhibit broader signals at $g \approx 4.1–5.6$.

**FIG. 5.** Temperature-dependent EPR spectral resonances of *C. tepidum* Rd. A, magnetic field (149–164 mT) of the aerobically purified Rd at 4.7–16.0 K. B, magnetic field (62–78 mT) of aerobically purified Rd at 4.7–16.0 K. Each feature was shown at the same value of intensity. The EPR parameters were: center field, 156 mT (A) and 70 mT (B); microwave power, 10 milliwatts; modulation amplitude, 0.505 mT (A) and 0.201 mT (B); the receiver gain, $2 \times 10^3$ at 4.7 K and $5 \times 10^3$ at 15.0 K (A) and $1 \times 10^4$ (B); microwave frequency, 9.45 GHz; modulation frequency, 100 kHz. C, temperature-dependent relative intensity of EPR spectra at the $g \approx 9.7$ region (●) and $g \approx 4.3$ region (○).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Acetyl-CoA</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>75.0</td>
<td>100</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Coenzyme A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rd</td>
<td>6.2</td>
<td>8.3</td>
</tr>
<tr>
<td>PFOR</td>
<td>1.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Complete but air conditions</td>
<td>34.5</td>
<td>46.0</td>
</tr>
<tr>
<td>Complete but denatured Rd</td>
<td>5.4</td>
<td>7.2</td>
</tr>
</tbody>
</table>

**TABLE I**
Acetyl-CoA production from pyruvate, catalyzed by *C. tepidum* PFOR

Acetyl-CoA production was measured at 25 °C under standard assay conditions. The 1-ml assay mixture contained 0.1 mM ThDP, 2.0 mM MgCl₂, 1.0 mM coenzyme A, 0.5 mM pyruvate, 0.5 mM coenzyme A, 0.1 mM ThDP, 1.0 mM MgCl₂, and 63 nM PFOR in 50 mM Tris-HCl, pH 7.8, under anaerobic conditions. Acetyl-CoA was determined using a coupled enzymatic reaction with malate dehydrogenase/citrate synthase. The consumption of pyruvate was determined using lactate dehydrogenase.

**FIG. 6.** A, pyruvate- and coenzyme A-dependent spectral changes of *C. tepidum* Rd catalyzed by PFOR. The 1-ml reaction mixture contained 5 mM pyruvate, 2.0 mM MgCl₂, 1.0 mM coenzyme A, 42 nM PFOR, and 90 μM Rd in 50 mM Tris-HCl, pH 7.8, under anaerobic conditions. The reaction was initiated by the addition of coenzyme A to the reaction mixture under anaerobic conditions. Interval spectra were shown every 30 s at 30 °C. B, acetyl-CoA production and pyruvate consumption by Rd-dependent PFOR activity. The 1-ml reaction mixtures contained 5 μM pyruvate, 0.5 mM coenzyme A, 0.1 mM ThDP, 1.0 mM MgCl₂, and 63 nM PFOR in 50 mM Tris-HCl, pH 7.8, under anaerobic conditions. Acetyl-CoA was determined using a coupled enzymatic reaction with malate dehydrogenase/citrate synthase. The consumption of pyruvate was determined using lactate dehydrogenase.
CO2 assimilation or anabolic reaction where acetyl-CoA is re- 
addition, the same enzyme that catalyzes PFOR activity (Equa-

tion was further indicated by the fact that as little as 167 nM 
Rd or Fd from the indicated source, all in 50 mM phosphate buffer, pH 7.0, containing 1.0 mM acetyl-CoA, 2.0 mM MgCl2, 0.1 mM ThDP, 10 mM 
β-mercaptoethanol, and 5 mM [14C]-NaHCO3, (2 μCi of H14CO3-) in a 
1-ml reaction mixture. The reaction was initiated with bicarbonate 
after preincubation of the reaction mixture for 30 min and was run at 48 °C for 30 min under a nitrogen atmosphere at a light intensity of 
10,000 lux. The reaction was then quenched by the addition of propionic 
acid, and the acid stable pyruvate was counted by liquid scintillation spectrometry.

The g value is consistent with a δ = 1/2 subspectrum for the g = 
9.7 region and with a δ = 3/2 subspectrum for the g = 4.3 
region. Because the EPR signals of Rd disappeared and the 
molecule was completely reduced upon the addition of the 
complete PFOR reaction mixture, it would appear that a major 
function of Rd was as an electron acceptor for PFOR. This was 
supported by the Rd-dependent oxidation of pyruvate to acetyl-
CoA and was further buttressed by the UV-visible spectral 
changes, which are coenzyme A-dependent, when Rd was re-
duced via the activity of PFOR. Under the assay conditions 
described, oxidized Rd appeared to be the favored electron 
acceptor for the PFOR reaction, because its specificity was 30- 
and 64-fold greater, respectively, than two different Fds puri-

ified from this organism. The specificity for PFOR in this 
reaction was further indicated by the fact that as little as 167 nM 
PFOR was capable of completely reducing 800 μM of Rd within 
15 min at 25 °C. Therefore, the results obtained indicate a 
potentially important role for this small protein in controlling 
anabolic and catabolic metabolism at the level of PFOR/PS. 
However, future genetic and physiological experiments must be 
performed to establish the actual role for Rd in 
C. tepidum. Potential of Rd from 

C. tepidum Rd and PFOR activity (58 °C).

It is clear that this phototrophic green sulfur bacterium 
metabolizes both pyruvate and acetate and that the formation 
of a key intermediate like acetyl-CoA via PFOR must be an 
important regulatory step. The fact that Rd may serve as an 
electron acceptor for PFOR could thus be very important as an 
appreciation of the metabolic control profile of C. tepidum 
develops. Several efforts have been made to assign specific func-
tions to redox carriers such as Rd, Fd, and flavodoxin or other 
proteins such as cytchrome c550 and cytchrome c553, all of 
which have recently been purified from C. tepidum. C. tepi-
dum is an organism for which a functional genetic system 
exists (34); thus it should be feasible to elucidate the physio-
logical role of Rd and other electron carriers, especially since 
the genomic sequence of this organism has recently reached the 
completion stage. Such studies will nicely complement bio-
chemical studies with purified proteins, e.g., Rd and PFOR. 
Certainly, the fact that Rd participates in the PFOR reaction 
but is not efficiently used in the PS reaction of this bifunctional 
protein under the reported conditions suggests that PFOR/PS 
catalyzes two fundamentally different reactions, with different 
catalytic mechanisms, for diverse physiological purposes.

Acknowledgment—We express our appreciation to Dr. Chulhwan 
Kim for determining the reduction potential of Rd by cyclic 
voltammetry.

REFERENCES

Arch. Microbiol. 156, 81–90


Sci. U. S. A. 55, 928–934


178, 3363–3368


Biochem. 61, 510–513


128, 223–230


167, 275–279

180, 1119–1128


273, 20196–20204


1420–1425

Chem. 273, 15404–15411

J. Biol. Chem. 273, 5514–5519

243, 203–216

155, 1224–1237


15151–15152

122–124

228, 988–1002


30. Gomes, C. M., Silva, G., Oliveira, S., LeGall, J., Liu, M.-Y., Xavier, A. V., 
22502–22508


Biochem. Biophys. 228, 80–88

C. tepidum Rd and PFOR

Rubredoxin from the Green Sulfur Bacterium *Chlorobium tepidum* Functions as an Electron Acceptor for Pyruvate Ferredoxin Oxidoreductase
Ki-Seok Yoon, Russ Hille, Craig Hemann and F. Robert Tabita

doi: 10.1074/jbc.274.42.29772

Access the most updated version of this article at [http://www.jbc.org/content/274/42/29772](http://www.jbc.org/content/274/42/29772)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 53 references, 22 of which can be accessed free at [http://www.jbc.org/content/274/42/29772.full.html#ref-list-1](http://www.jbc.org/content/274/42/29772.full.html#ref-list-1)