An unusual thiol-driven fumarate reductase was purified from cell extracts of *Methanobacterium thermotogicum* and partially characterized. Two coenzymes previously isolated from cell extracts, 2-mercaptoethanesulfonic acid (HS-CoM) and N-(7-mercaptoheptanoyl)threonine-O-phosphate (HS-HTP), were established as direct electron donors for fumarate reductase. By measuring the consumption of free thiol, we determined that fumarate reductase catalyzed the oxidation of HS-CoM and HS-HTP; by the direct measurement of succinate and the heterodisulfide of HS-CoM and HS-HTP (CoM-S-S-HTP), we established that these compounds were products of the fumarate reductase reaction. A number of thiols-containing compounds did not function as substrates for fumarate reductase, but the enzyme had high specific activity when HS-CoM and HS-HTP were used as electron donors. HS-CoM and HS-HTP were quantitatively oxidized by the fumarate reductase reaction, and results indicated that this reaction was irreversible. Additionally, by measuring formylmethanofuran, we demonstrated that the addition of fumarate to cell extracts activated CO₂ fixation for the formation of formylmethanofuran. Results indicated that this activation resulted from the production of CoM-S-S-HTP (a compound known to be involved in the activation of formylmethanofuran synthesis) by the fumarate reductase reaction.

Six unusual coenzymes have been discovered in cell extracts of *Methanobacterium thermotogicum*, and studies on these coenzymes have been crucial for understanding the physiology of methane-producing archaeabacteria (1, 2). Two of these coenzymes, 2-mercaptoethanesulfonic acid (HS-CoM), coenzyme M, and N-(7-mercaptoheptanoyl)threonine-O-phosphate (HS-HTP), are required for the final step of CO₂ reduction to methane and are together involved in the regulation of CO₂ fixation in vitro. HS-CoM was shown to function as a methyl carrier and 2-(methylthio)ethanesulfonic acid (CH₃-S-CoM) was established as the terminal intermediate of methanogenesis (3). HS-HTP was found to be the direct electron donor for the reductive demethylation of CH₃-S-CoM (5), and the oxidized form of HS-HTP, the heterodisulfide of HS-CoM and HS-HTP (CoM-S-S-HTP), was identified as a co-product of the methylreductase reaction: CH₃-S-CoM + HS-HTP → CoM-S-S-HTP + CH₄ (5, 6). Interestingly, CoM-S-S-HTP greatly stimulated methanogenesis from CO₂ in cell extracts (7). This stimulation resulted from the activation of an early step of methanogenesis from CO₂, formylmethanofuran (formyl-MFR) synthesis (7), a novel reaction that fixes CO₂ to the primary amine of the coenzyme methanofuran (MFR).

In this paper we establish that HS-CoM and HS-HTP can also function as electron donors for purified fumarate reductase and that CoM-S-S-HTP is a product of the fumarate reductase reaction. These findings established new metabolic roles for HS-CoM and HS-HTP, further defined the activation of formyl-MFR synthesis by CoM-S-S-HTP, and revealed an example of a thiol-driven fumarate reductase.

**EXPERIMENTAL PROCEDURES**

**Growth of Cells and Preparation of Cell Extracts—** *M. thermotogicum* strain ΔH (ATCC 29096, DSM 1053) was cultivated and harvested as previously described (8). To prepare cell extract, cells were suspended in 50 mM K'PIPES buffer at pH 6.2 in 10 mM 2-mercaptoethanol, and cells were broken with a French pressure cell using anoxic procedures (9). G-25 enzyme preparation was obtained by depleting cell extract of low molecular weight cofactors with anaerobic Sephadex G-25 gel filtration chromatography (10, 11). Cells, cell extract, and G-25 enzyme preparation were stored at -20°C under N₂ until used.

**Fumarate Reductase Assays—** One assay for the fumarate reductase measured the fumarate-dependent oxidation of reduced benzyl viologen, Aₚ₅₀ was monitored (τ₉₀ = 7800 m⁻² cm⁻¹) and the assay was performed as described (12). A second assay for the fumarate reductase measured the fumarate-dependent oxidation of HS-CoM and HS-HTP and employed 5,5'-dithiobis-(2-nitrobenzoic acid) to measure free thiol. The standard components of this assay were 100 mM Tris-HCl, pH 8.3, 10 mM MgCl₂, 2.5 mM HS-CoM, 2.5 mM HS-HTP, 5 mM disodium fumarate, enzyme as desired, and distilled H₂O to a volume of 200 µL. Inside an anaerobic chamber, the standard assay components (except fumarate) were combined in a 5-µL vial which was then sealed with a 20-mm red rubber stopper. The sealed assay vial was removed from the anaerobic chamber and the headspace gas was replaced with O₂-free N₂ by use of gassing and vent needles (3). The assay mixtures were heated from room temperature to 60°C, and the addition of an anoxic solution of disodium fumarate by syringe was used to initiate the fumarate reductase reaction. Portions of the reaction mixtures (20
were used to reduce the benzyl viologen and was prepared as described (13). Absorbance measurements were performed using a lambda 3B spectrophotometer (Perkin-Elmer).

The fumarate reductase assays were obtained from Sigma and were handled without special precautions, but with the following exceptions. HS-HTP was synthesized and quantified as described (14) and then stored as a dry solid desiccated at -20°C under N2. Prior to use, HS-HTP and HS-CoM were dissolved in anoxic 5 mM sodium acetate buffer pH 7.4. The resulting solutions were stable for several months, if they were handled using anoxic procedures and were stored frozen at -20°C under 202 kPa of N2.

**Fumarate Reductase Purification**—The procedure employed was a modification of a published method (12). Because of the O2 sensitivity of the fumarate reductase, anaerobic procedures for chromatography, centrifugation, sample handling, and sample storage were employed (9, 10). Anoxic cell extracts were prepared as described (9) except that prior to cell breakage cells were suspended in buffer which contained 50 mM sodium phosphate at pH 7.0, 50 mM KCl, 50 mM disodium EDTA, 10 mM 2-mercaptoethanol, 5 mM sodium fumarate, and 2 mM dithiothreitol (DTT). Cell extract was heated in a water bath at 75°C for 4 h under 202 kPa of N2 and the proteins which precipitated were pelleted by centrifugation for 20 min at 30,000 × g. An anoxic, saturated solution of ultrapurammonium sulfate (Schwarz/Mann) containing 10 mM 2-mercaptoethanol and 2 mM DTT was added to the supernatant to start the extraction (1 ml of ammonium sulfate solution/ml of heat-treated extract). No pH adjustment was necessary, and the enzyme preparation was stirred for 2 h at room temperature after the addition of ammonium sulfate was complete. Precipitated proteins were pelleted by centrifugation for 30 min at 30,000 × g, and the pellet was resuspended in buffer containing 20 mM Tris-HCl, pH 8.0, 1.0 mM KCl, 10 mM 2-mercaptoethanol, and 2 mM DTT. A 6 × 5-cm column of phenyl-Sepharose CL-4B (Pharmacia LKB Biotechnology Inc.) was equilibrated with buffer containing 20 mM Tris-HCl, pH 8.0, 400 mM KCl, 10 mM 2-mercaptoethanol, and 2 mM DTT. The resuspended ammonium sulfate pellet was applied to the column (235 mg of protein/ml of resin), and the A280 of the column effluent was monitored with a UV-1 single path monitor (Pharmacia LKB Biotechnology Inc.). The column was washed with 2 bed volumes of the equilibration buffer and eluted with a 5-bed volume linear gradient from 400 to 500 mM NaCl in the same buffer. Fractions containing the highest fumarate reductase activity were pooled, and a 30-μm filter membrane was placed over the 400-ml stirred cell (Amicon) were used to concentrate and exchange these fractions into the equilibration buffer to be used in the next chromatographic step. Hydroxylapatite chromatography was performed on a Phenyl-Sepharose (Bio-Rad) column (12), and active fractions were pooled and concentrated. For the final purification step, a fast protein liquid chromatography system consisting of a Mono Q HR 5/5 column with two P-500 pumps and a G-250 gradient programmer (Pharmacia LKB Biotechnology Inc.) was employed. Buffer A contained 50 mM sodium phosphate, pH 7.0, 50 mM NaCl, 10 mM 2-mercaptoethanol, and 2 mM DTT in 10% glycerol. Buffer B was similar to buffer A, but contained 1 M NaCl. The Mono Q column was equilibrated with buffer A. About 200 μg of protein was applied to the column, and the column was eluted with a 20-ml linear gradient from 0 to 100% buffer B at a flow rate of 1 ml/min while A280 was monitored. Protein was determined using Protein Assay Reagent (Pierce Chemical Co.), a reagent for quantifying proteins by a procedure based on the method of Bradford (15).

Native and denaturing polyacrylamide gel electrophoresis (PAGE) were performed as described (16). Native gels were 10% (w/v) polyacrylamide, and denaturing gels were 12% (w/v) polyacrylamide. Following electrophoresis, Comassie Brilliant Blue R-250 was used to stain protein (17). To perform denaturing PAGE on bands excised from polyacrylamide gels, the procedure of Rousier was used (18), and anaerobic PAGE was performed as described (19). To locate fumarate reductase activity, anaerobic PAGE was performed on chosen samples, and the polyacrylamide gel (while still inside the anaerobic chamber) was cut into strips which corresponded to gel lanes. Each gel strip was placed inside a 2.5 × 15-cm test tube, and 25 μl of a solution containing 50 mM sodium phosphate at pH 7.0, 1 mM benzyl viologen, 5 mM sodium thiocyanate, and 1 mM sodium ascorbate was used to reduce the benzyl viologen in the test tube. The gel strips were placed outside the anaerobic chamber. The gel strips were incubated at 50°C, with gentle stirring, until they turned dark blue. A syringe was used to add 100 μl of 100 mM sodium fumarate to the benzyl viologen mixture, and incubation at 50°C was continued. The gel strips were periodically checked for zones of clearing indicative of fumarate reductase.

**Products of the Fumarate Reductase Reaction**—For succinate and Co-M-S-HTP determinations, fumarate reductase reactions were performed as described above, but reactions were sealed up as necessary and the reactions were buffered with 100 mM K2Pipes at pH 6.2.

For succinate quantitation, 40-μl portions of fumarate reductase assay mixtures were placed on ice and air-exposed for 1 h. This procedure terminated the reactions. Samples were stored at -20°C prior to the quantitation of succinate by a previously described method (21). The reagents needed for succinate determination were obtained commercially (Sigma) with the exception that succinate thiokinase was purified from cell extracts of Escherichia coli (22).

Co-M-S-HTP was quantified by analyzing 40-μl portions of fumarate reductase reaction mixtures by high performance liquid chromatography (HPLC) as described (6). For controls that tested for the possibility of chemical formation of Co-M-S-HTP by thiol disulfide interchange, fumarate reductase reaction mixtures were changed to include the homodisulfides of HS-CoM (Co-M-S-CoM) and HS-HTP (HS-S-HTP, 1.25 mM each, but enzyme was omitted. Co-M-S-HTP, HS-S-S-HTP, and Co-M-S-CoM were synthesized and quantified as described (7). Co-M-S-HTP was stored desiccated as a dry solid at -20°C, and solutions of Co-M-S-HTP were prepared in 5 mM sodium acetate buffer at pH 4.0 for the day of use.

**Activation of Formyl-MFR Synthesis**—An HPLC assay was used to quantify formyl-MFR (23). Modifying this method by use of a 3.9 × 150 mm Bondpak C8 column which was eluted with a 10-min linear gradient from 0 to 100% buffer B shortened the analysis time. MFR eluted at 11.1 min and formyl-MFR at 12.4 min. MFR, for use as a chromatographic standard, was purified from boiled cell extracts as described (24), and formyl-MFR was synthesized by the formula of MFR with p-nitrophenylformate (25).

**RESULTS**

**Fumarate-dependent Oxidation of HS-CoM and HS-HTP by Cell Extracts**—Previous studies of fumarate reductase from Methanobacterium failed to identify its physiological electron donor; compounds giving negative results included the reduced forms of flavins, nicotinamide coenzymes and coenzyme F420 (12, 26). By measurement of free thiol, we found that cell extract catalyzed the fumarate-dependent oxidation of HS-CoM and HS-HTP. In assay mixtures containing cell extract, fumarate, HS-CoM, and HS-HTP, thiol was lost at a rate of 0.3 μmol/min/mg cell extract protein. These results suggested that HS-CoM and HS-HTP might be physiologically important electron donors for fumarate reductase.

**Purification of the Fumarate Reductase**—A modification of the method of Khandekar and Eirich (12) was used to purify fumarate reductase from Thiobacillus thioparatus...
fumarate reductase (Table I). The ammonium sulfate precipitation as well as the phenyl-Sepharose and Mono Q chromatography were not previously employed fractionation steps. During the the phenyl-Sepharose chromatography the fumarate reductase eluted at 375 mM KCl, but the majority of the protein eluted at higher salt concentrations. By Mono Q chromatography, the fumarate reductase eluted as a sharp, symmetrical peak with 400 mM NaCl. The modified purification procedure improved the final yield of fumarate reductase from 7 to 25%.

Enzyme preparations obtained throughout the purification also catalyzed the fumarate-dependent oxidation of HS-CoM and HS-HTP. We observed that a ratio of specific activities (specific activity of HS-CoM and HS-HTP oxidation/specific activity benzyl viologen oxidation) decreased following two purification steps. The total decrease in this ratio subsequent to ammonium sulfate precipitation and phenyl-Sepharose chromatography was approximately 7-fold (from 0.30 to 0.043). It was possible that these fractionation steps resolved the enzyme preparation for a factor stimulatory to the fumarate-dependent oxidation of HS-CoM and HS-HTP, but not stimulatory to benzyl viologen oxidation by the fumarate reductase; however, attempts to increase the thiol-oxidizing activity of the fumarate reductase by reconstitution were unsuccessful.

Following the Mono Q chromatography, results of PAGE indicated fumarate reductase was highly purified. Coomassie staining revealed a single protein band of \( R_F \ 0.28 \) when 4 \( \mu \)g of the fumarate reductase preparation was analyzed by anaerobic PAGE (Fig. 1, lane 1). When similar gels were loaded with higher amounts of protein (10–20 \( \mu \)g), a second protein band, at \( R_F \ 0.56 \), was also observed (data not shown); however, an activity stain indicated that both bands were multimeric forms of fumarate reductase (data not shown). The protein bands at \( R_F \ 0.28 \) and 0.56 were excised from the native gels and, along with 4 \( \mu \)g of the enzyme preparation originally applied to the anaerobic polyacrylamide gel, were run in a second dimension by denaturing PAGE. Results indicated that each protein sample contained polypeptides of 50 and 59 kDa (data not shown), indicating that the two protein bands observed on anaerobic polyacrylamide gels were different multimeric forms of the fumarate reductase and were not aggregates of the fumarate reductase with contaminating proteins. Moreover, we found that the slower migrating form of the fumarate reductase was converted to the faster migrating form by \( \text{O}_2 \) exposure (Fig 1). We also performed additional fractionation methods after fumarate reductase appeared homogeneous by anaerobic PAGE including Superoxide 12 fast protein liquid chromatography gel filtration, DEAE-Sepharose CL-6B (Pharmacia LKB Biotechnology Inc.) chromatography, and preparative gel electrophoresis. Following these procedures, the specific activity of fumarate reductase remained constant (with the exception that specific activities were not determined following preparative electrophoresis), and denaturing PAGE again indicated a two-subunit composition (data not shown).

Importantly, purified fumarate reductase catalyzed the oxidation of HS-CoM and HS-HTP with relatively high specific activity (107.4 units/mg) at the optimal pH of 8.3 and retained the majority of its activity at pH 7.0 (75.2 units/mg). These results strongly indicated that HS-CoM and HS-HTP were the direct electron donors for fumarate reductase.

**Products of the Fumarate Reductase Reaction**—Using an enzyme assay that employed succinate thiokinase, we quantified the succinate formed during the time course of a fumarate reductase assay (Fig. 2A). We found that, on average, 1.97 mol of thiol was lost per mol of succinate formed. Succinate formation required HS-CoM, HS-HTP, fumarate, and fumarate reductase. These results established that fumarate reductase coupled the oxidation of HS-CoM and HS-HTP to the reduction of fumarate to succinate.

The possible products of HS-CoM and HS-HTP oxidation included CoM-S-S-HTP, CoM-S-S-CoM, and HTP-S-S-HTP. During a fumarate reductase reaction we quantified CoM-S-S-HTP as well as thiol (Fig. 2B). We found that, on average, 2.08 mol of thiol was consumed per mol of CoM-S-S-HTP produced. After a 30-min incubation 91% of the added HS-CoM and HS-HTP had been oxidized to CoM-S-S-HTP. No HTP-S-S-HTP was detected. For CoM-S-S-HTP determinations, the fumarate reductase reaction was performed at pH 6.2. At this pH, the reaction proceeded at 30% of its rate at the optimal pH of 8.3, but the lower pH was necessary to minimize the production of CoM-S-S-HTP by thiol-disulfide interchange. Controls showed that <1 nmol of CoM-S-S-HTP could have been formed per min by thiol-disulfide interchange under the assay conditions employed (data not shown), which was <1% of the observed enzymatic rate of CoM-S-S-HTP formation. Neither the formation of CoM-S-S-HTP nor the loss of thiol was detected unless reaction mixtures included HS-CoM, HS-HTP, enzyme, and fumarate. These results established that CoM-S-S-HTP was a product of the fumarate reductase reaction and ruled out the possibility that either CoM-S-S-CoM or HTP-S-S-HTP were reaction products.

**Specificity and Reversibility of the Fumarate Reductase Reaction**—Fumarate reductase did not catalyze the oxidation of DTT, 2-mercaptoethanol, cysteine, lipoic acid, or glutathione (each up to 5 mM) when one of these thiol compounds was used to replace both HS-CoM and HS-HTP in fumarate reductase assay mixtures. Additionally, none of these thiol compounds replaced either HS-CoM or HS-HTP with the exception that cysteine substituted for HS-CoM. The specific activity of the fumarate reductase in the presence of 2.5 mM each of HS-HTP and cysteine was 10.1 pmol min" " mg" -1. Notably, cysteine and HS-CoM have structural similarities. In each assay, 10 \( \mu \)g of purified fumarate reductase was used. For the determinations that gave negative results, the concen-
Thiol-driven Fumarate Reductase

![Graph](image)

**Fig. 2. Products of the fumarate reductase reaction.** A, in a reaction mixture that contained 25 µl of purified fumarate reductase (5 µg of protein), 1.5 µmol of HS-CoM, 1.5 µmol of HS-HTP, 3 µmol of disodium fumarate, 60 µmol of K+ PIPES buffer at pH 6.2, and 6 µmol of MgCl₂ in a total volume of 600 µl, the oxidation of HS-CoM and HS-HTP was monitored by measuring the consumption of free thiol (●) and succinate was monitored using an enzymatic determination (○). B, in a similar reaction mixture, free thiol (●) and CoM-S-S-HTP were quantified (○) over a 30-min time period. Thiols determinations were corrected for background.

**TABLE I**

The activation of formyl-MFR synthesis in cell extracts by fumarate.

The 200-µl assay mixtures contained 40 nmol of MFR, 20 nmol of HS-CoM, 20 nmol of HS-HTP, 100 µl of G-25 enzyme preparation (3 mg of protein), 20 µmol of K⁺ PIPES buffer at pH 6.2, and 2 pmol of MgCl₂. Formyl-MFR was quantified by HPLC as described (23). Higher rates of formyl-MFR synthesis were difficult to determine because of limiting amounts of MFR. G-25 enzyme preparation was cell extract depleted of low molecular weight cofactors by Sephadex G-25 gel filtration chromatography.

| Assay component omitted | Inhibitors added | Rate of formyl-MFR synthesis (nmol/min) |
|-------------------------|------------------|----------------------------------------|
| None                    |                  | >18                                    |
| Fumarate                |                  | 0                                      |
| HS-HTP                  |                  | 0                                      |
| HS-CoM                  |                  | 1                                      |
| G-25 enzyme             |                  | 0                                      |
| None                    | DTT + HO-Cbl     | 0                                      |
| None                    | BES              | >18                                    |
| None                    | Chloramphenicol  | >18                                    |

The concentration of thiol was varied between 1 and 5 mM and reactions were followed for 30 min.

When a fumarate reductase assay mixture was prepared omitting HS-CoM, HS-HTP, and fumarate, but adding succinate (6–50 mM) and 5 mM CoM-S-S-HTP, no measurable increase in thiol was observed during a 30-min incubation, showing that the fumarate reductase reaction was irreversible under the assay conditions employed. Previously, attempts to measure this reverse reaction using ferricyanide as an artificial electron acceptor also gave negative results (12).

**Activation of Formyl-MFR Synthesis by Fumarate**—Previously, fumarate was found to stimulate CO₂ reduction to methane in cell extracts (27). Using an HPLC assay for formyl-MFR, we demonstrated that the addition of fumarate to cell extracts activated formyl-MFR synthesis (Table II). In addition to fumarate and cell extract, the requirements of this activation included HS-HTP and HS-CoM, indicating that CoM-S-S-HTP was involved. The finding that DTT + HO-Cbl inhibited the activation of formyl-MFR synthesis by fumarate supported the involvement of CoM-S-S-HTP; previously, it was shown that DTT + HO-Cbl chemically reduced CoM-S-S-HTP and specifically inhibited the activation of formyl-MFR synthesis by this disulfide (23). The methylreductase inhibitors 2-bromoethanesulfonic acid and chloramphenicol did not inhibit the activation of formyl-MFR synthesis by fumarate but did inhibit the activation by CH₃-S-CoM (data not shown). This result precluded the possibility that fumarate activated formyl-MFR synthesis by providing a C₁ unit that was reducible to methane, a possibility previously suggested to account for the stimulation of CO₂ reduction to methane by fumarate (27).

Additionally, we found that oxaloacetate and malate activated formyl-MFR synthesis in cell extracts, and the requirements of this activation as well as the effects of inhibitors were similar to those for fumarate (data not shown), with the exception that NADPH was also required when oxaloacetate was used to activate formyl-MFR synthesis. Because earlier work has indicated that oxaloacetate and malate can be converted to fumarate by a partial reductive tricarboxylic acid

![Scheme 1](image)
Thiol-driven Fumarate Reductase

cycle (26), we suggest that oxaloacetate and malate activated formyl-MFR synthesis via the fumarate reductase reaction.

**DISCUSSION**

Previously, fumarate reductase was purified from *M. thermoautotrophicum*. Denaturing PAGE of this enzyme showed a “ladder of bands” at apparent molecular masses of 22, 40, 58, and 78 kDa, and it was proposed that a ladder resulted because the denaturing procedure used prior to electrophoresis did not completely separate the subunits of the highly heat-stable fumarate reductase (12). We repeatedly found that fumarate reductase had subunits of 50 and 59 kDa, and this subunit composition remained unchanged even when samples were boiled for up to 10 min in the denaturing procedure used prior to electrophoresis (data not shown). We made extensive efforts to ensure the purity of our enzyme preparations (see “Results”), and we are currently uncertain of the reason a different subunit composition was previously observed.

HS-CoM and HS-HTP were originally identified as factors required to reconstitute methanogenesis in cell extracts depleted of soluble, low molecular weight cofactors (3, 14), and the physiological roles for these coenzymes in methanogenesis were determined (3–5). This study has established that HS-CoM and HS-HTP also function as the electron donors for the fumarate reductase reaction of *M. thermoautotrophicum*. Purified fumarate reductase catalyzed the reaction shown (Scheme 1) with high specific activity and high specificity, indicating that HS-CoM and HS-HTP are physiologically important electron donors. This has established anabolic roles for HS-CoM and HS-HTP and has defined a new function for HS-CoM as an electron carrier.

Previous work indicated that the steady-state level of CoM-S-S-MTP determined the rate of formyl-MFR synthesis in cell extracts (7). Results presented herein indicated that the fumarate reductase reaction, a component of a partial reductive tricarboxylic acid cycle, can influence CoM-S-S-HTP levels in cell extracts. CoM-S-S-HTP was quantitatively produced by the fumarate reductase reaction, and the addition of fumarate to cell extracts activated formyl-MFR synthesis. Previously, the methylreductase, which catalyzes the terminal reaction of methanogenesis was shown to produce CoM-S-S-HTP and activate formyl-MFR synthesis (7, 9) and an H2-dependent CoM-S-S-HTP reductase was found to regenerate HS-CoM and HS-HTP and inactivate formyl-MFR synthesis (7) Additionally, CoM-S-S-HTP was proposed as a component in chemiosmotic energy generation (5), in which case its steady-state levels could be related to a membrane potential. We would speculate that the relative influence of these processes on CoM-S-S-HTP levels may vary with environmental or physiological conditions, and that under certain conditions CoM-S-S-HTP production by the fumarate reductase reaction ensures an optimal activation of formyl-MFR synthesis.

Additionally, this work has further characterized a fumarate reductase previously shown to have unusual properties including high heat stability and O2 sensitivity (12). As noted above, fumarate reductase may be important to the regulation of CO2 fixation as well as energy conservation. Moreover, to our knowledge, this is a first example of a thiol-driven fumarate reductase.

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