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Biochemistry

Photoactivation of the 2-(methylthio)ethanesulfonic acid reductase from *Methanobacterium*

(methanogen/methane/coenzyme F430)

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ABSTRACT **Inactive 2-(methylthio)ethanesulfonic acid (CH_3-S-CoM) reductase was partially activated by exposure to light. This simplified system replaces the complex enzymatic system of protein components A2, A3a, A3b, and ATP, which previously represented the only available means of reactivating the enzyme. Components necessary for light activation include *N*-(7-mercaptoheptanoyl)-L-threonine O^3-phosphate (HS-HTP), CH_3-S-CoM, titanium(III) citrate [Ti(III)Cit], and light above 400 nm. Photoactivation was inhibited by known inhibitors of methanogenesis: 2-bromoethanesulfonate (BES), *N*-(6-mercaptohexanoyl)-L-threonine O^3-phosphate, *N*-(8-mercaptooctanoyl)-L-threonine O^3-phosphate, and sodium dithionite. Methanogenesis continued when the light-activated reaction mixture was incubated in the dark. Although the specific activity was low (35 nmol of CH_4 per h per mg of protein) the reaction products methane and the unsymmetrical disulfide of 2-mercaptoethanesulfonate (HS-CoM) and HS-HTP were identified. We were unable to photoactivate a reaction mixture containing the isolated prosthetic group, native F430, or its analogues.**

Methanogenic archaebacteria (archaea) are anaerobes that biocatalytically convert H_2 and CO_2 to methane (1, 2). The terminal step of methanogenesis involves the reductive demethylation of 2-(methylthio)ethanesulfonic acid (CH_3-S-CoM) with reducing equivalents from *N*-(7-mercaptoheptanoyl)-L-threonine O^3-phosphate (HS-HTP) according to the following equation (3, 4):

$$CH_3\text{-S-CoM} + \text{HS-HTP} \rightarrow CH_4(\uparrow) + \text{CoM-S-S-HTP}.$$

The yellow enzyme that catalyzes this reaction is the CH_3-S-CoM reductase (A_{418}, ε = 23,100 $M^{-1}\cdot cm^{-1}$). The enzyme (M_r 300,000) is composed of six subunits with two molecules of a nickel-containing tetrahydrocorphin, F430, bound to the enzyme (5, 6). This coenzyme has been proposed to be the site of catalysis (7). Thauer and coworkers (4) have purified active reductase from *Methanobacterium thermoautotrophicum* (strain Marburg) under anaerobic conditions. The enzyme was found to catalyze the reduction of CH_3-S-CoM to form CH_4 with a specific activity of up to 75 nmol of CH_4 per min per mg of protein (8). The reductase from *M. thermoautotrophicum* (strain ΔH), however, has not been active when highly pure (9). The question of how cells maintain an "active pool" of the reductase is important, since all methanogens examined possess the reductase (10). A model has been proposed for the reductive activation of the reductase (11). Implicit in this concept of activation is the reduction of Ni(II) to Ni(I) of native F430. This reaction is dependent on a multicomponent enzyme system A2, A3a, and A3b. Only A2 and the reductase have been purified to homogeneity. Other difficulties associated with mechanistic studies of this activation include O_2 sensitivity of A3a and A3b and the absence of an assay for each individual protein fraction. We describe here a simplified system that reactivates the reductase from both strains when exposed for long periods to high light intensity.

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MATERIALS AND METHODS

Bacterial Cells. *M. thermoautotrophicum* (strains ΔH, DSM 1053; Marburg, DSM 2133) were grown at 60°C and prepared as described (12).

Analytical Methods. Reverse-phase HPLC (RP-HPLC) was performed on a Waters HPLC system. Eluates were monitored with a Varian 2050 variable wavelength detector. Thiol concentration was determined with Ellman's reagent (13), and phosphate was determined as described (14). ^1H NMR was performed on a Nicolet 360-MHz spectrometer. Mass spectra were obtained with a ZAB-SE 10-kV mass spectrometer. GC/MS was performed with a capillary column under splitless operating conditions. The injection port was at 150°C, and the column was 15 m long. The oven was isothermal at 20°C. The mass spectrometer was a 70VSE with an acceleration potential of 8 kV. Methane was measured as described (11). The injection volume (20–30 μl) was sufficient to detect <0.1 nmol of methane by GC.

Light Source. The lamp and power supply (Research Arc lamp source, model 66024) were from Oriel (Stratford, CT). Filters were purchased from Oriel. The light source was a 1000-W Hg/Xe bulb (Hanovia 977B0010; Newark, NJ). Unless indicated, each sample exposed to the light was 25 cm from the bulb.

Assay Vessels. Components of the reaction mixture were dispensed into cuvettes or tubes in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI). Each cuvette was sealed with a 20-mm red rubber serum stopper (Wheaton Industries, Millville, NJ). Two plastic cuvettes (Markson Science, Phoenix) could be placed in the light source simultaneously. Modified EPR tubes (14 cm × 3 mm) also were used. To keep the contents anaerobic, each tube was fitted with a small piece of rubber tubing and sealed with a small quartz test tube (3 cm × 3 mm). The capped tubes were then removed from the chamber and immediately wrapped with strips of parafilm. After photoactivation, the headspace gas was sampled by puncturing the tubing with the needle of a gas-tight Hamilton syringe. The amount of methane in the headspace gas was quantitated by GC. The headspace gas of the cuvettes and tubes was the same atmosphere as the

Abbreviations: HS-CoM (coenzyme M), 2-mercaptoethanesulfonate; CH_3-S-CoM, 2-(methylthio)ethanesulfonic acid; BES, 2-bromoethanesulfonate; HS-HTP, *N*-(7-mercaptoheptanoyl)-L-threonine O^3-phosphate; CoM-S-S-HTP, unsymmetrical disulfide of HS-CoM and HS-HTP; RP-HPLC, reverse-phase high-performance liquid chromatography; Ti(III)Cit, titanium(III) citrate.

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anaerobic chamber (approximately 95% N_2/5% H_2). The volumes of the cuvette and of the tube were determined to be 1.71 ml and 1.19 ml, respectively.

Purification of the CH_3-S-CoM Reductase. The following purification steps were performed aerobically. The bacterial cells were broken by passage through a French pressure cell at 110 MPa. The suspension was centrifuged for 1 h at 25,000 × *g* in a Sorvall SS-34 rotor. Ammonium sulfate was slowly added to the supernatant until 65% saturation was reached. After stirring overnight at 5°C, the solution was centrifuged again at 25,000 × *g* for 1 h and subjected to YM100 membrane ultrafiltration (M_r cutoff, 100,000; Amicon). The enzyme was applied to a phenyl-Sepharose CL-4B column (Pharmacia LKB) (45 × 1.5 cm), equilibrated with 2 M KOAc in 20 mM KP_i buffer (pH 7.0). The enzyme was detected in the void volume (9). The protein was concentrated with a YM100 membrane. The retained solution was diluted 1:15 with distilled H_2O and applied to a column of DE-52 cellulose (Whatman; 45 × 1.5 cm), equilibrated in 20 mM KP_i (pH 7.0). After washing with 3 bed vol of this buffer, a linear gradient from 100 ml of 20 mM KP_i (pH 7.0) to 100 ml of 1.0 M NaCl in 20 mM KP_i (pH 7.0) eluted the protein at 0.5–0.6 M NaCl. The enzyme was desalted with a YM100 membrane. The retained solution was applied to a column (1 × 13 cm) of methotrexate resin (Pierce) equilibrated in 20 mM KP_i (pH 7.0) (15). A linear gradient to 1 M KOAc with 20 mM KP_i (pH 7.0) eluted the reductase at 0.9 M KOAc. The protein was concentrated with an Amicon PM30 membrane.

Anion-exchange chromatography with FPLC was also used to obtain homogeneous enzyme. The system included a Mono Q 5/5 column (Pharmacia). The conditions of the gradient were as follows: 3 ml of buffer A and then a 20-min linear gradient of buffer A to buffer B [buffer A = 20 mM KP_i (pH 7.0); buffer B = 1.6 M KOAc/20 mM KP_i, pH 7.0]. The flow rate was 1 ml/min and the effluent was monitored at 280 nm. The retention times of the reductases were as follows: strain ΔH, 21 min; strain Marburg, 22.8 min. The A_{274}/A_{430} was ≈8.0 but varied slightly in different preparations. Protein concentrations were determined as described (16). Bovine serum albumin was used as the standard. The reductase was transferred to a vial (25 ml), flushed under H_2 for 30 min, and sealed under H_2. The enzyme was stored at 5°C.

Analytical PAGE. Denaturing and nondenaturing analytical discontinuous PAGE was performed in slab gels at pH 6.8–8.8 (17). The gel was 10% polyacrylamide [acrylamide/bisacrylamide (30:1)]. Alternatively, purity was monitored by native gel (8–25%) or SDS gel electrophoresis with the PhastSystem (Pharmacia). Coomassie brilliant blue R-250 solution was used to stain the gels (18).

Reagents. Titanium(III) citrate [Ti(III)Cit] was prepared from 20% $TiCl_3$ (Fisher Scientific) and sodium citrate as described (19). A 20 mM solution at pH 6.61 has been determined to have an $E^{\circ\prime}$ = −403.9 mV as measured with a platinum/calomel electrode ($E^{\circ\prime}$ represents standard electrode potential at pH 7) (20). The solution was stored at 5°C. CH_3-S-CoM was synthesized as described (21). HS-HTP was synthesized as described (22). The heterodisulfide CoM-S-S-HTP was synthesized as described (23). *N*-(6-mercaptohexanoyl)-L-threonine O^3-phosphate was synthesized in an analogous fashion as HS-HTP (22) with the following modifications: thiourea was refluxed with 6-bromohexanoic acid (Aldrich). After coupling the resultant ester with phospho-L-threonine, 10 mg of palladium on activated carbon (Aldrich) was added to reduce the homodisulfide. The reaction mixture was transferred to a vial and pressurized to 3 atm with H_2 (1 atm = 101.3 kPa). Before chromatography, the solution was filtered through a 0.45-μm filter (Schleicher & Schuell). A Waters C18 μBondapak column (7.8 mm × 30 cm) was used to purify the compound. The conditions were as follows: a 10-min isocratic application of 20 mM NH_4OAc (pH 5.0) and then a 20-min linear gradient to 20 mM NH_4OAc (pH 5.0) in 80% MeOH. The flow rate of 2 ml/min was monitored at 215 nm. The final product, which eluted at t_R = 20.7 min, was confirmed by ^1H NMR and fast atom bombardment (FAB) cation MS. A positive ion at m/z = 330.0783 was observed by high-resolution MS. The Δ millimers unit (mmu) = −0.7, and the composition was determined to be $C_{10}H_{21}O_7NPS$. Synthesis of *N*-(8-mercaptooctanoyl)-L-threonine O^3-phosphate followed that described for *N*-(6-mercaptohexanoyl)-L-threonine O^3-phosphate, except the thiourea was refluxed with 8-bromooctanoic acid (Aldrich). The final product, which eluted at 24.6 min, was confirmed by ^1H NMR and FAB cation MS. A positive ion at m/z = 358.1078 was observed by high-resolution MS. The Δmmu = 1.1, and the composition was determined to be $C_{12}H_{25}O_7NPS$. Native coenzyme F430 and 12,13-diepimer F430 and 12,13-didehydro-F430 (F560) were purified by anion-exchange chromatography and RP-HPLC as described (24). Concentrations of these cofactors were determined by using the molar absorptivities of the structurally related methyl ester derivatives (25, 26).

Temperature. The temperature optimum of the enzyme (from both strains) is 60°C–65°C (27, 28). The temperature directly in front of the filter (with an air stream to cool it) was ≈50°C.

RESULTS

Methane was identified as a product of photoactivation by GC and by GC/MS. Evidence that the minimal system required for the photoactivation of the enzyme includes the CH_3-S-CoM reductase, HS-HTP, CH_3-S-CoM, Ti(III)Cit, and light (above 400 nm) is discussed below.

The Holoenzyme Is Required for Photoactivation and Methanogenesis. Four reaction mixtures were simultaneously exposed to light (Table 1). The results show that in a 5-h light exposure the amount of CH_4 produced is enzyme concentration dependent. No methane was detected in the tube lacking the protein, nor was any methane produced by a complete reaction mixture wrapped in foil. No difference was observed by SDS gel electrophoresis between the subunits of the enzyme before and after photoactivation (data not shown).

The enzyme could not be replaced by isolated F430. We attempted to photoactivate native F430, the diepimer of F430 and the didehydro species, since these have different chemical properties (24, 29, 30). When Ti(III)Cit (3.6 μmol) was added to the cuvette containing the purple 12,13-didehydro-F430 (F560; 45 nmol), the solution immediately turned yellow independent of light. After light exposure, analysis of a sample of this solution by RP-HPLC, which is capable of separating the various stereoisomers (24), indicated that the bulk of the product (>80%) was native F430. This was not surprising, since Eschenmoser and coworkers (26) had shown that the purple dehydrogenation product can be

Table 1. Photoactivation of the CH_3-S-CoM reductase from *M. thermoautotrophicum*

Tube	Reductase added, nmol	*hν*	Methane formed, nmol
1	10	+	12.6
2	5	+	5.1
3	0	+	0.0
4	10	−	0.0

The tubes were modified EPR tubes. Each tube contained 0.36 mM HS-HTP, 4.1 mM CH_3-S-CoM, and 7.4 mM Ti(III)Cit. The CH_3-S-CoM reductase (strain Marburg) was used. A filter was used to cut off wavelengths below 400 nm. Tube 4 was wrapped in aluminum foil. Tubes were illuminated for 5 h.

diastereoselectively reduced with Zn in AcOH to give native F430 as the main product.

Fig. 1*a* shows the absolute dependence of methanogenesis on protein concentration. At a very high concentration of protein (189 μM), less methane is produced than at a more optimal concentration of protein (38 μM; A_{418}, 1.7).

Addition of HS-HTP Is Required for Photoactivation and Methanogenesis. Two identical reaction mixtures were prepared (see Fig. 2 for concentrations). HS-HTP was added to one cuvette. After overnight exposure to light, the cuvette containing HS-HTP contained 14 times as much methane as the cuvette without HS-HTP (total, 575 vs. 42 nmol of CH_4). It has been reported that the reductase has HS-HTP bound to it (31); this may explain the background levels of methane observed.

We were interested to know whether or not HS-HTP is required for activation of the reductase. Each cuvette described above was flushed with H_2. Additional CH_3-S-CoM (1.25 μmol) was added. The cuvettes were placed in the dark, and the reaction was started by increasing the temperature of the cuvettes to 65°C. At the times indicated in Fig. 2, gas samples were analyzed for CH_4 by GC. The complete reaction mixture, which previously had been incubated in light, showed methane production after a short lag, the rate being 90 nmol of CH_4 per h and with a specific activity of 35 nmol of CH_4 per h per mg of protein. The other cuvette, which had previously been incubated in the light without HS-HTP, produced no methane. At t = 1224 sec, HS-HTP was added to the cuvette from which HS-HTP had been omitted. Only a small amount of methane was produced. When an endpoint was taken, the cuvette photoactivated with HS-HTP present had 10.3 times more methane than the cuvette without HS-HTP.

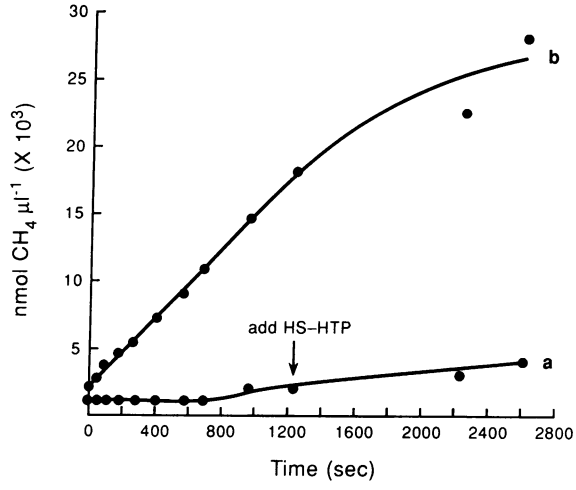

FIG. 2. Methanogenesis in the dark after photoactivation. Both cuvettes contained 3 mg of reductase (strain ΔH), 1.2 μmol of CH_3-S-CoM, 9 μmol of dithiothreitol, and 4 μmol of Ti(III)Cit. Dithiothreitol was added to keep a reduced pool of HS-HTP present (4). Only cuvette b contained HS-HTP (0.93 μmol) during photoactivation. After photoactivation, both cuvettes were flushed with H_2. Then 1.25 μmol of CH_3-S-CoM was added to each cuvette. Methanogenesis was started by raising the temperature to 65°C. At the time indicated, HS-HTP (4.65 μmol) was added to cuvette a.

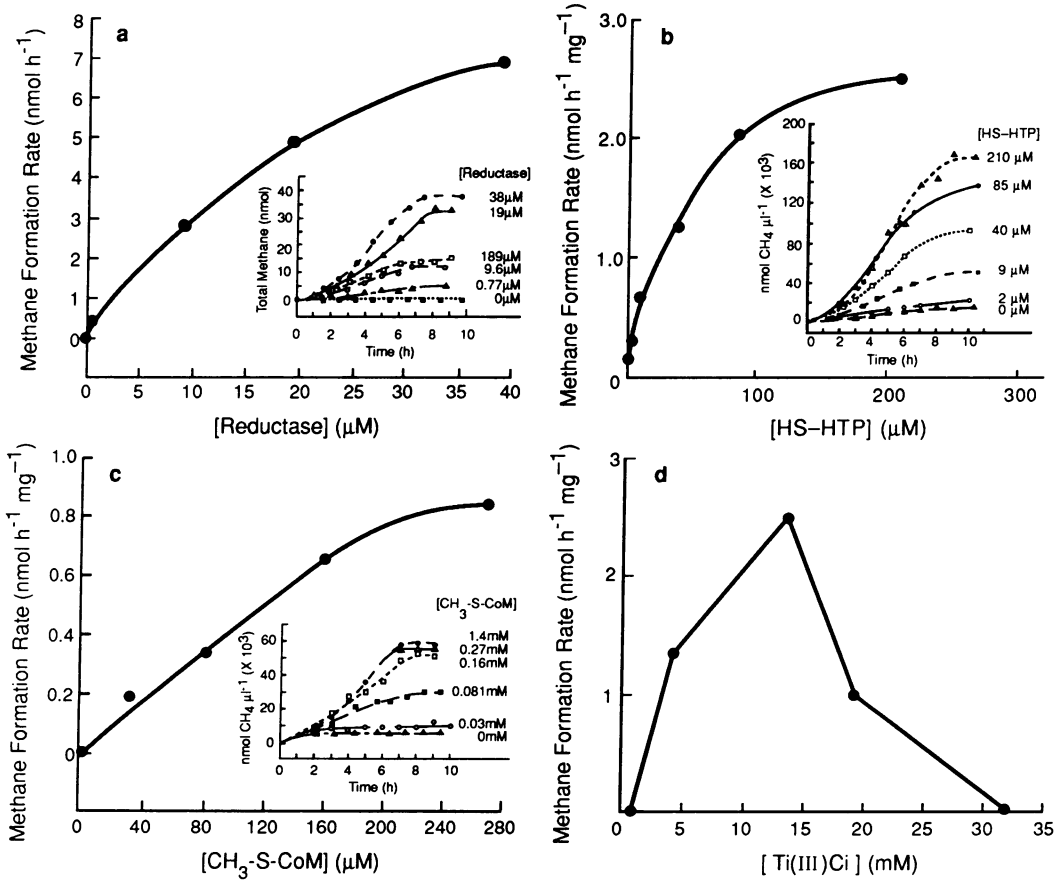

FIG. 1. Dependence of methanogenesis (strain ΔH) on protein concentration (*a*), HS-HTP concentration (*b*), CH_3-S-CoM concentration (*c*), and Ti(III)Cit concentration (*d*). For these experiments only, the tubes were ≈35 cm from the light source. (*a*) Each cuvette contained 200 μM HS-HTP, 10 mM Ti(III)Cit, 4 mM KP_i (pH 7.0), and 2 mM CH_3-S-CoM. (*b*) Each cuvette contained 10 mM Ti(III)Cit, 29 μM reductase, 4 mM KP_i (pH 7.0), and 2 mM CH_3-S-CoM. (*c*) Each cuvette contained 10 mM Ti(III)Cit, 30 μM reductase, 140 μM HS-HTP, and 4 mM KP_i (pH 7.0). (*d*) Each cuvette contained 50 μM HS-HTP, 2 mM CH_3-S-CoM, 4 mM KP_i (pH 7.0), and 13 μM reductase.

N-(6-mercaptohexanoyl)-L-threonine O^3-phosphate and *N*-(8-mercaptooctanoyl)-L-threonine O^3-phosphate, structural analogues of HS-HTP, are effective inhibitors of methanogenesis (4). We added each analogue to a separate reaction mixture before exposing them to light. The mixtures contained HS-HTP (71 μM), CH_3-S-CoM (3 mM), Ti(III)Cit (6 mM), and reductase (10 nmol). The lowest concentrations of the analogues tested, the *N*-6 analogue at 10.2 μM and the *N*-8 analogue at 72 μM, completely inhibited methanogenesis.

Fig. 1*b* shows the absolute dependence of methanogenesis by photoactivation on HS-HTP concentration.

Addition of CH_3-S-CoM Is Required for Photoactivation and Methanogenesis. No methane was generated in an otherwise complete reaction mixture without CH_3-S-CoM, even after a 12-h exposure to light. When CH_3-S-CoM was added after light exposure, no methane was detected after incubation in the dark, indicating that CH_3-S-CoM must be present during light exposure for activation to occur. A structural analogue of this coenzyme, 2-bromoethanesulfonate (BES), was found to be an inhibitor of methane formation (32). This analogue is also a potent inhibitor of the photoactivation process; as shown in Fig. 3, addition of 5 μM BES to a reaction mixture inhibited methanogenesis dramatically. Dithionite (13 μM) is known to cause a 50% reduction in the rate of methanogenesis (16). This molecule, which has similarities to CH_3-S-CoM, is also an effective inhibitor of photoactivation; 62 μM completely inhibited light activation. Fig. 1*c* shows the absolute dependence of methanogenesis by photoactivation on CH_3-S-CoM.

Coenzyme M (HS-CoM) Does Not Replace CH_3-S-CoM in Photoactivation. Because free HS-CoM is found in methanogens (33), it seemed conceivable that exposure of the reaction mixture to light with added HS-CoM might activate the enzyme. The reaction mixture contained 4.8 mg of protein, 4.1 mM Ti(III)Cit, 1.3 mM HS-CoM, 71 μM HS-HTP, and 6 mM KP_i (pH 7.0) with no CH_3-S-CoM. The vial was exposed to light for 7 h. No methane was detected. CH_3-S-CoM was added, and the cuvette was incubated in the dark at 60°C for 24 h. Again, no methane was detected, indicating that HS-CoM cannot substitute for CH_3-S-CoM during photoactivation.

Ti(III)Cit Is Required for Photoactivation and Methanogenesis. When Ti(III)Cit was deleted from an otherwise complete mixture, no methane was observed after overnight exposure to light. Dithiothreitol (0.5 M) and sodium citrate (0.1 M) could not substitute for Ti(III)Cit in the reaction mixture. As shown in Fig. 1*d*, the optimum concentration of Ti(III)Cit is ≈10 mM (A_{422}, 0.96).

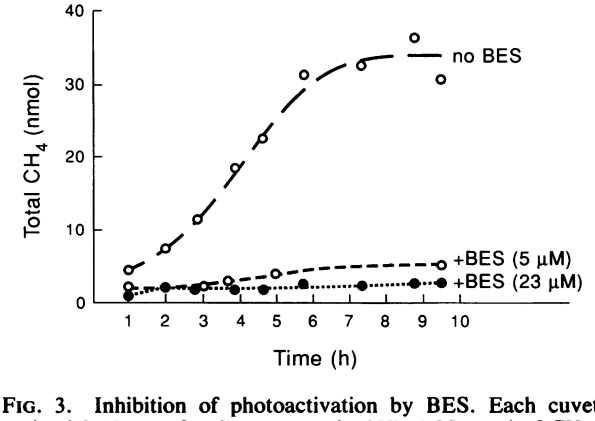

FIG. 3. Inhibition of photoactivation by BES. Each cuvette contained 2.15 mg of reductase (strain ΔH), 1.25 μmol of CH_3-S-CoM, 0.93 μmol of HS-HTP, 9 μmol of dithiothreitol, 3.6 μmol of Ti(III)Cit, and 4.6 mM KP_i (pH 7.0). The concentrations of BES are shown.

Light Is Required for Activation. We were unable to activate enzyme from either strain without light. Tubes that were wrapped in foil were used as controls. To ensure that the photoactivation was not a thermal reaction, covered tubes were placed in an incubator (55°C) overnight. Under these conditions, we were unable to detect methane. To test the effective range of light wavelengths one complete reaction mixture was exposed to light that had passed through a filter that absorbed wavelengths below 515 nm. Another identical reaction mixture was exposed to light that had passed through a filter that absorbed wavelengths below 400 nm. After 12 h of incubation we observed 30 times more methane in the cuvette with the 400-nm filter. The effective wavelengths of light lie between 400 and 515 nm.

The Unsymmetrical Disulfide of HS-CoM and HS-HTP Is a Product of the Photoactivated Reaction. To identify the heterodisulfide product of methanogenesis, a reaction mixture in a sealed tube was illuminated for 12 h (see legend to Fig. 4 for concentrations). The solution was then placed in tubes, and ethanol was added until the protein precipitated (60%; vol/vol). The precipitate was removed by centrifugation in a Beckman Microfuge E for 5 min. The supernatant was transferred and spun for 2 h until almost dry in a Savant SpeedVac concentrator. The contents were resuspended in H_2O. The heterodisulfide product was detected by RP-HPLC; the ^1H NMR spectrum (Fig. 4) matched the previously published spectrum (4). We tested the possibility that the reductase had the disulfide bound to it. We precipitated

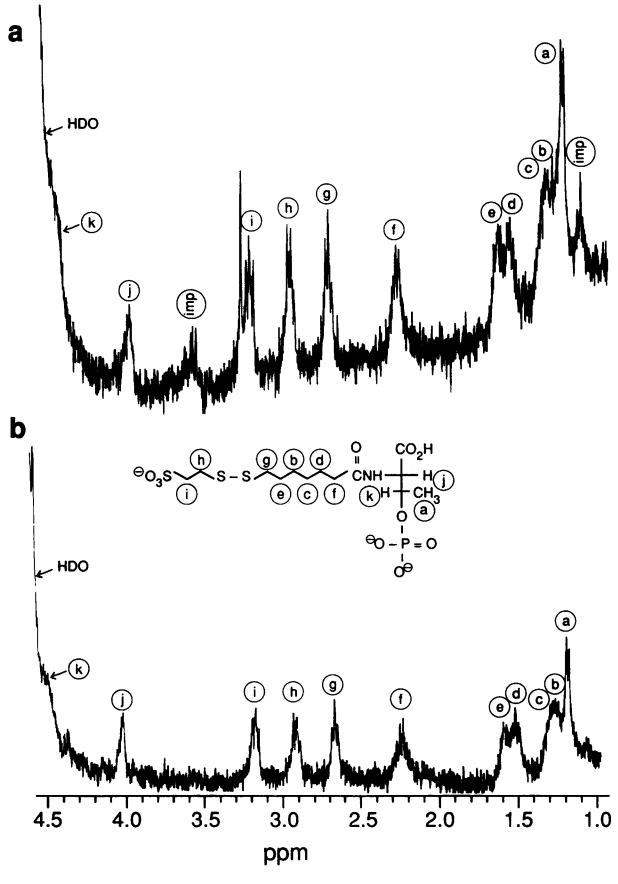

FIG. 4. ^1H NMR spectra (360 MHz) of the unsymmetrical disulfide of HS-CoM and HS-HTP (CoM-S-S-HTP). (*a*) The disulfide obtained after photoactivation. The reaction mixture contained 2.2 mM HS-HTP, 2.8 mM CH_3-S-CoM, 6 mg of reductase (ΔH), 10 mM Ti(III)Cit. The time of light activation was 12 h. The final liquid vol was 900 μl. (*b*) The chemically synthesized disulfide. The signals are assigned. imp, Sample impurity. Number of acquisitions: *a*, 512; *b*, 32.

an equivalent amount of protein with ethanol. Under these conditions, no CoM-S-S-HTP could be found by RP-HPLC.

DISCUSSION

The life of the excited state of the component that absorbs a photon may be on the order of picoseconds. The excited state is predicted to lead to the formation of Ni(I), which then requires HS-HTP and CH_3-S-CoM for catalysis to occur. Thus, the formation of methane by the four-component system [the reductase with two tetrapyrrole centers, Ti(III)Cit, HS-HTP and CH_3-S-CoM] is dependent on the improbable event that all necessary components will be in their correct positions when a photon induces an excited state. Since all components are in a relatively dilute solution, it becomes clear why such intense radiation for such a long time is required. It is likely that most of the excited states decay without initiating catalysis. Certainly, the overall quantum yield is not very high, and this is reflected in the relatively low rate. Attempts to observe the Ni(I) state of the protein by laser spectroscopy have so far been unsuccessful.

Evidence that the light-driven reaction is similar to the normal reductase reaction is provided by (*i*) the specificity of the enzyme; (*ii*) the requirement of CH_3-S-CoM and HS-HTP; (*iii*) the effective inhibition of BES, dithionite, *N*-(6-mercaptohexanoyl)-L-threonine O^3-phosphate, and *N*-(8-mercaptooctanoyl)-L-threonine O^3-phosphate; and (*iv*) the formation of products CH_4 and CoM-S-S-HTP.

A filter was used to absorb the more energetic wavelengths below 400 nm. However, it must be emphasized that we have not yet conclusively shown which component of the reaction mixture is actually absorbing energy [both the reductase and Ti(III)Cit absorb light above 400 nm]. Indeed, upon illumination, the color of Ti(III)Cit changed to a dark green. Neither HS-HTP nor CH_3-S-CoM absorbs light above 400 nm. We were unable to observe a Ni(I) signal by EPR. This was expected because of the low activity of methanogenesis. Also, there is a very broad and intense signal at $g = 2$ due to Ti(III)Cit, as previously noted (34).

Complete reaction mixtures that were light activated were subsequently found to be competent for methanogenesis in the dark. The specific activity was low at 35 nmol of CH_4 per h per mg of protein. The rate was ≈90 nmol of CH_4 per h. The specific activity is ≈1% of the maximal activity observed by Thauer and coworkers (4) with anaerobically purified reductase (strain Marburg). We were not able to activate the reductase without CH_3-S-CoM and HS-HTP in the reaction mixture; in addition, there was no methane production in the dark if these coenzymes were added after light exposure. HS-CoM could not substitute for CH_3-S-CoM. These data indicate that the activation process and methanogenesis are intimately associated. A major problem when working with the reductase is that the purified form is not very active, even when purified under strict anaerobic conditions (strain Marburg). The specific activity of the enzyme drops to 1% upon cell breakage (4). Even though the rate of methanogenesis is low in the photoactivated enzyme, this simplified system may help us to understand the mechanism of how methanogens activate the enzyme *in vivo*. This is a most important question of the terminal step of methanogenesis.

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