Holler, E., Baltzinger, M., & Favre, A. (1981) *Biochemistry 20*, 1139–1147.
Hurd, R. E., & Reid, B. R. (1979) *Biochemistry 18*, 4005–4011.
Jones, C. R., & Kearns, D. R. (1975) *Biochemistry 14*, 2660–2665.
Jones, C. R., Bolton, P. H., & Kearns, D. R. (1978) *Biochemistry 17*, 601–607.
Kearns, D. R. (1976) *Prog. Nucleic Acid Res. Mol. Biol. 18*, 91.
Kim, S. H., Suddath, F. L., Quigley, G., McPherson, A., Sussman, N., Wang, A. H. L., & Rich, A. (1974) *Science (Washington, D.C.) 185*, 435–440.
Krauss, G., Riesner, D., & Maas, G. (1976) *Eur. J. Biochem. 68*, 81–93.
Liebman, M., Rubin, J., & Sandaralingam, M. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4821–4825.
Olmsted, J., & Kearns, D. R. (1977) *Biochemistry 16*, 3647–3654.
Pachmann, U., Cronvall, E., Rigler, R., Hirsch, R., Wintermeyer, W., & Zachau, H. G. (1973) *Eur. J. Biochem. 39*, 265–273.
Rich, A., & Schimmel, P. R. (1977) *Nucleic Acids Res. 4*, 1649–1665.
Risler, J. L., Zelwer, C., & Brunie, S. (1981) *Nature (London) 292*, 384–386.
Robertus, J. D., Ladner, J. E., Finch, J. T., Rhodes, D., Brown, R. S., Clark, B. F. C., & Klug, A. (1974) *Nature (London) 250*, 546–551.
Rosa, J. J., Rosa, M. D., & Sigler, P. B. (1979) *Biochemistry 18*, 637–647.
Sakai, T. T., & Cohen, S. S. (1976) *Prog. Nucleic Acid Res. Mol. Biol. 17*, 15.
Schimmel, P. R., & Soll, D. G. (1979) *Annu. Rev. Biochem. 48*, 601–648.
Schulman, L. H., & Pelka, H. (1977) *Biochemistry 16*, 4256–4265.
Schulman, L. H. Valenzuela, D., & Pelka, H. (1981) *Biochemistry 20*, 6018–6023.
Shinitzky, M. (1972) *J. Chem. Phys. 56*, 5979–5981.
Starzyk, R. M., Koontz, S. W., & Schimmel, P. R. (1982) *Nature (London) 298*, 136–140.
Stryer, L. (1978) *Annu. Rev. Biochem. 47*, 819–846.
Tao, T., Nelson, J. H., & Cantor, C. R. (1970) *Biochemistry 9*, 3514–3524.
Thomas, G., Fourrey, J. L., & Favre, A. (1978) *Biochemistry 17*, 4500–4514.
Thomas, J. C., Schurr, J. M., & Hare, D. R. (1984) *Biochemistry 23*, 5407–5413.
Tsai, C. C., Jain, S. C., & Sobell, H. M. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 628–632.
Waring, M. J. (1965) *J. Mol. Biol. 13*, 269–282.
Wells, B. D., & Cantor, C. R. (1977) *Nucleic Acids Res. 4*, 1668–1680.
Wetzle, R., & Soll, D. (1977) *Nucleic Acids Res. 4*, 1681–1694.
Wintermeyer, W., & Zachau, H. G. (1979) *Eur. J. Biochem. 98*, 465–475.
Woo, N. H., Roe, B. A., & Rich, A. (1980) *Nature (London) 286*, 346–351.
Wrede, P., Woo, N. H., & Rich, A. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3289–3293.
Zelwer, C., Risler, J. L., & Brunie, S. (1982) *J. Mol. Biol. 155*, 63–81.

# Intermediates in the Biosynthesis of Coenzyme M (2-Mercaptoethanesulfonic Acid)†

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ABSTRACT: The early steps in the formation of coenzyme M (2-mercaptoethanesulfonic acid) were examined in purified extracts of *Methanobacterium formicicum*. Extracts incubated with phosphoenolpyruvate (PEP), bisulfite, and cysteine were found to readily catalyze the enzymatic formation of the coenzyme; incubation with pyruvate instead of PEP produced no coenzyme. Extracts were incubated with PEP, bisulfite, and cysteine and analyzed for the presence of sulfonic acids by gas chromatography–mass spectrometry (GC–MS) of suitable derivatives. Three of the proposed intermediates in the conversion of these products into coenzyme M were identified, i.e., sulfolactic acid, sulfopyruvic acid, and sulfoacetaldehyde. Sulfopyruvic acid was also shown to be readily converted into sulfoacetaldehyde and coenzyme M by the extracts. These results are consistent with the initial step in coenzyme M biosynthesis being the condensation of PEP with sulfite to form sulfolactic acid. The sulfolactic acid is then oxidized to sulfopyruvic acid which is decarboxylated to form sulfoacetaldehyde. After reaction with cysteine to form a thiazolidine intermediate, the sulfoacetaldehyde is subsequently converted into enzyme M by a series of known reactions (R. H. White, unpublished results).

The structure of coenzyme M (2-mercaptoethanesulfonic acid), one of several recently described coenzymes involved in the biological production of methane (Escalante-Semerena et al., 1984), was determined in 1974 by Taylor and Wolfe. It is unique among coenzymes in that it occurs only in methanogenic bacteria (Balch & Wolfe, 1979). It is also the smallest organic coenzyme ($M_r$ 142), it contains the highest percentage of sulfur (45%), and it is one of the few sulfonic acids found in nature. The methylation of coenzyme M to

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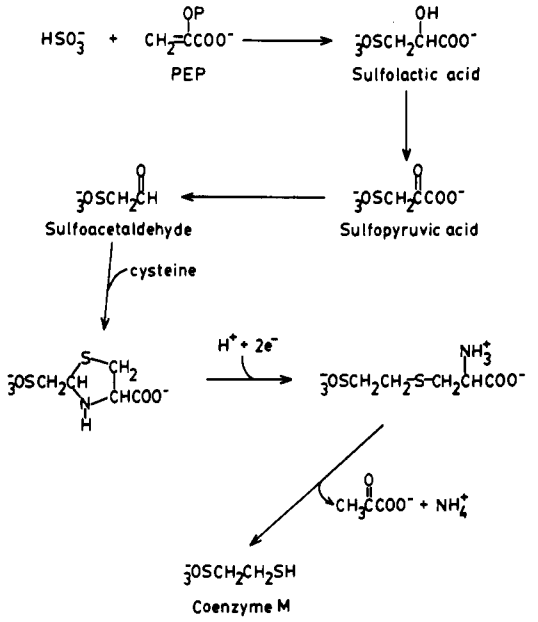


FIGURE 1: Proposed pathway for the biosynthesis of coenzyme M.

*S*-methyl coenzyme M, which undergoes a subsequent reductive cleavage to methane and coenzyme M, is involved in the production of methane from both hydrogen and carbon dioxide, methanol, and methylamines (Shapiro & Wolfe, 1980) as well as acetate (Nelson & Ferry, 1984; Lovley et al., 1984). Recent work on the biosynthesis of coenzyme M (White, 1985) has shown that it most likely arises by the series of reactions shown in Figure 1. Up to now, only the last series of reactions in this scheme, i.e., the conversion of sulfoacetaldehyde to coenzyme M, have been shown to occur in cell-free extracts of methanogenic bacteria (R. H. White, unpublished results). I now wish to report on the remaining reactions.

## Materials and Methods

*Growth of Organism. Methanobacterium formicicum* was grown at 39 °C in a 2-L Multigen fermenter (New Brunswick Scientific Co., Inc., Edison, NJ) which was continuously supplied with a gas mixture consisting of $H_2/CO_2$ (80/20). The medium used was the same as that described by Schauer and Ferry (1982) but without the addition of yeast extract or trypticase. Cells were removed from the medium by centrifugation under nitrogen at the end of log-phase growth and were washed twice with an anaerobic assay buffer, 50 mM in 2-[[tris(hydroxymethyl)methyl]amino]ethanesulfonic acid (TES), 10 mM in $MgCl_2$, and 2 mM in mercaptoethanol.

*Preparation and Purification of Cell Extracts.* All operations were performed under nitrogen with solutions degassed with nitrogen in order to assure anaerobic conditions throughout the procedure. The cell pellet isolated above was suspended in a volume of assay buffer equivalent to its weight in grams and the resulting suspension sonicated at 3 °C. The extent of cell lysis was determined by measuring the release of coenzyme $F_{420}$ at 420 nm (Eirich et al., 1978) and was found to be at a maximum after two 30-s, 50-W bursts from a Sonifier cell disruptor with a micro tip. After centrifugation for 20 min at 10000*g*, 3 mL of the resulting clear, greenish solution (10–15 mg of protein/mL) was transferred under anaerobic conditions to a 1.5 × 24.5 cm column of Sephacryl S-200 equilibrated with the above anaerobic buffer and kept at 3 °C. Those fractions containing the highest activity for the conversion of sulfoacetaldehyde into coenzyme M (i.e., fractions 11–14, Figure 2) were collected under hydrogen for incubation with the desired substrates. The ability of the fractions to produce coenzyme M was measured by determining the extent of the conversion of [2,2-$^2H_2$]sulfoacetaldehyde into coenzyme M as described by White (1985).

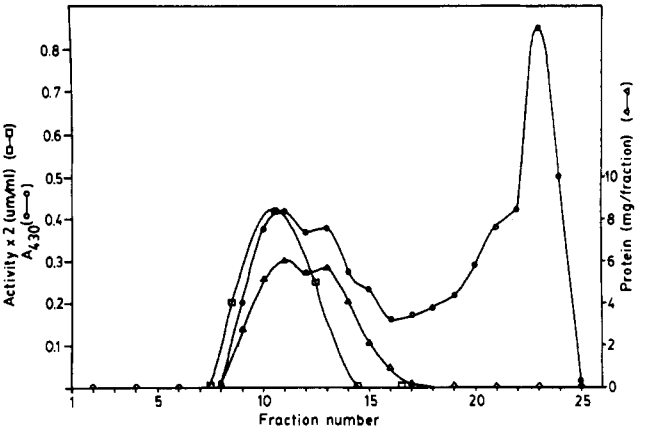


FIGURE 2: Elution profile for an extract of *M. formicicum* which was passed through a Sephacryl S-200 column. Activities are expressed as micromoles of coenzyme M produced per milliliter of extract over a 6-h period when incubated with 24 mM sulfoacetaldehyde and 21 mM cysteine. Blue dextran ($M_r > 2 \times 10^6$) eluted at fraction 9; $F_{420}$ and coenzyme M eluted at fraction 23. Calibration of the column with hemoglobin and blue dextran showed that the maximum activity eluted with an apparent molecular weight of 100 000.

Table I: Production of Coenzyme M in Purified, Cell-Free Extracts of *M. formicicum*

| substrates | observed intensity: *m/z* 74/*m/z* 88 | nmol of CoM/mg of protein | intermediates identified[b] |
|---|---|---|---|
| PEP (30 mM), $HSO_3^-$ (20 mM), Cys (20 mM) | 0.29 | 11.3 | + |
| PEP (30 mM), $HSO_3^-$ (20 mM), Cys (20 mM) (no extract) | 0.0[a] | 0.0[a] | – |
| pyruvate (31 mM), $HSO_3^-$ (38 mM), Cys (14 mM) | 0.0[a] | 0.0[a] | – |
| sulfopyruvate (8.7 mM), Cys (8.7 mM) | 0.15 | 5.7 | + |
| sulfopyruvate (9.4 mM) | 0.11 | 4.4 | + |
| sulfopyruvate (30 mM) (no extract) | 0.0[a] | 0.0[a] | +[c] |

[a] The intensity of the *m/z* 88 ion for the *S*-ethyl coenzyme M internal standard was measured in all of the experiments, even those experiments in which the *S*-methyl coenzyme M was not observed. [b] A positive sign indicates that sulfolactic acid, sulfopyruvic acid, and sulfoacetaldehyde were also identified as products of the incubation; these were identified as the derivatives shown in Figure 3. A negative sign indicates that these products were not found. [c] Only sulfopyruvic acid was identified.

*Enzymatic Incubations.* Concentrated (50×), degassed solutions of the indicated substrates were added to 1–2 mL of the combined, active fractions to give the concentrations indicated in Table I; the reactions were then allowed to proceed at 39 °C with shaking under 30 psi of $H_2$. The individual reactions were then terminated after 3 h by the addition of 0.2 mL of 6 M HCl.

*Preparation and Analysis of Sulfonic Acid Derivatives.* To the above acidified reaction mixture was added 100 μL of a 5.35 mM solution of ammonium 2-(ethylthio)ethanesulfonate which served as an internal standard for the quantitation of coenzyme M. The reaction mixture was then centrifuged to remove the precipitated proteins, and the coenzyme M present in the reaction mixture was alkylated with methyl iodide to yield 2-(methylthio)ethanesulfonate as described by Lovley et al. (1985). The sulfonic acids present in the solution were

isolated by applying the sample to a 4 × 40 mm column of Dowex 1-X8 $Cl^-$ and eluting the sulfonic acids with 3 M HCl as was previously described for the isolation of 2-(methylthio)ethanesulfonate from similar mixtures (White, 1985). After evaporation of the HCl with a stream of nitrogen, the resulting sulfonic acids were dissolved in methanol and reacted with an excess of diazomethane in diethyl ether. This procedure converted both the sulfonic acids (Heywood et al., 1958) and the carboxylic acids into methyl esters. In addition, the hydroxyl group of sulfolactic acid was methylated, and the $\alpha$-keto acids were converted into a series of products which include the methyl ester, the methyl ester epoxide derivative, and the dimethyl ketal derivative of the methyl ester (Simmonds et al., 1967). Sulfoacetaldehyde was converted into the methyl ester dimethyl acetal. All products were confirmed by gas chromatography–mass spectrometry (GC–MS) of knowns derivatized in the same manner as the isolated samples. A VG 70-70HF mass spectrometer was used to obtain a mass spectrum for each of the derivatives at 70 eV by scanning the mass range from $m/z$ 50 to 500 at the top of each peak as it eluted from the gas chromatographic column discussed later. The mass spectra of the derivatives of the known compounds were identical with those isolated from the incubation mixtures which are shown in Figure 3. The formation of *O*-methylsulfolactic acid from sulfolactic acid can be explained by the methylation of the hydroxyl group of the sulfolactic acid by diazomethane in the presence of the sulfonic acid. The methylation of hydroxyl groups with diazomethane in the presence of Lewis acids is well documented (Caserio et al., 1958; Müller & Rundel, 1958). After evaporation of the solvents with a stream of nitrogen, the residue was dissolved in methylene chloride and assayed by GC–MS. The derivatives were separated on a 0.3 × 183 cm glass column containing 3% SE-30 on 80/100 mesh Supelcoport (AW, DMCS) from Supelco, Inc. (Bellefonte, PA), programmed from 90 °C at 10 °C $min^{-1}$. Under these GC conditions, the methyl ester derivatives of the dimethyl acetal of sulfoacetaldehyde, methyl coenzyme M, ethyl coenzyme M, *O*-methylsulfolactic acid, the epoxide derivative of sulfopyruvic acid, and the dimethyl ketal of sulfopyruvic acid eluted with retention times of 4.34, 5.23, 6.24, 6.54, 7.60, and 8.2 min, respectively.

*Evaluation of the Mass Spectra of the Derivatives.* The assignment of the ions in the mass spectrum of the methyl 2-(methylthio)ethanesulfonate used in the identity of the coenzyme M in the extracts has been previously published (Lovley et al., 1984). The assignment of many of the ions in the mass spectra of the derivatives of the other sulfonic acids was based on the high-resolution mass measurement of the ions as well as on the presence or absence of a $^{34}S$ isotope peak. Thus, the methyl ester dimethyl acetal derivative of sulfoacetaldehyde (Figure 3a) showed an $M^+ - OCH_3$ ion at $m/z$ 153 with a mass corresponding to $C_4H_9O_5S$, an $M^+ - [OCH_3 + HOCH_3]$ ion at $m/z$ 121 with a mass corresponding to $C_3H_5O_4S$, and an ion at $m/z$ 75 corresponding to $[CH_3OCHOCH_3]^{\cdot+}$. The methyl ester epoxide derivative of sulfopyruvic acid (Figure 3b) showed an $M^+$ ion at $m/z$ 210 with a mass corresponding to $C_6H_{10}O_6S$, an $m/z$ 179 ion corresponding to $M^+ - OCH_3$, an $m/z$ 151 ion corresponding to $M^+ - COOCH_3$, and an ion at $m/z$ 85 corresponding to $C_4H_5O_2$. The derivative of sulfolactic acid (Figure 3c) showed an $M^+$ at $m/z$ 212 corresponding to $C_6H_{15}O_6S$, an $M^+ - COOCH_3$ ion at $m/z$ 153 with a mass corresponding to $C_4H_9O_5S$, an $M^+ - [COOCH_3 + HOCH_3]$ ion at $m/z$ 121 with a mass corresponding to $C_3H_5O_4S$, and an ion at $m/z$ 75 corresponding to $[CH_3OCHOCH_3]^{\cdot+}$. The dimethyl acetal

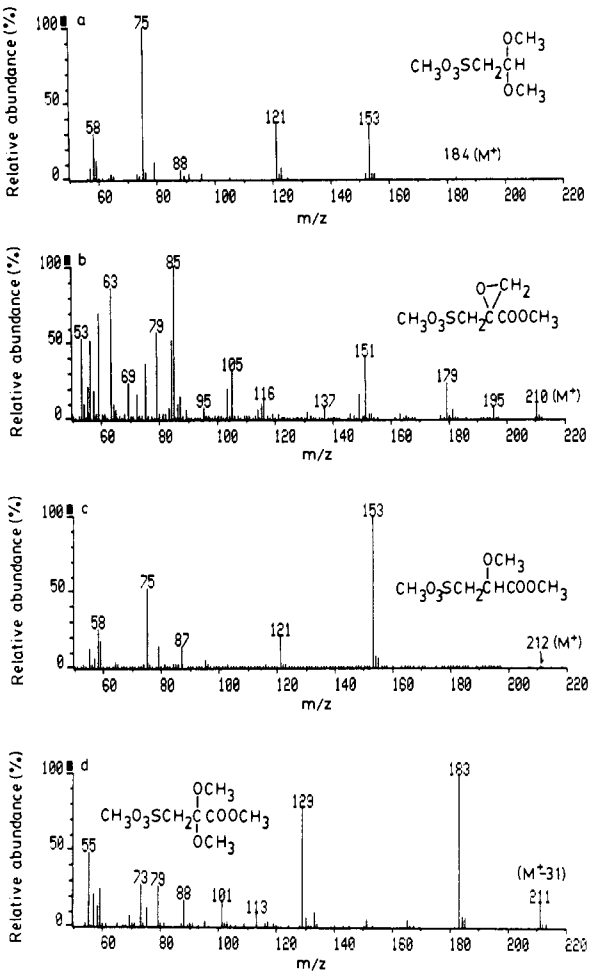


FIGURE 3: Mass spectra of the derivatives of the sulfonic acids involved in the biosynthesis of coenzyme M. (a) The methyl ester of the dimethyl acetal of sulfoacetaldehyde, (b) the dimethyl ester epoxide derivative of sulfopyruvic acid, (c) the *O*-methyl ether dimethyl ester of sulfolactic acid, and (d) the dimethyl acetal dimethyl ester of sulfopyruvic acid. All spectra were recorded by GC–MS of the respective compound or group of compounds.

derivative of sulfopyruvic acid (Figure 3d) showed an $M^+ - OCH_3$ ion at $m/z$ 211 corresponding to $C_6H_{11}O_6S$ and an $M^+ - COOCH_3$ ion at $m/z$ 183 corresponding to $C_4H_9O_5S$.

*Measurement of the Total Amount of Coenzyme M Formed.* The amount of coenzyme M in each derivatized sample was quantitated by GC–MS from the ratio of methyl coenzyme M to ethyl coenzyme M with the ethyl coenzyme M serving as an internal standard. By comparison of the ratio of the intergrated intensity of the base peak ion at $m/z$ 74 in the methyl coenzyme M derivative to the intergrated intensity of the $m/z$ 88 base peak ion in the ethyl coenzyme M in each of these samples with the ratios of samples containing known amounts of coenzyme M, it was possible to quantitate the amount of coenzyme M produced.

*Synthesis and Characterization of Sulfopyruvate.* After 0.84 g (5 mmol) of bromopyruvic acid was dissolved in 5 mL of water, 1.26 g (10 mmol) of sodium sulfite was added with stirring. [Sodium hydroxide (2 M) was added to keep the pH to less than 8.1.] After 10 min, all the sulfite dissolved; after an additional 1.5 h, the solution was diluted with 5 volumes of water, and the sodium ions were removed by passing the solution through a column of Dowex 50W-X8 $H^+$. The resulting solution was treated with a freshly prepared suspension of silver hydroxide (5 mmol) in water, and the resulting silver bromide was removed by filtration. After concentration of the solution to half of its original volume in vacuo at 80 °C,

0.9 g (5 mmol) of dicyclohexylamine dissolved in 3 mL of ethanol was added and the solution concentrated to dryness. The solid residue was dissolved in warm acetone, filtered, and crystallized by the addition of diethyl ether. After three recrystallizations from acetone, 600 mg of colorless crystals was obtained. The crystals melted at 143–145 °C and had infrared absorbances at 2950, 2825, 1730, 1460, 1220, and 1040 $cm^{-1}$ when pressed into a KBr pellet. Passing a sample of the salt dissolved in methanol down a column of Dowex 50W-X8 $H^+$ in methanol and treatment of the effluent dissolved in a small volume of methanol with $CH_2N_2$ in ether gave the diazomethane reaction products b and d shown in Figure 3.

*Synthesis of S-Ethyl Coenzyme M.* Ammonium 2-(ethylthio)ethanesulfonate was prepared by alkylation of coenzyme M with ethyl iodide as described by Gunsalus et al. (1978).

## Results and Discussion

Attempts to establish the conversion of phosphoenolpyruvate (PEP) and bisulfite or sulfopyruvate into coenzyme M using crude cell-free extracts of *Methanobacterium formicicum* were complicated by the presence of large amounts of anionic compounds which interfered with the GC–MS analyses. Often, the coenzyme M derivative could not be positively identified in the GC–MS data, and in those cases where it could be identified, establishing that new synthesis had actually occurred was made difficult because of the large amount of coenzyme M present in the extracts at the start of the incubation. Although the identity of these interfering compounds was not established, they appear to consist, in part, of a series of polycarboxylic acids which elute off the Dowex 1 column with the sulfonic acids. This work with the crude cell-free extracts did, however, establish the formation of sulfolactic acid from PEP and bisulfite. The sulfolactic acid was isolated and identified as the methyl ether dimethyl ester derivative (spectrum c, Figure 3).

These analytical problems were finally avoided by removing the small molecular weight molecules from the crude extracts prior to their incubation with the substrates. This was accomplished by separating the extracts on a Sephacryl S-200 column (Figure 2). Those fractions containing the maximum specific activity for the conversion of sulfoacetaldehyde into coenzyme M were found to be free of coenzyme M and the anionic compounds which interfered with the GC-MS analysis; thus, any coenzyme M found after incubation with substrates had to result from newly biosynthesized coenzyme M.

The conversion of PEP, bisulfite, and cysteine into coenzyme M was readily demonstrated by using extracts fractionated on the Sephacryl S-200 column (Table I). The total amount of coenzyme M produced per milligram of protein (11.3 nmol) was about 20% of that previously observed for the conversion of sulfoacetaldehyde and cysteine into coenzyme M by cell-free extracts of *Methanobacterium formicicum* (R. H. White, unpublished results). The presence of the coenzyme M derivative was confirmed in each experiment by both its GC retention time and its mass spectrum. Of equal importance is the identification, by GC–MS, of three of the proposed intermediates in the conversion of PEP into coenzyme M, i.e., sulfolactic acid, sulfopyruvic acid, and sulfoacetaldehyde. These compounds were isolated from the incubation mixtures along with the methyl and ethyl coenzyme M derivatives and identified as the derivatives shown in Figure 3. The gas chromatographic traces (not shown) of these isolated sulfonic acids were very clean in that all of the major peaks observed originated from a combination of the derivatives of sulfolactic acid, sulfopyruvic acid, and sulfoacetaldehyde and/or methyl coenzyme M and ethyl coenzyme M. Neither coenzyme M nor any intermediate was observed in control experiments that contained all of the substrates and either no extract or boiled extract. Likewise, none of these products were found to be produced when pyruvate was substituted for PEP, indicating that PEP and not pyruvate combines with bisulfite to form sulfolactic acid (Table I).

If the conversion of PEP into coenzyme M is as outlined in Figure 1, then we would expect each of the indicated intermediates to be converted into coenzyme M by the cell-free extracts. The first intermediate, sulfolactic acid, was shown to convert into coenzyme M when fed to growing, whole cells of *Methanobacterium formicicum* (White, 1985); the second intermediate, sulfopyruvate, was shown to convert into coenzyme M in that 4.4 nmol of coenzyme M/mg of protein was produced when extracts were incubated with sulfopyruvate (Table I). This conversion was found to be stimulated by the addition of cysteine to the incubation mixture, which was expected since cysteine has been shown to react with sulfoacetaldehyde to form a thiazolidine adduct which is then converted into coenzyme M via sulfoethylcysteine (R. H. White, unpublished results). A large amount of sulfopyruvate was identified in these experiments since this material was added as the substrate. In addition, sulfoacetaldehyde was found along with a small amount of sulfolactic acid which was formed by the reduction of the sulfopyruvate during the preparation of the derivatives. The production of coenzyme M was not observed unless the substrates were incubated in the presence of an extract.

The above results provide a logical metabolic origin for the reduced thiol sulfur in coenzyme M; i.e., it originates from cysteine which is derived from sulfide and serine (White, 1985). The cysteine sulfur can be derived directly from either the sulfide or elemental sulfur in the growth medium since either of these two sulfur sources can serve as the sole source of sulfur for the growth of the methanogenic bacteria *M. thermoautotrophicum* and *Methanobacterium* strain ivanov (Bhatnagar et al., 1984).

The question then arises as to the origin of the oxidized bisulfite sulfur used in the biosynthesis of the sulfonic acid portion of coenzyme M. In these experiments, this sulfur could not be taken up as bisulfite or some intermediate oxidation state of sulfur other than sulfate, i.e., thiosulfate, bisulfite, or dithionate, since these are not present in the medium. Moreover, in experiments where they were added to the medium, they were found to strongly inhibit cell growth (Bhatnagar et al., 1984; R. H. White, unpublished results). Sulfate, on the other hand, which is present in the medium, neither supports the growth of methanogenic bacteria (Bhatnagar et al., 1984) nor is it incorporated into coenzyme M by growing cells of a methanogenic bacteria (White, 1985). These results indicate that methanogenic bacteria are not able to assimilate and reduce inorganic sulfate. This, in turn, indicates that they contain neither adenylyl sulfate (APS) nor 3′-phosphoadenylyl sulfate (PAPS) and are thus unable to form bisulfite by the reduction of APS or PAPS or by the transfer of a sulfate group from APS to a thiol receptor catalyzed by APS:thiol sulfotransferase (Schmidt et al., 1974). [The reduction of APS and of PAPS is catalyzed by adenyl sulfate reductase (Dickerson & Timkovich, 1975) and PAPS reductase (Fujimoto & Ishimoto, 1961; Wilson et al., 1961; Pasternak et al., 1965), respectively.] Thus, cells of methanogenic bacteria must generate bisulfite by the oxidation of sulfide. This oxidation can be accomplished by the $P_{590}$ enzyme isolated from *M. barkeri* which was shown to possess sulfite reductase activity

by Moura et al. (1982). Since the average redox potential for the six-electron transfer in the sulfite reductase reaction is −120 mV (Wagner et al., 1974), then the reverse reaction, i.e., the oxidation of sulfide to bisulfite, could readily occur under physiological conditions if an appropriate electron acceptor is present. This reverse functioning of $P_{590}$ was, in fact, proposed by Moura et al. (1982) as the means of providing the metabolic source of the oxidized sulfur used in the biosynthesis of coenzyme M. This same reverse functioning was postulated for the siroheme sulfite reductase which was isolated from *Thiobacillus dentrificans*, a facultative anaerobe that oxidizes sulfide or sulfur in the absence of molecular oxygen (Aminuddin & Nicholas, 1973; Schedel & Truper, 1979).

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**Registry No.** PEP, 138-08-9; bisulfite, 15181-46-1; L-cysteine, 52-90-4; sulfolactic acid, 38769-05-0; sulfoacetaldehyde, 32797-12-9; sulfopyruvic acid, 98022-26-5; coenzyme M, 45127-11-5.

REFERENCES

Aminuddin, M., & Nicholas, D. J. D. (1973) *Biochim. Biophys. Acta 325*, 81–93.

Balch, W. E., & Wolfe, R. S. (1979) *J. Bacteriol. 137*, 256–263.

Bhatnagar, L., Henriquet, M., Zeikus, J. G., & Aubert, J.-P. (1984) *FEMS Microbiol. Lett. 22*, 155–158.

Caserio, M. C., Roberts, J. D., Neeman, M., & Johnson, W. S. (1958) *J. Am. Chem. Soc. 80*, 2584–2585.

Dickerson, R. E., & Timkovich, R. (1975) *Enzymes (3rd Ed.)*, 395–547.

Eirich, L. D., Vogels, G. D., & Wolfe, R. S. (1978) *Biochemistry 17*, 4583–4593.

Escalante-Semerena, J. C., Leigh, J. A., & Wolfe, R. S. (1984) in *Microbial Growth on $C_1$ Compounds* (Crawford, R. L., & Hanson, R. S., Eds.) pp 191–198, American Society for Microbiology, Washington, DC.

Fujimoto, D., & Ishimoto, M. (1961) *J. Biochem. (Tokyo) 50*, 535–537.

Gunsalus, R. P., Romesser, J. A., & Wolfe, R. S. (1978) *Biochemistry 17*, 2374–2377.

Heywood, A., Mathias, A., & Williams, A. E. (1970) *Anal. Chem. 42*, 1272–1273.

Lovley, D. R., White, R. H., & Ferry, J. G. (1984) *J. Bacteriol. 160*, 521–525.

Moura, J. J. G., Moura, I., Santos, H., Xavier, A. V., Scandellari, M., & LeGall, J. (1982) *Biochem. Biophys. Res. Commun. 108*, 1002–1009.

Müller, E., & Rundel, W. (1958) *Angew. Chem. 70*, 105.

Nelson, M. J. K., & Ferry, J. G. (1984) *J. Bacteriol. 160*, 526–532.

Pasternak, C. A., Ellis, R. J., Jones-Mortimer, M. C., & Crichton, C. E. (1965) *Biochem. J. 96*, 270–275.

Schauer, N. L., & Ferry, J. G. (1982) *J. Bacteriol. 150*, 1–7.

Schedel, M., & Truper, H. G. (1979) *Biochim. Biophys. Acta 568*, 454–467.

Schmidt. A., Abrams, W. R., & Schiff, J. A. (1974) *Eur. J. Biochem. 47*, 423–434.

Shapiro, S., & Wolfe, R. S. (1980) *J. Bacteriol. 141*, 728–734.

Simmonds, P. G., Pettitt, B. C., & Zlatkis, A. (1967) *Anal. Chem. 39*, 163–167.

Taylor, C. D., & Wolfe, R. S. (1974) *J. Biol. Chem. 249*, 4879–4885.

Wagner, G. C., Kassner, R. J., & Kamen, M. D. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 253–256.

White, R. H. (1985) *Biochemistry 24*, 6487–6493.

Wilson, L. G., Asahi, T., & Bandurski, R. S. (1961) *J. Biol. Chem. 236*, 1822–1829.