

# Component C of the Methylreductase System of *Methanobacterium*\*

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Component C of the methyl coenzyme M methylreductase system of *Methanobacterium thermoautotrophicum* has been purified to homogeneity with a 17% recovery of initial units. The native protein has a molecular weight of 300,000 and is composed of three different subunits with masses of 68,000, 45,000, and 38,500. They are present in equal proportion, suggesting a stoichiometry of  $\alpha_2\beta_2\gamma_2$  in the native protein. The amino acid composition reveals a preponderance of acidic amino acid residues. The protein is yellow, having an absorption maximum at 425 nm and a shoulder at 455 nm. Reconstitution of the methyl coenzyme M methylreductase activity was linearly dependent on added component C. Component C has been detected in cell extracts of other methanogens.

The terminal step of methane formation requires the participation of three components: component A, an oxygen-sensitive,  $M_r = 500,000$  protein complex having hydrogenase activity; component B, an oxygen-sensitive, colorless cofactor; and component C, an oxygen-stable acidic protein (1). Component C has been identified recently as the 2-(methylthio)ethanesulfonic acid methylreductase (2). In the presence of  $Mg^{2+}$ , ATP, and hydrogen, these components reduce  $CH_3-S-CoM$  to methane and  $HS-CoM$ . Here we report the purification and physical properties of component C; a preliminary report in abstract form has appeared (3).

## EXPERIMENTAL PROCEDURES<sup>2</sup>

**Materials** - Trizma base, Tes, Coomassie R250, dansyl chloride, dansylated amino acid standards, dextran sulfate, and all protein standards were purchased from Sigma. Cheng-Chin polyamide plates were obtained from Pierce. Acrylamide, N,N'-methylenebisacrylamide and SDS were purchased from BioRad. DEAE-cellulose (DE52) was the product of Whatman; all other chromatographic gels were obtained from Pharmacia. Gases used in these studies were obtained from Linde, Division of Union Carbide.

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<sup>1</sup> The abbreviations used are:  $CH_3-S-CoM$ , 2-(methylthio)ethanesulfonic acid;  $HS-CoM$ , 2-mercaptoethanesulfonic acid; Tris base, tris(hydroxymethyl)aminomethane; Tes, N-[tris(hydroxymethyl)methyl-2-amino]ethanesulfonic acid; Tea, triethanolamine; SDS, sodium dodecyl sulfate; DEAE-cellulose, diethylaminoethylcellulose; dansyl, 5-dimethylaminonaphthalene-1-sulfonyl.

<sup>2</sup> Portions of this paper (including "Experimental Procedures," Figs. 1 to 8, and Table I) are presented in miniprint as prepared by the author. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 80M-2314, cite author(s), and include a check or money order for \$4.80 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

**Growth of Organisms** - *Methanobacterium thermoautotrophicum* was grown at 55° C on hydrogen and  $CO_2$  as energy and carbon sources (4). All other organisms were grown according to published procedures (5) and were the gift of John Leigh.

**Enzyme Assay** - Aliquots from each column fraction were assayed for the presence of component C by combination with saturating quantities of components A and B in the standard methylreductase assay (1). When aerobic fractions were assayed for component C activity, the reaction mixture and aerobic enzyme were flushed with oxygen-free  $H_2$  for 40 min prior to the addition of oxygen labile components. The specific activity of component C at various stages of purification was determined only after dialysis at 5° C against 250 volumes of 50 mM Tris-Cl at pH 7.2 containing 0.1 M NaCl and 10 mM 2-mercaptoethanol. Specific activity is expressed as  $\mu mol\ CH_4$  produced  $mg^{-1}\ h^{-1}$ .

**Protein Determination** - The protein concentration of each column fraction was estimated by measuring the turbidity of a sample in 20% trichloroacetic acid at 400 nm. More accurate protein determinations were made according to Lowry et al. (6). Bovine serum albumin was used as a standard.

**Conductivity Measurements** - The salt concentration of each column fraction was estimated with a Radiometer conductivity meter (Copenhagen, Denmark) that had been calibrated with appropriate standards.

**Electrophoresis** - Nondissociating polyacrylamide gel electrophoresis was performed routinely with the Tea-Tes buffer system of Orr et al. (7) or in some cases, by the method of Davis (8). Sodium dodecyl sulfate gel electrophoresis of protein was performed using the buffer system of Laemmli (9). In some cases the sodium phosphate system of Weber and Osborn was substituted (10). Proteins in all gels were stained with Coomassie Blue R250 as outlined by Fairbanks et al. (11).

**Molecular Weight Determination** - The molecular weight of the native protein was determined electrophoretically by the method of Hedrick and Smith (12) using the buffer system of Orr et al. (7). The ratio of acrylamide to N,N'-methylenebisacrylamide was maintained at 38:1. Standards used for calibration were ovalbumin ( $M_r$  43,500), bovine serum albumin ( $M_r$  68,000), aldolase ( $M_r$  160,000), catalase ( $M_r$  250,000) and apoferritin ( $M_r$  450,000). Verification of the molecular weight of component C was obtained by gel filtration on Sephadex G200 (13). The column (1.0 cm x 90 cm) was equilibrated with 50 mM Tris-Cl at pH 7.2 containing 0.2 M NaCl and 10 mM 2-mercaptoethanol. The column was calibrated with the following standards: ovalbumin, bovine serum albumin, yeast alcohol dehydrogenase ( $M_r$  150,000), catalase and Jack bean urease ( $M_r$  483,000). After calibration pure component C was passed through the column, and the elution of this protein was determined both by absorbance at 280 nm and by activity in the methylreductase assay.

Molecular weights of the subunits of component C were determined by SDS gel electrophoresis as described above. The data were treated according to the method of Weber and Osborn (10) with the following standards: bovine serum albumin; ovalbumin; and  $\alpha$ -chymotrypsinogen A ( $M_r$  25,000). Samples were reduced and denatured by boiling in the SDS sample buffer of Laemmli (9) for 5 min followed by incubation at 37° C overnight.

**Sedimentation Velocity Centrifugation** - A 200  $\mu g$  sample of pure component C in 10 mM potassium phosphate buffer (pH 7) containing 100 mM NaCl and 10 mM 2-mercaptoethanol was centrifuged at 40,000 rpm for 2 h at 20° C in a Beckman Model E ultracentrifuge. The progress of the moving boundary was followed with a photoelectric scanner. The data for determination of the  $s$  value were treated as outlined by Schachman and Edelstein (14). The  $s$  value was corrected to water at 20° C (15).

**Amino Acid Composition** - The amino acid composition of component C was determined after hydrolysis with 6 N HCl at 110° C in an evacuated, sealed ampule (16). Each ampule contained 200  $\mu g$  of component C; hydrolysis times of 24, 48, 72 and 96 h were used in this study. Cysteine was determined as cysteic acid after hydrolysis for 24 h in 6 N HCl containing dimethylsulfoxide (17); aspartic acid and alanine served as internal controls of recovery in this case. Values obtained were corrected for slow hydrolysis of acid labile amino acids.

**N-Terminal Analysis** - Individual subunits of component C were separated by SDS gel electrophoresis and then extracted from the appropriate portions of gel by the method of Weiner et al. (18) to yield isolated subunits. As described by Gros and Labouze (19), each subunit was treated with dansyl chloride and then hydrolyzed in 5.5 M HCl to release the dansylated amino acids. Separation and identification of the dansylated amino acids was carried out on polyamide sheets with suitable standards according to Gray (20).

**Preparation of Antibodies to Component C** - Antibodies to component C were elicited in mice (21). Control sera were prepared by the injection of Freund's complete adjuvant and saline (9:1, vol/vol) alone. The  $\gamma$  fraction was further purified by Dextran sulfate precipitation (22) followed by three precipitations with ammonium sulfate at 5° C (23).

**Immunodiffusion** - Representative samples (5  $\mu l$ ) of various cell-free extracts from different methanogens were allowed to diffuse for 24 h at 20° C toward antibodies directed against purified component C. Nonprecipitated protein was removed from the plate by washing for 48 h with 4 liters of 0.05 M Tris-Cl, pH 8.6 containing 0.9% NaCl. The plate was stained with Coomassie blue as described above.

**Immunoreplicate Electrophoresis** - Immunoreplicate electrophoresis was performed as described by Shove et al. (24) with the following modifications: (i) aliquots of various methanogen cell-free extracts and purified component C were first separated on a 10% polyacrylamide slab with the Tea-Tes buffer system described above; and (ii) the polyacrylamide slab was placed on a 1% agarose slab containing antibodies against purified component C. The overlay was allowed to develop for 24 h at 37° C in a humid environment. After this time the polyacrylamide slab was removed and stained for protein. The agarose gel was washed to remove nonprecipitated proteins, pressed, stained for protein and compared with the polyacrylamide gel.

**Purification of Component C** - Cell-free extracts of *M. thermoautotrophicum* were prepared as described previously (1). The anaerobic extract was applied to a DEAE-cellulose (DE52, Whatman) column (2.5 x 20 cm) equilibrated anaerobically with 0.05 M Tris-Cl, pH 7.0 containing 10 mM 2-mercaptoethanol (buffer A). Component C was eluted in a batchwise manner (2). Fractions containing active component C were pooled and concentrated anaerobically to 15 ml by ultrafiltration (PM 10 membrane). All steps hereafter were performed anaerobically in the cold (5° C). Component C was further purified through the use of gradient sievesorptive chromatography (25).

A DEAE-Sephadex A-25 column (2.5 cm x 58 cm) was thoroughly equilibrated with 0.1 M NaCl in buffer A. Prior to the application of the sample, an 80 ml linear gradient from 0.1 to 0.4 M NaCl in buffer A was loaded onto the column. Next the concentrated sample of component C, whose conductivity was adjusted with solid NaCl to be equal to 0.8 M NaCl in buffer A, was loaded. The column was washed finally with 1.0 M NaCl in buffer A at a rate of 10 ml  $h^{-1}$ . Fractions (5 ml) were collected. Each fraction was assayed for protein, salt concentration, and component C activity. Fractions containing component C were pooled, concentrated anaerobically and salt adjusted as described above.

The concentrated sample of component C was applied to a DEAE-Sephadex A-25 column (2.5 cm x 65 cm) which had been thoroughly equilibrated with 0.16 M NaCl in buffer A. After application of the sample, the column was washed with 1.0 M NaCl in buffer A. Conductivities, protein concentrations and component C activity were monitored as described above.

A third DEAE-Sephadex A-25 column (2.5 cm x 65 cm) was equilibrated with 0.2 M NaCl in buffer A. Prior to sample application, a 90 ml linear gradient from 0.2-0.3 M NaCl in buffer A was loaded on the column. The concentrated sample of component C was applied and eluted as described previously. Peak fractions contained pure component C as judged by nondissociating gel electrophoresis. Occasionally a peak fraction from this column was slightly contaminated with other proteins and required rechromatography on DEAE-Sephadex A-25 to achieve final purification as described for the third DEAE-Sephadex A-25 column.

## RESULTS

**Purification of Component C**—Component C of the  $\text{CH}_3\text{-S-CoM}$  methylreductase system of *Methanobacterium thermoautotrophicum* was purified to homogeneity as judged by its behavior upon nondissociating gel electrophoresis and sedimentation velocity centrifugation. As shown in Fig. 1 when component C was subjected to electrophoresis on polyacrylamide gels of varying porosity at either pH 7 or pH 9, only one major band was detected on gels stained for protein. Sedimentation velocity centrifugation of component C revealed a single, symmetrical sedimenting-boundary with a corrected  $s$  value of 15.05.

The purification scheme employed for the purification of component C relied heavily upon the use of gradient sievesorptive chromatography (25). Through the use of this chromatographic method, it was possible to remove extraneous proteins and yet maintain an active component C. Conventional methods of protein purification were either ineffective or fatal to the purification of component C. The progress of one representative purification is summarized in Table I, and an example of gradient sievesorptive chromatography as applied to purification of component C is shown in Fig. 2. The purified protein was enriched only 8.3-fold over cell-free extract with a recovery of 17% of the initial units. The protein has been purified both aerobically and anaerobically without any pronounced differences in activity or physical properties. The purified protein was stable for months on ice.

**Physical Properties of Component C**—The molecular weight of purified component C has been determined by two methods to be approximately 300,000. When the data obtained from Fig. 1 were plotted with those obtained for suitable standards according to the method of Hedrick and Smith (12), an  $M_r$  of 330,000 was found for component C (Fig. 3). Similarly, chromatography of component C on a calibrated Sephadex G-200 column gave a  $M_r = 300,000$  (Fig. 4).

The amino acid composition of component C has been examined; based on an  $M_r$  of 300,000 the moles of amino acid/mol of protein values were: Asp, 272; Thr, 126; Ser, 117; Glu, 347; Pro, 149; Gly, 259; Ala, 272; Val, 214; Met, 60; Ile, 138; Lys, 45; Tryp, not determined; Cys, 29. The most unusual feature of the amino acid composition was a 2:1 preponderance

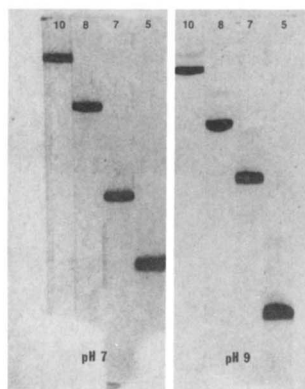


FIGURE 1. Analytical Polyacrylamide Gel Electrophoresis of Component C.

Purified component C (10  $\mu\text{g}$ ) was subjected to polyacrylamide gel electrophoresis as described in "Experimental Procedures" at either pH 7 or at pH 9. In each case the gel porosities were 10%, 8%, 7% and 5% polyacrylamide.

TABLE I  
Purification of Component C From *Methanobacterium*

Step or Treatment	Volume (ml)	Total Protein (mg)	Specific Activity ( $\mu\text{mol mg}^{-1} \text{h}^{-1}$ )	Recovery (%)
1. Cell-free extract	146	2493	1.2	100
2. DEAE-cellulose	16	871	2.3	81
3. DEAE Sephadex A25	4.3	277	4.2	47
4. DEAE Sephadex A25	3.8	136	4.6	25
5. DEAE Sephadex A25	2.5	50	10.0	16.7

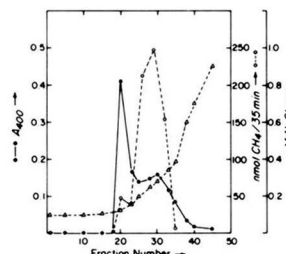


FIGURE 2. Gradient Sievesorptive Chromatography of Component C.

Crude component C from DEAE-cellulose column (16 ml) was chromatographed on a DEAE-Sephadex A-25 column containing an 80 ml preformed gradient from 0.1M - 0.4M NaCl in buffer A as described in "Experimental Procedures." Component C activity was determined on a 25  $\mu\text{l}$  aliquot of each fraction in the standard methyl reductase assay.

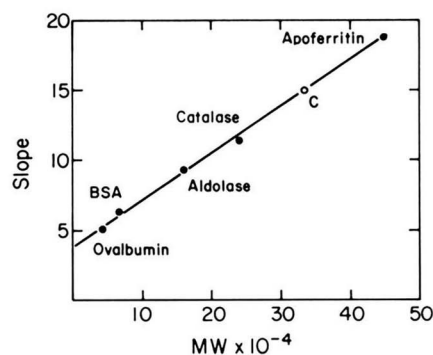


FIGURE 3. Molecular Weight Determination of Component C by Polyacrylamide Gel Electrophoresis.

The relative mobility of component C and various proteins of known molecular weight to bromophenol blue were determined by electrophoresis on 10, 8, 7 and 5% polyacrylamide gels at pH 7. The slope obtained for each protein was plotted according to the method of Hedrick and Smith (12). The standards are indicated.

of acidic amino acids to basic amino acids, a fact supporting the observation that the protein complex bound rather tightly to anionic ion exchange resins. The ratio of polar to nonpolar amino acids was 1.36, and  $\bar{p}$  calculated from the amino acid composition was 0.71.

The subunit composition of component C after SDS-gel electrophoresis is shown in Fig. 5. The native protein is composed of three different subunits:  $M_r = 68,000$ ,  $M_r = 45,000$ , and  $M_r = 38,500$ . The estimated molecular weight of the two smaller subunits varied slightly, i.e.  $\pm 3,000$ . The fact that each subunit was present in equal molar amount in component C is supported by densitometer scans. Graphical integration of each peak area gave the following molar subunit stoichiometries on a total mass basis: 2.1 for the  $M_r = 68,000$  subunit; 1.9 for the  $M_r = 45,000$  subunit, and 2.15 for the  $M_r = 38,500$  subunit in the native protein of  $M_r = 300,000$ . Thus, the native protein contains two copies of each subunit.

Evidence to support the presence of three different subunits in the native protein was obtained through  $\text{NH}_2$ -terminal analysis. Component C was separated into its constituent subunits by SDS-gel electrophoresis, the band corresponding to each of the subunits was excised, eluted, and subjected to electrophoresis again to assure its purity. These separated subunits were dansylated, hydrolyzed, and then chromatographed on polyamide sheets with the appropriate standards. Methionine was the  $\text{NH}_2$ -terminal amino acid of the  $M_r = 68,000$  subunit whereas the two smaller subunits contained

alanine at this position. Treatment of the native protein with dansyl chloride followed by hydrolysis and chromatography gave only two spots on polyamide sheets, corresponding to methionine and alanine. The alanine spot had greater fluorescence intensity when viewed under long wavelength ultraviolet light. Thus, only three subunits were found for component C.

The protein had a characteristic nonfluorescent, yellow color, the spectrum of which is shown in Fig. 6. The protein had an absorbance maximum at 425 nm and a shoulder at 455 nm. There were no peaks in the 300 nm region as would be expected for a typical flavin. Over a 2-h incubation period, none of the common reductants such as ascorbate, dithionite, or sodium borohydride or oxidants such as ferricyanide brought about any significant change in the visible spectrum of the protein. Attempts to perturb the visible spectrum of component C by addition of either hydrogenase or hydrogenase and component B were not successful.

**Reconstitution of  $\text{CH}_3\text{-S-CoM}$  Methylreductase System—**The ability of purified component C to reconstitute the  $\text{CH}_3\text{-S-CoM}$  methylreductase system is shown in Fig. 7. The rate of methanogenesis was linearly dependent on added component C over a 5-fold concentration range, i.e. 133 nM to 670 nM. The slight amount of activity seen in the absence of added component C was due to residual enzyme adhering to the much cruder hydrogenase. This was readily demonstrated by immunodiffusion experiments where both hydrogenase and purified component C were allowed to diffuse toward antibodies to component C. The immunoprecipitation line obtained for the hydrogenase fused with that found for the purified protein (data not shown).

**Immunodiffusion: Detection of Component C in Other Methanogens—**Immunodiffusion experiments were undertaken to determine whether antibodies to component C of *M. thermoautotrophicum* would react with extracts of other

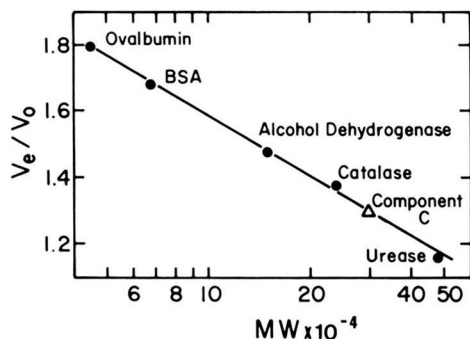


FIGURE 4. Molecular Weight Determination of Native Component C on Sephadex G200.

The molecular weight of component C was determined by gel filtration as described in "Experimental Procedures." Standards are indicated.

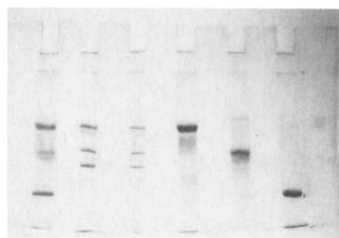


FIGURE 5. SDS Gel Electrophoresis of Component C.

The subunit composition of component C was examined by SDS gel electrophoresis on a 10% polyacrylamide slab as described in "Experimental Procedures." From left to right: lane 1, 5  $\mu\text{g}$  each of bovine serum albumin ( $M_r$  68,000), ovalbumin ( $M_r$  43,500), and  $\alpha$ -chymotrypsinogen-A ( $M_r$  25,000); lane 2, 4  $\mu\text{g}$  of purified component C; lane 3, 2  $\mu\text{g}$  of purified component C; lane 4, 10  $\mu\text{g}$  of bovine serum albumin; lane 5, 10  $\mu\text{g}$  of ovalbumin and lane 6, 10  $\mu\text{g}$  of  $\alpha$ -chymotrypsinogen A.

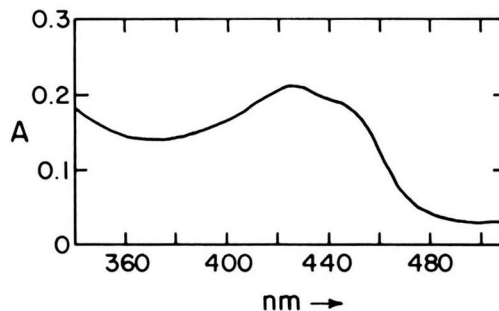


FIGURE 6. Visible Spectrum of Component C.

The visible spectrum of component C (2 mg/ml in 0.05M Tris-Cl, pH 7) was recorded with a Cary 14 Recording Spectrophotometer.

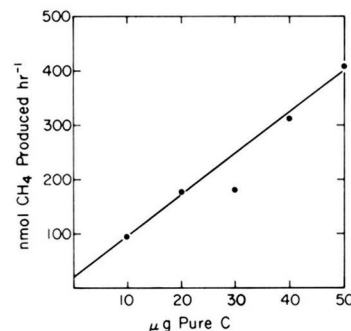


FIGURE 7. Reconstitution of Methylreductase: Rate Dependence on Added Component C.

Each reaction vial contained the standard reaction mixture (Experimental Procedures), 30  $\mu\text{g}$  of pure cofactor B, 0.5 mg of crude component A, and an amount of component C as indicated. Reactions were initiated by transfer to a 60°C water bath; aliquots of the gas phase were assayed for  $\text{CH}_4$ . Initial rates were measured.



FIGURE 8. Immunoreplicate Electrophoresis of Component C and Cell Extracts of Other Methanogens.

Component C and cell extracts of various methanogens were first subjected to polyacrylamide gel electrophoresis (A) and then overlaid on top of an agarose gel containing antibodies to purified component C to obtain the immunoreplicate (B). Both gels were treated as described in "Experimental Procedures." Lanes 1 and 8 contained 2  $\mu\text{g}$  of purified component C; other lanes contained cell extract as indicated: lane 2, *M. thermoautotrophicum* (25  $\mu\text{g}$ ); lane 3, *M. marisnigri* (25  $\mu\text{g}$ ); lane 4, *M. ruminantium* (25  $\mu\text{g}$ ); lane 5, *M. formicicum* (25  $\mu\text{g}$ ); lane 6, *M. hungatei* (25  $\mu\text{g}$ ); and lane 7, *M. bryantii* str. MOHG (25  $\mu\text{g}$ ).

methanogens to produce immunoprecipitation. Clarified cell-free extracts of the following methanogens were prepared: *M. thermoautotrophicum*; *Methanospirillum hungatei*; *Methanobacterium formicicum*; *Methanobacterium bryantii* str. MOHG; *Methanobrevibacter ruminantium* and *Methanogenium marisnigri*. All methanogens tested gave immunoprecipitation lines that fused with component C except *M. marisnigri* and *M. ruminantium* even though these extracts had fully active methylreductase activity.

**Immunoreplicate Electrophoresis—**Because of the tendency of component C to associate with other proteins in cell extracts, it seemed wise to examine the cross-reactivity of these methanogen cell-free extracts after resolution by non-

dissociating gel electrophoresis on a 10% polyacrylamide slab. The results are shown in Fig. 8. Clearly *M. marisnigri* and *M. ruminantium* have protein bands with the same mobility as purified component C, but as described above, these failed to cross-react with antibodies to component C.

#### DISCUSSION

Since component C is an acidic protein, ion exchange chromatography appeared to be a logical technique to use for purification. Chromatography of component C on DEAE-cellulose under a variety of conditions gave negligible purification. The use of the stronger ion exchange resin DEAE-Sephadex A-25 resulted in nearly total inactivation of recoverable component C activity. Poor purification was obtained with Sephacryl S300 or Bio-Gel P200. A method of purification was needed that would remove contaminating proteins and still yield an active component C; gradient sievesorptive chromatography fulfilled these requirements. Furthermore component C emerged from these columns in a concentrated band of activity and its behavior was reproducible from column to column. This technique may be ideal for the purification of other large, multisubunit proteins where stability is a problem.

The low increase in specific activity upon purification of component C is puzzling. However, as seen in Fig. 7, component C is one of the major soluble proteins released upon cell breakage. Whether or not it is 12% of the total protein remains to be seen. It also is possible that one or more of the proteins removed during the purification of component C may play a role in efficiently integrating component C into the methylreductase system. Experiments to clarify this possibility are in progress.

When it became evident in 1967 (26) that the major metabolic system used by most methanogens was an anaerobic respiration in which hydrogen was oxidized and carbon dioxide was reduced to methane, we concluded that ATP synthesis in these organisms must occur by electron transport phosphorylation; ATP pools and the effect of uncouplers were studied (27, 28). Recently, excellent evidence has been presented by Doddema and Vogels (29, 30) as well as by Sauer *et al.* (31, 32) that intact vesicles of methanogens oxidize hydrogen, producing ATP by electron transport phosphorylation with the reduction of carbon dioxide to methane. The membranous vesicles of Sauer *et al.* (32) produced only a slight increase in methane formation when ATP or  $\text{CH}_3\text{-S-CoM}$  were added, and only a fraction of the methyl moieties of added  $\text{CH}_3\text{-S-CoM}$  was converted to methane by these intact vesicles. To us there appears to be a reasonable explanation for these results; the membranous vesicles may represent a highly integrated system in which ATP and  $\text{CH}_3\text{-S-CoM}$  could be generated in nearly saturating amounts either on or inside the membrane environment. Thus, externally added ATP or  $\text{CH}_3\text{-S-CoM}$ , both highly charged molecules, may not penetrate readily to the appropriate enzyme sites in the membrane.

To understand the enzymology of this multi-enzyme, multi-coenzyme system, we have elected to study specific reactions the components of which can be fractionated in solution. (Whether or not these proteins are truly soluble is another question.) We have focused on the  $\text{CH}_3\text{-S-CoM}$  methylreductase, and by providing ATP and  $\text{CH}_3\text{-S-CoM}$  have simplified the system, component C being the first protein to be purified to homogeneity. The native protein as purified contains six subunits having a stoichiometry of  $\alpha_2$ ,  $\beta_2$ , and  $\gamma_2$ . The molec-

ular weight of about 300,000 is slightly greater than twice that reported by Gunsalus and Wolfe (1). Although no evidence of an  $M_r = 135,000$  component C was found during any stage of purification; it is possible that their protein was a trimer composed of  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits. Component C has a distinct nonfluorescent, yellow color which can be attributed to an acid- or heat-extractable chromophore. Neither the structure nor function of this chromophore is known at the present time. Preliminary observations suggest that the chromophore is the nickel-containing factor  $F_{430}$  (33).

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