Component A3 of the Methylcoenzyme M Methylreductase System of *Methanobacterium thermoautotrophicum* ΔH: Resolution into Two Components

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Component A3 of the methylcoenzyme M methylreductase system of *Methanobacterium thermoautotrophicum* (strain ΔH) has been resolved into two fractions. One, named component A3a, was defined as the fraction required along with components A2 and C to produce methane from 2-(methylthio)ethanesulfonate when titanium(III) citrate was used as the sole source of electrons. The second one, named component A3b, was required when H_2 and 7-mercapto-*N*-heptanoyl-*O*-phospho-L-threonine were provided as the dual source of electrons. Component A3a was a large iron-sulfur protein aggregate (M_r 500,000) and is most likely involved in providing electrons at a low potential for the reductive activation of component C.

In *Methanobacterium thermoautotrophicum* (strain ΔH), the enzymatic complex which reductively demethylates 2-(methylthio)ethanesulfonate (methylcoenzyme M, CH_3-S-CoM) to produce CH_4 with H_2 as the source of electrons has been shown to be composed of at least four enzymatic fractions (components A1, A2, A3, and C) (18) and one cofactor, 7-mercapto-*N*-heptanoyl-*O*-phospho-L-threonine (HS-HTP) (3, 20). The properties of the methylreductase system were recently reviewed (25).

Previously we have shown that the titanium(III) citrate (Ti^{III})-driven reductive demethylation of CH_3-S-CoM requires only three enzymatic fractions, components C, A2, and A3 (22). When HS-HTP was used as the reductant to drive the demethylation of CH_3-S-CoM, H_2 was also absolutely required, a likely role being to reductively reactivate component C, the methylreductase enzyme per se (22). *Methanobacterium thermoautotrophicum* contains two hydrogenases. One is able to reduce factor F_{420}, the deazaflavin present in methanogenic bacteria (F_{420}-hydrogenase), as well as the artificial electron acceptor methylviologen (MV). The other one only reduces MV and is called the methylviologen hydrogenase (MV-hydrogenase) (11). In the H_2 + HS-HTP-driven assay with components A2, A3, and C, only the MV-hydrogenase was present (in A3), indicating that it was the hydrogenase involved in the reductive activation of component C. This fraction, purified on the basis of its ability to complement components A1, A2, and C in an H_2-driven assay, had routinely been found to contain the MV-hydrogenase, although purified preparations of MV-hydrogenase did not contain component A3 activity (unpublished data). This led us to believe that the MV-hydrogenase was only one of the constituents of component A3. Use of Ti^{III} as the sole source of electrons bypassed the requirement for H_2, although fraction A3 was still required.

Here we present the resolution of fraction A3 into two fractions. One, component A3a, contained little MV-hydrogenase and complemented components A2 and C in a Ti^{III}-driven assay. The second one, A3b, contained most of the MV-hydrogenase and was required, in addition to components A2, A3, and C, for the H_2 + HS-HTP-driven demeth-

ylation of CH_3-S-CoM. The partial purification and characterization of component A3a are also reported. The names A3a and A3b replace the terms A3 per se and A4, respectively, used previously (P. E. Rouvière, C. H. Kuhner, and R. S. Wolfe, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, abstr. I20, p. 220).

MATERIALS AND METHODS

Abbreviations. The following abbreviations are used: HS-CoM, coenzyme M, 2-mercaptoethanesulfonic acid; CH_3-S-CoM, methylcoenzyme M, 2-(methylthio)ethanesulfonic acid; HS-HTP, 7-mercapto-*N*-heptanoyl-*O*-phospho-L-threonine; (S-HTP)$_2$, the homodisulfide of HS-HTP; CoM-S-S-HTP, the mixed disulfide of HS-CoM and HS-HTP; F_{420}, (*N*-L-lactyl-*L*-glutamyl)-L-glutamic acid phosphodiester of 7,8-didemethyl-8-hydroxy-5-deazariboflavin-5′-phosphate; CN-Cbl, cyano-cob(III)alamin; Ti^{III}, titanium(III) citrate; DEAE, diethylaminoethyl; buffer I, 20 mM potassium phosphate (pH 7.0) plus 10 mM 2-mercaptoethanol; MV, methylviologen; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid).

Growth of cells and extract preparation. *Methanobacterium thermoautotrophicum* ΔH was grown in a 200-liter fermentor as described before (9). The cell extract was prepared as described before (18).

Preparation of (S-HTP)$_2$, HS-HTP, and Ti^{III}. The disulfide of HS-HTP [(S-HTP)$_2$] and HS-HTP were synthesized as described previously (19). HS-HTP was stored at −20°C under N_2. Ti^{III} was prepared from titanium chloride and sodium citrate as described before (6) and kept under an N_2 atmosphere.

Preparation of enzymatic fractions. Enzyme purification steps were performed anaerobically as described before (10). Homogenous component C was prepared by ion-exchange chromatography on DEAE-cellulose as described before (23), followed by hydrophobic interaction chromatography on phenyl-Sepharose CL-4B equilibrated in 1 M potassium acetate in buffer I (D. P. Nagle, Jr., and R. S. Wolfe, Abstr. Annu. Meet. Am. Soc. Microbiol., 1983, I14, p. 142). Additional purification was attained by the same technique, but the component C pool was brought to 2 M potassium acetate concentration and loaded onto a phenyl-Sepharose

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resin equilibrated with 2 M potassium acetate in buffer I. Under these conditions, component C did not bind to the resin. Component A2 was purified by affinity chromatography on ATP-agarose as described before (23). It was further purified by anion-exchange chromatography on a Mono Q HR 10/10 column with a fast protein liquid chromatography apparatus (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.) (22). The resolution of the other A components is described in the Results section.

Methanogenic assays. Assays were performed in calibrated, stoppered vials as described before (23). Air-stable components (PIPES, pH 6.3, 16 μmol; CH_3-S-CoM, 0.5 μmol; Mg^{2+}, 4 μmol; ATP, 0.8 μmol; CN-Cbl, 17 nmol) were added to the reaction vials before transfer into the anaerobic chamber, where air-sensitive components (enzymatic fractions and Ti^{III}, 1.7 μmol; $(S-HTP)_2$, 5 nmol; HS-HTP, 0.5 μmol) were added as required. The volume of the reaction mixture was 200 μl. The Ti^{III}-driven methane assay required the presence of components A2, A3a, and C (22) and therefore could be used to detect component A3a with components A2 and C used as reagents. Since this assay could also detect "active" component C (3), which did not require any other enzyme components to produce methane from CH_3-S-CoM and HS-HTP (7), bathophenanthroline disulfonate (BPDS), which specifically inhibited component A3a, could be used to eliminate the ambiguity of the assay (see Results).

Hydrogenase assays. F_{420}- and MV-hydrogenases were assayed spectrophotometrically in temperature-controlled sealed anaerobic cuvettes. Each cuvette contained 3 ml of 100 mM potassium acetate in buffer I (MV-hydrogenase assay) or 2 M potassium acetate in buffer I (F_{420}-hydrogenase assay). An enzymatic fraction was added to each cuvette in the anaerobic chamber, and the cuvettes were sealed. The headspace of each cuvette was flushed with O_2-free H_2 for 3 min, and the cuvette was incubated at 60°C. The reaction was started by the addition of anoxic solutions of oxidized F_{420} or MV. Activities were calculated from extinction coefficients of 40,000 M^{-1} at 420 nm and 11,300 M^{-1} at 601 nm for F_{420} and MV, respectively.

Anaerobic preparative polyacrylamide gel electrophoresis. Component A3a was subjected to preparative electrophoresis on a 1-mm-thick nondenaturing discontinuous polyacrylamide slab gel which was prepared as described before (14). The acrylamide concentration in the stacking gel and in the resolving gel was 5%. Once polymerized, the gel was transferred into the anaerobic chamber. The Tris-glycine running buffer at pH 8.8 was made anaerobic by being sparged with O_2-free nitrogen and by adding 2-mercaptoethanol to a concentration of 10 mM. Protein samples (1 to 2 mg) to be loaded on the gel were mixed with 400 μl of the following solution: Tris hydrochloride at pH 6.8, 0.5 M; glycerol, 50%; and $Na_2S_2O_4$, 100 mM. Since bromophenol blue or pyronin Y turned colorless upon reduction by dithionite, the electrophoretic front was monitored by the migration of small brown iron-sulfur proteins or the utilization of Fe^{2+} chelated with BPDS placed in a well next to the protein sample. Once the electrophoresis was completed, the gel was sliced horizontally into 0.5-cm strips. Each strip was crushed in about 1.5 ml of buffer I containing 100 mM potassium acetate and placed in a 20-ml air-tight serum vial under an N_2 atmosphere. Proteins were allowed to diffuse out of the gel by gentle stirring at 4°C overnight. Component A3a activity was detected by the Ti^{III}-driven methane assay.

Molecular weight determination of component A3a. The molecular weight of component A3a was estimated by gel filtration on a Sephacryl S300 column (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.) (1.5 by 90 cm) which was equilibrated with buffer I containing 100 mM potassium acetate and developed anaerobically at a flow rate of 10 ml h^{-1}. The column was calibrated with 1 ml of a solution of each standard protein at a concentration adjusted to give an A_{280} of 10. The protein standards and their molecular weights were: horse heart cytochrome c, 13,000; bovine pancreas α-chymotrypsinogen A, 24,000; chicken ovalbumin, 45,000; bovine serum albumin, 66,000; yeast alcohol dehydrogenase, 150,000; bovine catalase, 250,000; *Methanobacterium thermoautotrophicum* component C, 300,000; horse spleen ferritin, 443,000; bovine thyroglobulin, 669,000; and blue dextran, 2,000,000. Component A3a (30 mg) obtained from the phenyl-Sepharose chromatography (see below) was loaded onto the column, and A3a activity was detected with the Ti^{III}-driven methane assay.

Inhibition of component A3a by air. The sensitivity of the individual components to air was tested as follows. In a three-component, Ti^{III}-driven methane assay, one of the fractions A2, A3a, or C was dispensed with the air-stable cofactors and exposed to air for 5 min. Then the vials were transferred into the anaerobic chamber, where the other components were added. Each component was added as described in Table 1, footnote a. The methane assays were performed as described above.

Inhibition of component A3a by BPDS. BPDS was added to the methane assay vials with the air-stable components. To test inhibition of an enzymatic component by BPDS, the component was incubated anaerobically with BPDS for 10 min at 60°C; then, 20 μl of a 25 mM anoxic $Fe(NH_4)_2SO_4$ solution was added anaerobically to the vial to trap any unreacted BPDS. Other protein components and Ti^{III} were then added anaerobically with a syringe, and the methane assay was performed as described above.
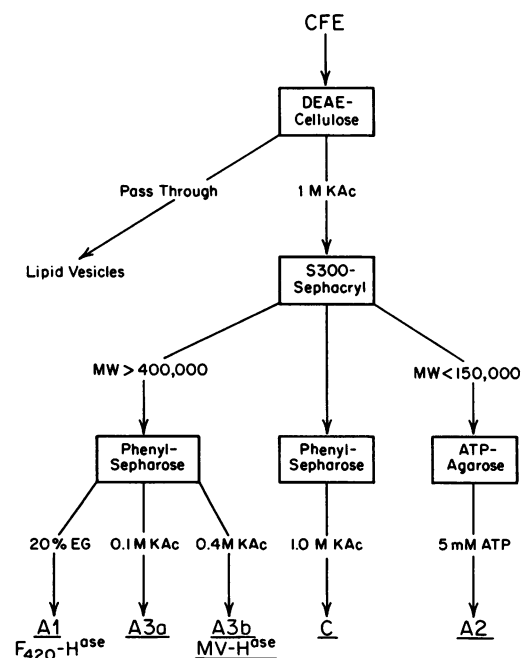

FIG. 1. Purification scheme for the resolution of the methyl-coenzyme M methylreductase system into five components. Abbreviations: CFE, cell-free extract; KAc, potassium acetate; MW, molecular weight; EG, ethylene glycol; H^{ase}, hydrogenase.
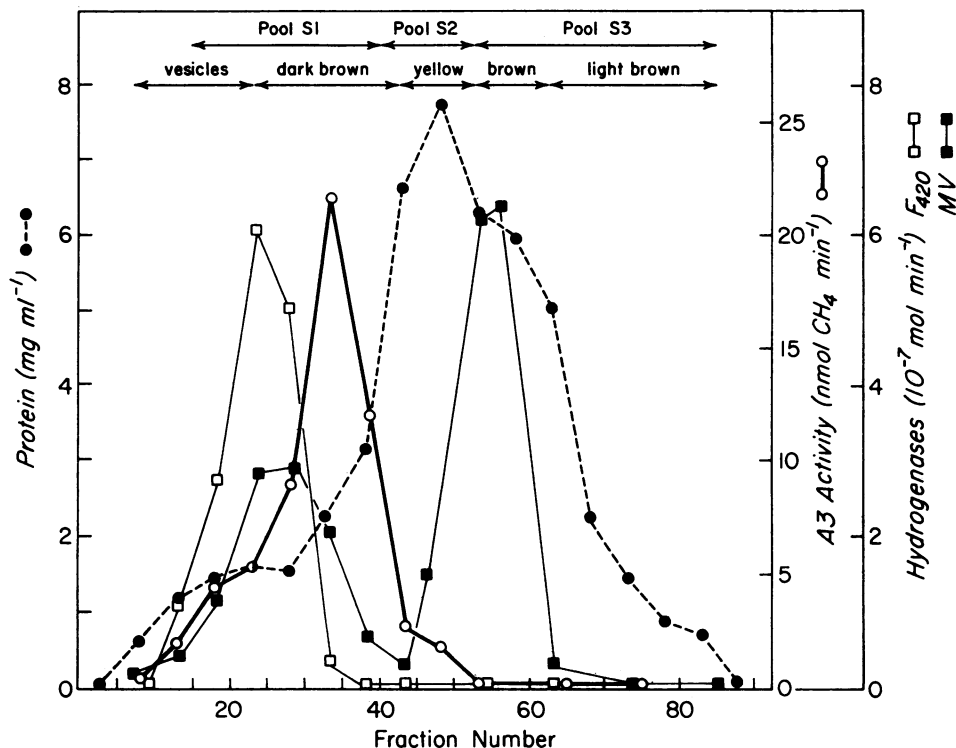
FIG. 2. Fractionation of the methylreductase system by Sephacryl S300 gel filtration. A total of 1,900 mg of the active pool from the DEAE-cellulose column (35 mg ml^{-1}) was loaded on a column (5 by 55 cm) equilibrated with buffer I containing 100 mM potassium acetate. The flow rate was 40 ml h^{-1}, and 7-ml fractions were collected. Component A3, MV-hydrogenase, and F$_{420}$-hydrogenase were assayed as described in Materials and Methods. Component C was detected by polyacrylamide gel electrophoresis. Fractions were pooled as shown (pools S1 to S3) and concentrated anaerobically by ultrafiltration on a PM30 membrane. Pool S1 contained A1, A3a, and A3b activities, pool S2 contained component C, and pool S3 contained component A2.

RESULTS

Fractionation of components A1 and A3. The scheme for the resolution of the different components of the methylreductase complex is shown in Fig. 1. Cell extract (8 g of protein) was applied to an 800-ml DEAE-cellulose column (10 by 12 cm) to separate soluble enzymes from lipid vesicles, which did not bind to the resin. The various components of the methylreductase complex were eluted with 1 M potassium acetate in buffer I. The eluate was concentrated anaerobically on a PM30 ultrafiltration membrane (Amicon Corp., Danvers, Mass.), and about one-third was applied to a Sephacryl S300 gel filtration column as described in the legend to Fig. 2. The fractions were combined in three separate pools (S1 to S3) (Fig. 2). The low-molecular-weight pool, S3 (M_r < 150,000; fractions 52 to 85), contained component A2. The yellow intermediate-molecular-weight pool, S2 (M_r from 150,000 to 400,000; fractions 41 to 51), contained most of component C, which was further purified by phenyl-Sepharose chromatography as described in Materials and Methods. The dark brown, high-molecular-weight pool, S1 (M_r > 400,000; fractions 16 to 40), contained all of the component A3 activity as assayed by the Ti^{III} methane assay. It also contained one-third of the MV-reducing hydrogenase activity and all of the F$_{420}$-hydrogenase activity. The remaining two-thirds of the MV-hydrogenase activity was contained in the low-molecular-weight pool S3. Pool S1 complemented components A2 and C in an H$_2$-driven assay, indicating that it contained component A1 as well as component A3. The S300 gel filtration chromatography was very reproducible and is shown in Fig.

2. Components A1 and A3 from pool S1 were further resolved by phenyl-Sepharose chromatography by eluting the column with a decreasing salt gradient (Fig. 3). Resolution was achieved by extensive washing of the column with 1.0, 0.2, and 0.0 M potassium acetate in buffer I. Four protein peaks were eluted and pooled separately (Fig. 3). Contaminating component C eluted in 1.0 M potassium acetate (pooled fractions 5 to 25). Component A3 had previously been defined as the fraction complementing components C and A2 in the Ti^{III}-driven assay (22). This fraction eluted in 0.1 M potassium acetate (pooled fractions 130 to 162), contained little MV-hydrogenase activity, and was named A3a. The MV-hydrogenase eluted in 0.4 M potassium acetate (pooled fractions 45 to 90), whereas most of the F$_{420}$-reducing hydrogenase eluted in 20% ethylene glycol (pooled fractions 185 to 218) as described before (4, 18).

It was not possible to measure the specific activity of component A3a; however, since at each step the entirety of the A3a activity was pooled, the amount of protein resolved from the A3 pool is an estimation of the purification process. The purification factors with respect to the amount of protein recovered at each step were 1.2-, 3.1-, and 7.5-fold (for the DEAE-cellulose, S300-Sephacryl, and phenyl-Sepharose chromatography steps, respectively), indicating that component A3 had been resolved from at least 95% of the proteins.

Definition of resolved fractions A3a and A3b. Various fractions were combined in an H$_2$-driven methane assay. As shown in Table 1, the pool of fractions 45 to 90, which we named A3b, was absolutely required in addition to the known components of the methylreductase system: purified
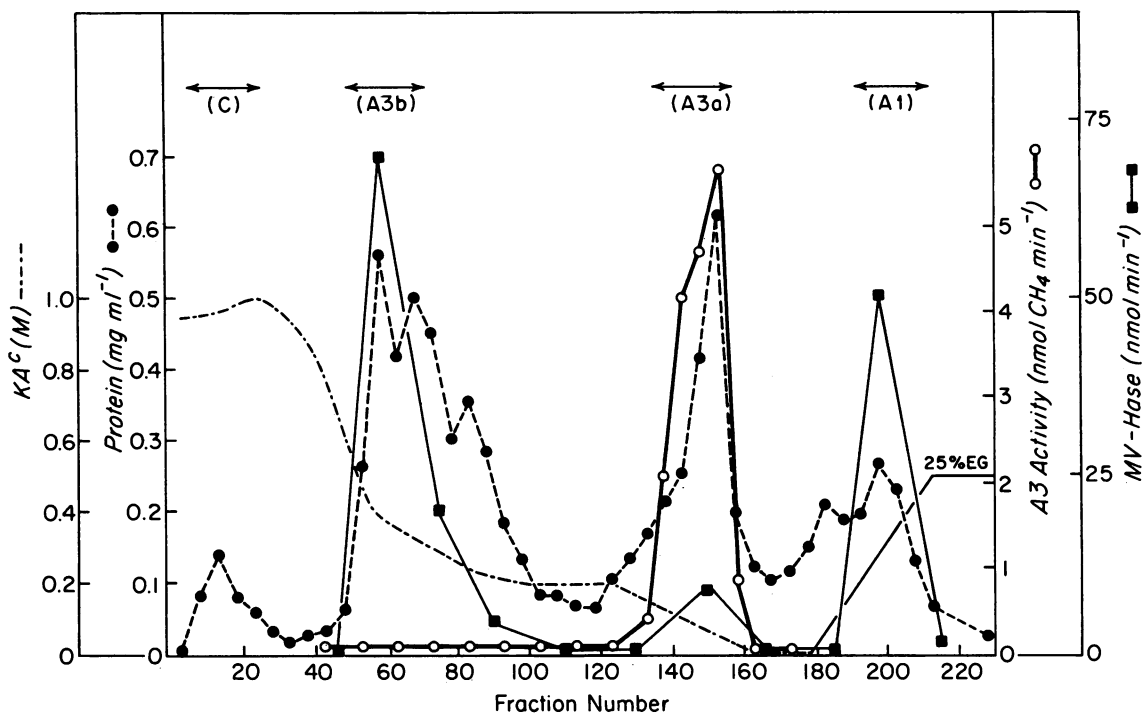
FIG. 3. Resolution of components A1, A3a, and A3b by phenyl-Sepharose chromatography. Pool S1 (360 mg), adjusted to a concentration of 1 M potassium acetate (KA^c), was loaded on a phenyl-Sepharose column (40 by 2.5 cm). The column was developed with an appropriate decreasing potassium acetate gradient with the pumps and gradient programmer of a fast protein liquid chromatography system (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.). The flow rate was 50 ml h^{-1}, and 6-ml fractions were collected. Component A1 was eluted with an increasing ethylene glycol (EG) gradient as shown. Enzymatic activities were detected as described in Materials and Methods. Protein peaks were pooled as shown. The F$_{420}$-hydrogenase (Hase) eluted by ethylene glycol was found in the last peak.

components C and A2, component A3a (defined above by its requirement in the Ti^{III}-driven assay), and component A1 (defined as the fraction eluting in ethylene glycol [18]). Fraction A3b contained most of the MV-hydrogenase, indicating that the component A3 previously known for this activity had now been resolved into two components, A3a and A3b. In this five-component system, addition of flavin adenine dinucleotide had no effect. Although F$_{420}$ was not absolutely required, it was stimulatory and decreased the lag time of the reaction by 20 min.

When we tested the requirement for individual fractions in a methane assay similar to that of Table 1 but driven by the dual source of electrons, H$_2$ and HS-HTP (500 nmol) (22), in

TABLE 1. Resolution of component A3 into two fractions: detection in an H$_2$-driven assay[a]

Vial no.	Component omitted	CH$_4$ (nmol min^{-1})
1	None	12.8
2	C	0.0
3	A1	3.0
4	A2	0.0
5	A3a	3.1
6	A3b	0.0

[a] Pooled fractions from the phenyl-Sepharose chromatography step were assayed to reconstitute the methylreductase system. Assays were performed as described in the text. Each vial contained PIPES, pH 6.3, 16 μmol; Mg^{2+}, 4 μmol; ATP, 1 μmol; CH$_3$-S-CoM, 0.5 μmol; and (S-HTP)$_2$, 5 nmol. Enzymatic components were added where indicated: component C, 200 μg; component A2, 20 μg; component A1 (fractions 185 to 218), 95 μg; component A3a (fractions 130 to 162), 115 μg; component A3b (fractions 45 to 90), 150 μg.

the absence of component A1. The rate of methane formation in the vial that contained components C, A2, A3a, and A3b was 20.2 nmol min^{-1}; no methane was detected when N$_2$ was substituted for H$_2$ with HS-HTP as the sole source of electrons. Reaction mixtures in which one of the components (C, A2, A3a, or A3b) was omitted produced no detectable methane, indicating that the newly resolved fraction, A3b, was not involved in the regeneration of HS-HTP. In this system, neither F$_{420}$ nor flavin adenine dinucleotide had any effect.

Characterization of component A3a. As shown in Fig. 2, component A3a activity, as detected by the Ti^{III}-driven assay, eluted after the F$_{420}$-hydrogenase (M_r 800,000) (26) and before component C (M_r 300,000) on a Sephacryl-S300 preparative gel filtration column. Its apparent molecular weight was more accurately determined by analytical gel filtration chromatography and found to be about 500,000 (Fig. 4). Attempts were made to further purify component A3a from the phenyl-Sepharose chromatography step by using anaerobic preparative polyacrylamide gel electrophoresis. Component A3a activity was recovered routinely. It was always collected in the upper part of the 5% gel (R_f, 0.2 to 0.4), confirming the very high molecular weight of component A3a. By comparison, component C migrated at the bottom of the gel (R_f, 0.8). However, when the eluted proteins were analyzed by native or denaturing polyacrylamide gel electrophoresis, it was not possible to assign component A3a activity to a single band without ambiguity.

Exposure of component A3a to air for 5 min produced a complete loss of activity. To test the hypothesis that component A3a might be an iron-sulfur protein, we used BPDS,
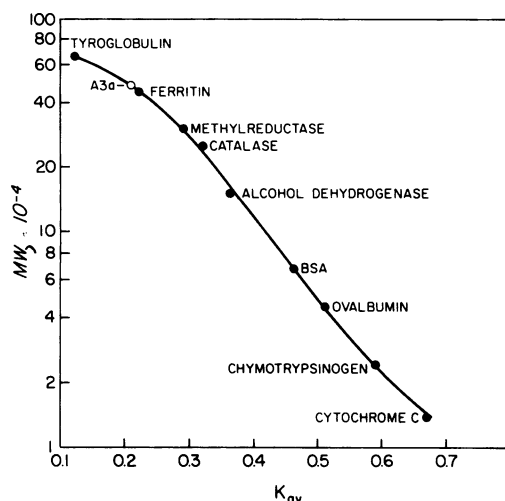
FIG. 4. Molecular weight (MW) determination of component A3a by analytical Sephacryl S300 gel filtration chromatography. BSA, Bovine serum albumin.

FIG. 6. Model for the functioning of the methylreductase system. Inactive component C (C_i) becomes activated by the reduction of its Ni(II) to Ni(I). The electrons from H_2 are provided by the MV-hydrogenase (H^{ase}) present in component A3b and transferred to the Ni through component A3a. This process requires ATP, probably through the activity of component A2. Active component C (C_a) can demethylate CH_3-S-CoM by using electrons from HS-HTP. The product of the methylreductase reaction, CoM-S-S-HTP, is then reduced by H_2 and component A1, which possibly includes the F_{420}-hydrogenase/F_{420}:CoM-S-S-HTP dehydrogenase system (not shown here).

a specific chelator of Fe^{2+} ions (15). When components A2 and C were preincubated at 60°C in the presence of BPDS (0.2 μmol) prior to the reconstitution of the complete Ti^{III}-driven reaction mixture, the rate of methane production dropped from 22.3 to 13.4 nmol min^{-1}. In contrast, when component A3a was preincubated similarly, no methane was produced. Preincubation of component A3a in the absence of BPDS only slightly decreased the rate of methane formation to 18.1 nmol min^{-1}, indicating a highly specific action of BPDS on component A3a. The inhibition kinetics of the most purified preparation of component A3a, obtained by preparative electrophoresis, are shown in Fig. 5. They were linear, and complete inhibition of 28.5 μg of component A3a was obtained with 20 nmol of BPDS. Since BPDS specifically inhibited fraction A3a in a Ti^{III}-driven assay, it could be used to determine whether the cell extract contained active or inactive component C. Preparations having active component C (C_a) did not require A2 or A3 and were not inhibited by BPDS (data not shown).

To aid the reader, we present our current concept of the H_2-driven methylreductase system in Fig. 6. At the upper left of the figure, H_2 is oxidized by the MV-reducing hydrog-

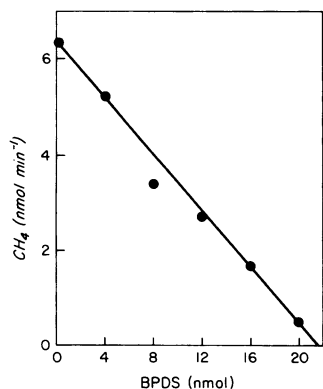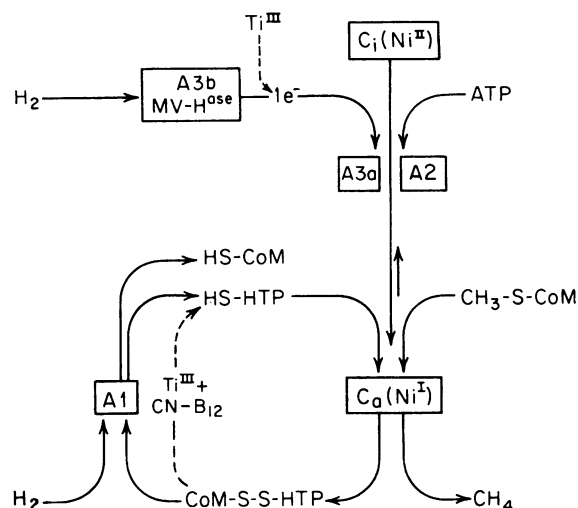enase in component A3b to provide a source of electrons for the conversion of inactive component C (C_i) to its active form (C_a) (Ti^{III} may bypass component A3b and provide an alternative source of electrons). Reduction of nickel in the tetrapyrrole (F_{430}) of component C from nickel(II) to nickel(I) required components A3a, A2, and ATP. C_a is the enzymatically active form of component C, which stoichiometrically converts HS-HTP and CH_3-S-CoM to CH_4 and CoM-S-S-HTP. The F_{420}-hydrogenase then provides electrons for the reduction of the heterodisulfide, CoM-S-S-HTP, to HS-CoM and HS-HTP. Component A1 may be bypassed by use of Ti^{III} and vitamin B_{12}, cyano-cob(III) alamin.

DISCUSSION

We have shown that the fraction known as component A3 consisted of at least two fractions. The first one, which we named component A3a, was required along with inactive component C and component A2 when Ti^{III} was used as the sole source of electrons. The second one, named component A3b, was required in addition to components C, A2, and A3a when H_2 and HS-HTP were used as dual electron sources to drive methanogenesis. In this four-component system, fraction A3b provided the MV-hydrogenase necessary for the utilization of H_2. Whether fraction A3b consists solely of the MV-hydrogenase is not known at this time. As shown previously (22), component A1 is involved in the transfer of electrons from H_2 to CoM-S-S-HTP, the product of the methylreductase reaction (5, 7). The other hydrogenase of *Methanobacterium thermoautotrophicum*, the F_{420}-hydrogenase, might also belong to the methylreductase system as part of component A1. The comigration of component A1 and the F_{420}-hydrogenase (18), the inhibition of the methylreductase by anti-F_{420}-hydrogenase antibodies (8), and the

FIG. 5. Kinetics of inhibition of component A3a by BPDS. A Ti^{III}-driven assay mix contained component C (260 μg), component A2 (10.5 μg), and component A3a (28.5 μg) obtained by anaerobic preparative polyacrylamide gel electrophoresis.

stimulatory effect of F_{420} in the five-component system as well as in cruder systems (18, 27) suggested the involvement of the F_{420}-hydrogenase in the regeneration of HS-HTP. This would then imply the existence of another protein, the F_{420}-dependent CoM-S-S-HTP-reducing dehydrogenase. At this time it is not known whether this enzyme is located in fraction A1 or A3b.

Gel filtration of component A3a showed a molecular weight of about 500,000, suggesting that component A3 was part of a large aggregate. This might explain the strange behavior of component A3 on fast protein liquid chromatography anion-exchange chromatography on a Mono Q column, where, by use of a shallow salt gradient, several peaks eluted at different salt concentrations, all of which had component A3a activity and presented similar electrophoretic profiles (data not shown). The large size of component A3a was confirmed when it was further purified by anaerobic preparative polyacrylamide gel electrophoresis; it migrated in the upper part of a 5% polyacrylamide gel. Homogeneity of component A3a has not yet been attained. Component A3a was air labile, suggesting that it possessed FeS centers. This was confirmed by the specific inhibition of component A3a by BPDS in the Ti^{III}-driven three-component methylreductase system, suggesting that component A3a is most likely involved in electron transfer reactions. If one assumes that component A3a (M_r 500,000) obtained by preparative electrophoresis approaches homogeneity, the chelation of one Fe^{2+} molecule per FeS center by 3 molecules of BPDS indicates that component A3a contains approximately 1 FeS center per 5,000 daltons. This order of magnitude is compatible with ferredoxinlike proteins. A possible role for component A3a is the transfer of electrons between the MV-hydrogenase and component C for its reductive activation (Fig. 6). The hypothesis that the active form of component C has the nickel atom of F_{430} in the Ni(I) state (2) has recently received confirmation by electron paramagnetic resonance studies of crude cell extracts in *Methanobacterium thermoautotrophicum* stain Marburg (1). Under aprotic conditions, Jaun and Pfaltz were able to reduce the nickel of factor F_{430} pentamethylester to Ni(I) by using a sodium amalgam and reported a midpoint potential of -1.32 V versus the ferrocenium/ferrocene electrode (-600 mV versus standard H_2 electrode) (12). The midpoint potential of complexed Ni might vary greatly according to the geometry and the nature of the ligand. In tetra-coordinated model complexes, midpoint potentials for the Ni(II)/Ni(I) couple have been reported to range from $-1,500$ to -560 mV versus the normal H_2 electrode (16). Since the electrons provided by the MV-hydrogenase are unlikely to reduce the Ni from Ni(II) to Ni(I), we propose that the role of ATP may be to induce a conformational change in either component A3a or C, which in turn would modify its redox potential. If component A3a were the substrate of the ATP utilization, its effect could be to lower the redox potential of A3a. A similar phenomenon takes place at the level of the Fe protein of the nitrogenase complex, where the binding of 2 molecules of ATP lowers the midpoint potential of its FeS centers from -250 to -400 mV (17). If this were the case, it would explain the inhibitory effect of dialdehyde of ATP on crude component A3 (24). A similar inhibitory effect of the dialdehyde of ATP has been observed on the ATP-dependent reductive activation of the methyltetrahydromethanopterin:CoM methyltransferase of *M. thermoautotrophicum* (13). One could envision the system composed of components A2, A3a, and A3b to be a general system for providing electrons at extremely low potential through the utilization of ATP. Very recently it was reported that the addition of Mg-ATP plus CH_3-S-CoM induced a shift of g values in the electron paramagnetic resonance signal of an FeS center in crude extracts of *Methanobacterium bryantii* (21). Unfortunately, the effects of CH_3-S-CoM and ATP have not been investigated independently. An alternative possibility could be that component A2 catalyzed the allosteric modification of component C, producing a more positive midpoint potential.

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