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Component A2 of the Methylcoenzyme M Methylreductase System from *Methanobacterium thermoautotrophicum*

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Component A2 of the methylcoenzyme M methylreductase system of *Methanobacterium thermoautotrophicum* has been purified 370-fold by liquid chromatography. Homogeneity was obtained by anaerobic preparative polyacrylamide gel electrophoresis. Component A2 is a colorless, air-stable protein consisting of a single polypeptide as indicated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The relative molecular mass of the native protein was determined by high-performance, size exclusion chromatography to be M_r 52,000; on sodium dodecyl sulfate-polyacrylamide gel electrophoresis a value of M_r 59,000 was obtained. When cell extract was subjected to N^6-ATP-agarose affinity chromatography the methylcoenzyme M methylreductase system was resolved into two fractions; one of them was component A2. This work provides a new operational definition for component A2, i.e., its characteristic chromatographic behavior on N^6-ATP-agarose. However, its functional definition is its ability to reconstitute the methylreductase activity with components A1, A3, and C. Several attempts to assign a role to component A2 are reported.

The CH_3-S-CoM methylreductase system of *Methanobacterium thermoautrophicum* is a multienzyme complex that was first separated into two crude protein fractions, components A and C (7). Component C was purified to homogeneity (3) and was proposed to be the site of the reductive demethylation of CH_3-S-CoM (2). Component A, which was shown to possess hydrogenase activity, was recently resolved into three protein fractions, A1, A2, and A3, each being required with component C for the production of methane from CH_3-S-CoM under an H_2 atmosphere (13). Although A1, A2, and A3 represented a resolution of component A, each of these fractions was composed of many proteins. To date, none of the proteins of these fractions has been purified to homogeneity, making it almost impossible to assign a defined enzymatic activity. For example, two hydrogenases have been isolated on the basis of their ability to reduce methylviologen or the cofactor F_{420} under H_2 (9). At the level of purity obtained after their resolution, fraction A1 contained F_{420}-linked hydrogenase activity (13) and fractions A2 and A3 contained non-F_{420}-linked hydrogenase. Similarly, at least one of the fractions contained the catalysts involved in the activation of the reaction by ATP (14, 17), but study of the role of ATP has been hindered by the fact that the A fractions possessed ATPase activity.

Analysis of this system is especially difficult, for the unstable enzymatic constituents of each A fraction must be maintained and stored as active reagents. Our strategy has been to purify each component for its ability to reconstitute the methylreductase system. We first chose to study fraction A2, the least oxygen sensitive fraction, by using fractions A1 and A3 and component C as reagents. We report here the purification of A2 to homogeneity, showing that A2 possesses only one protein that is involved in the methylreductase system. Although this protein is required for methanogenesis, we have been unable so far to define its enzymatic activity. That we now have two reagents to be used in the assay and purification of other enzymes from fractions A1 and A3 represents a significant advance in our study of this complex system.

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MATERIALS AND METHODS

Abbreviations. The following abbreviations are used throughout: CH_3-S-CoM, methylcoenzyme M [2-(methylthio)ethanesulfonic acid]; CFE; cell-free extract; TEAE, triethylaminoethyl; BCE; boiled-cell extract; 2-ME, 2-mercaptoethanol; PIPES, piperazine-*N*-*N*′-bis(2-ethanesulfonic acid); SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; factor F_{420}, (*N*-L-lactyl-L-glutamyl)-L-glutamic acid phosphodiester of 7,8-didemethyl-8-hydroxy-5-deazariboflavin-5′-phosphate; buffer I, 20 mM potassium phosphate–10 mM 2-ME (pH 7.0); buffer II: 1 M potassium acetate–20 mM potassium phosphate–10 mM 2-ME (pH 7.0).

Growth of cells and extract preparation. *M. thermoautotrophicum* strain ΔH was grown in a 200-liter fermentor as described elsewhere (5, 7). CFE and BCE were prepared as described previously (5, 13).

Preparation of enzymes for assay of component A2. CFE (190 ml, 2.8 mg of protein per ml) was prepared from 150 g of a cell slurry. Liquid chromatography of CFE was performed at room temperature under anoxic conditions as described previously (6). CFE was loaded onto a DEAE-cellulose (Whatman DE52) column (5 by 8 cm) that had been equilibrated with buffer I. The column was washed with the same buffer and developed with buffer I containing 500 mM potassium acetate. The eluate was pooled, and potassium acetate was added to a final concentration of 1 M. This pool (2.2 g of protein) was loaded onto a phenyl-Sepharose column (2.3 by 25 cm) that had been equilibrated with buffer II, and the column was washed with 2 bed volumes of buffer II. Component C of the CH_3-S-CoM methylreductase system did not bind to the resin (D. P. Nagle, Jr., and R. S. Wolfe, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, 114, p. 142). Fractions of component C were pooled, concentrated by ultrafiltration inside an anaerobic chamber on a PM30 membrane (Amicon Corp.), and stored at −20°C in 20% (vol/vol) ethylene glycol under 100 kPa of N_2. Component A was eluted with buffer I and concentrated by ultra-

filtration on a PM30 membrane. This pool (1.4 g of protein) was chromatographed on a TEAE-cellulose column (2.5 by 20 cm) as described previously (13). The nonbinding fraction contained components A1 and A3 and was used with component C to assay for component A2 activity. This reagent enzyme fraction, when stored at −70°C under 100 kPa of H_2, progressively lost its activity at a rate of 30% per month.

Methanogenic assay for component A2 activity. Assays were performed in calibrated, stoppered vials as described previously (7). Enzymes and BCE were added to the reaction mixture inside an anaerobic chamber, whereas all other components were added to the reaction vial before its transfer into the anaerobic chamber. The reaction mixture contained the following: PIPES (pH 6.3), 16 μmol; magnesium acetate, 4 μmol; ATP (pH 7.0), 0.8 μmol; CH_3-S-CoM, 0.5 μmol; BCE, 30 μl; component C, 0.25 mg of protein; fraction A1-A3, 0.3 mg of protein. Fractions having component A2 activity were added in sufficient amounts to sustain rates superior to 5 nmol of CH_4 per min. Buffer I was added to a final volume of 200 μl. The vials were transferred out of the anaerobic chamber, and the atmosphere of the headspace of each vial was exchanged for H_2. The reaction was initiated by transferring the vials into a 60°C water bath. Methane formation was followed as described previously (7). The formation of methane in a typical time course was sigmoidal. The rate of methane formation was calculated from data obtained at the inflection point. One unit of activity was defined as 1 nmol of methane produced per min in a standard assay. The lag of reaction was measured at the intercept of the abscissa and the tangent at the inflection point.

Purification of component A2. CFE was made from 380 g of cell slurry. To precipitate nucleic acids, anoxic 1 M $MnCl_2$ was added dropwise to the CFE inside an anaerobic chamber to a final concentration of 50 mM. Mn^{2+} ions in excess were precipitated by addition of potassium phosphate to a final concentration of 100 mM. This solution (5 g of protein) was applied onto a DEAE-Sephacel column (5 by 13 cm) equilibrated with buffer I. The column was washed with 1 bed volume of buffer I and developed with a 4-bed-volume linear gradient of potassium acetate, 0 to 1 M, in buffer I. The pool of active fractions was concentrated anaerobically on a PM30 membrane. Potassium acetate was then added to a final concentration of 1 M and loaded onto a phenyl-Sepharose column (5 by 11 cm) equilibrated with buffer II. The column was washed with 1 bed volume of buffer II and developed with a 5-bed-volume linear gradient of potassium acetate, 1 to 0 M, in buffer I. The fractions containing component A2 activity were pooled, desalted by ultrafiltration, and applied onto a TEAE-cellulose column (2.5 by 26 cm). Materials adsorbed on a column were eluted under the same conditions as those used for the DEAE-Sephacel step. The active fractions were pooled and then subjected to sievorptive chromatography (10). A Sephadex A25 column (1.5 by 93 cm) was equilibrated with 500 mM potassium acetate in buffer I. A 50-ml linear gradient, 0.5 to 1.0 M, was established in the column; the concentrated pool (4 ml) was applied in 1.5 M potassium acetate and chased with 1.5 M potassium acetate in buffer I. Chromatography was performed at a flow rate of 1.3 ml/h. For the next step an hydroxylapatite column (1.1 by 9 cm) equilibrated with a buffer that contained 10 mM potassium phosphate (pH 7.0) and 10 mM 2-ME was used. After the pooled component A2 fractions obtained from the sievorptive chromatography was loaded, the column was washed with 10 ml of equilibrating buffer and developed with a 100-ml linear gradient of potassium phosphate, 10 to 200 mM, (pH 7.0) that contained 10 mM 2-ME. Component A2 fractions were pooled and stored at 4°C under 100 kPa of H_2

ATP-agarose affinity chromatography. An alternative purification procedure for component A2 also was developed. A 2-ml ATP-agarose column (ATP linked through the N^6-amino group by an 8-carbon spacer; Sigma Chemical Co.) was equilibrated with buffer I inside the anaerobic chamber. A salt eluate fraction from DEAE-cellulose chromatography (13) with complete methylreductase activity was applied onto the column (19.5 mg of protein). The nonbinding fraction was recycled three times, and then the column was washed with buffer I and developed with the appropriate solutions (see below). Activity was assayed by reconstituting the methylreductase system with the nonbinding and eluate fractions. The reaction mixture contained PIPES, Mg^{2+}, BCE, ATP as described above for the assay of component A2 activity, nonbinding fraction (0.6 mg), and ATP-eluate in a quantity sufficient to sustain a rate superior to 5 nmol of CH_4 per min.

Analytical and preparative PAGE. Denaturing and nondenaturing analytical discontinuous PAGE was performed in slab gels with a pH 6.8 to 8.8 system (12). The acrylamide-bisacrylamide content was 4% for the stacking gel and 10% for the resolving gel, and in both cases the acrylamide/bisacrylamide ratio was 38:1. The slab gels were 1 mm thick, and the upper and lower buffers were 200 mM Tris-glycine (pH 8.8). When denaturing PAGE was performed, SDS was added to the gel and the buffer to a final concentration of 0.2%. Samples were boiled for 2 min in the presence of 20% glycerol–2% SDS–0.2% 2-ME before loading onto the gel. Proteins in slab gels were stained with Coomassie brilliant blue R-250 as described by Fairbanks et al. (4).

Anaerobic preparative electrophoresis was performed by using a tube gel electrophoresis apparatus inside an anaerobic chamber. The buffer was made anoxic by sparging it with N_2 (20 liters of N_2 per liter of buffer for 20 min). The gel (2 by 13 cm) was prepared, transferred into the anaerobic chamber, and allowed to equilibrate overnight. After electrophoresis, 1-mm-thick slices of gel were cut, and each slice was suspended in 5 ml of buffer I to allow the proteins to diffuse out of the gel. This buffer was changed three times at 24-h intervals. Component A2 activity was detected with the methanogenic assay.

Molecular weight determinations of component A2. The relative molecular mass of the native protein was determined by SDS-PAGE. The following standards (Sigma) were used: bovine serum albumin (M_r 66,000), ovalbumin (M_r 45,000), pepsin (M_r 34,700), and trypsinogen (M_r 24,000). The relative molecular mass of the native protein also was determined by high-performance size exclusion chromatography (8) with a TSK SW3000 column (Toyo Soda; 0.75 by 30 cm) connected to a 6,000-A model pump (Waters Associates, Inc.) and a UV detector equipped with a 280-nm filter (Kratos Inc., Schoeffel Instruments Co.). The buffer contained 10 mM potassium phosphate (pH 7.0), 300 mM potassium acetate, and 5% ethylene glycol; the flow rate was 0.5 ml/min with a back pressure of 10^6 Pa. Samples of the following standards (100 μl, 1 mg/ml) were used: blue dextran (M_r 1,000,000), bovine serum albumin, ovalbumin, lactalbumin (M_r 36,000), DNase I (M_r 31,000), and lysozyme (M_r 14,880). Component A2 activity was detected by the methanogenic assay, and its molecular size was calculated from its relative $K_{av} = V - V(\text{blue dextran})/V(\text{salt}) - V(\text{blue dextran})$, where V is the elution volume.

Electron transfer. To search for electron transfer activity the following electron carriers were studied (each listed compound is followed by the wavelength employed (nanometers) and the molar extinction coefficient of its absorbing form, oxidized or reduced): reduced methylviologen (601, 11,300); FAD (450, 11,300); FMN (450, 12,500); NADPH (340, 6,200); NADH (340, 6,200); oxidized methylene blue (600, 19,800); oxidized factor F_{420} (420, 40,000). Reduction was followed in a Bausch & Lomb Spectronic cell (light path, 1 cm). In an anaerobic chamber anoxic buffer I was added to a final volume of 3 ml, and each cell was stoppered with a red rubber stopper and transferred out of the anaerobic chamber. The headspace of each cell was exchanged for H_2 or N_2, and the cells were incubated for 5 min at 60°C. Reactions were started by injection of 1 to 10 μg of the enzyme being studied. The cell was placed in a water-jacketed holder of a Bausch & Lomb Spectronic 20 spectrophotometer, and changes in absorbances were followed.

ATPase activity. To check the ATPase activity of a fraction or component of the methylreductase system, the methanogenic assay (volume of reaction mixture, 200 μl) was performed in a Bausch & Lomb Spectronic cell. When methane production reached 60% of completion the cells were opened and allowed to cool to room temperature. To each tube was added the following: glucose, 0.1 mmol; Mg^{2+}, 15 μmol; NADP, 2 μmol; glucose 6-phosphate dehydrogenase, 10 U; and 20 mM potassium phosphate to a volume of 3 ml. The absorbance at 340 nm was read, and then 10 U of hexokinase was added. The change of absorbance was measured after 3 min, and the concentration of ATP in the cell was calculated.

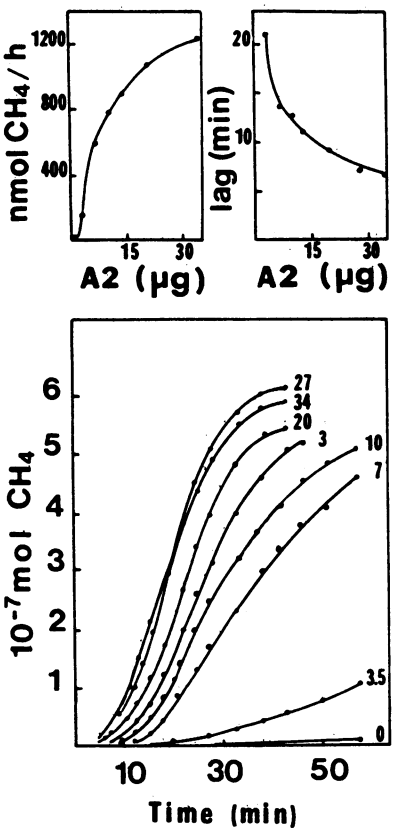

FIG. 1. Dependence of the methylreductase system on component A2. Time course of methane production (numbers refer to the micrograms of pooled A2 added); effect on the rate (top left) and on the lag (top right). The A2 fraction added was obtained by a Sephadex A25 sievorptive chromatography (protein concentration, 0.68 mg/ml), and component A2 represented about 5% of its protein as measured by densitometer (see lane 1 of Fig. 6). Reagent components A1, A3, and C and cofactors were added as described in the text.

TABLE 1. Purification of component A2

Purification step	Protein (g)	Total activity[a] (10^5 U)	Sp act (10^3 U/mg)	Purification (fold)
Cell extract (CFE)	7.1	5.3	0.077	1
Mn^{2+} Precipitation	5.0	8.3	0.17	2
DEAE-Sephacel	3.6	9.2	0.25	3
Phenyl-Sepharose	0.265	7.3	2.8	36
TEAE-cellulose	0.103	5.0	4.8	63
A25 sievorptive	0.026	2.0	7.5	97
Hydroxylapatite	0.007	2.0	28.3	368

[a] One unit produces 1 nmol of methane per min.

Protein determination. Protein concentrations were determined by measuring the turbidity of a sample at 400 nm in 20% trichloroacetic acid (11).

RESULTS

Reconstitution of the CH_3-S-CoM methylreductase system. Component C and fraction A1-A3, when incubated at 60°C under an atmosphere of H_2 in the methanogenic assay, did not catalyze the formation of methane from CH_3-S-CoM. Component A2 was defined as the protein fraction able to reconstitute methylreductase activity. A typical titration of component A2 is shown in Fig. 1. In contrast to component C, component A2 affected the lag as well as the rate of reaction. In other experiments, lags as long as 45 min were observed before any significant production of methane occurred. Component A2 from hydroxylapatite treatment (39% homogeneous) saturated the system at 1.4 μg of protein.

Purification of component A2. Component A2 was purified 368-fold by five liquid chromatography steps (Table 1). In the first step, DEAE-Sephacel, the activity was found in potassium acetate concentrations ranging from 250 to 600 mM with a maximum at 400 mM. In phenyl-Sepharose chromatography, the activity eluted between 300 and 150 mM potassium acetate with a maximum of 200 mM (Fig. 2). Components A1 and A3 also were assayed and were found to elute at 350 mM and 0 mM potassium acetate, respectively, with component A2 between them (Fig. 2). On TEAE-cellulose, component A2 activity was spread over the elution profile, from 400 to 600 mM. At this level of purification the active pool still had a light brown color and contained hydrogenase activity. More than 90% of this activity was separated from component A2 by sievorptive chromatography (Fig. 3). The pool containing component A2 after this step appeared colorless, and nondenaturing PAGE showed that it contained eight bands. Chromatography on hydroxylapatite increased the purity of component A2 3.8-fold. Three major bands were still present on nondenaturing PAGE. The band of component A2, as characterized later, corresponded to 39% of the protein content (Fig. 4). At this level of purity, component A2 could be exposed to air for 48 h without any loss of activity, which was not the case of the A2 active fraction after the TEAE-cellulose step.

Preparative PAGE of component A2. A sample containing 1.4 mg of protein of the component A2 fraction obtained by chromatography on hydroxylapatite was subjected to an-

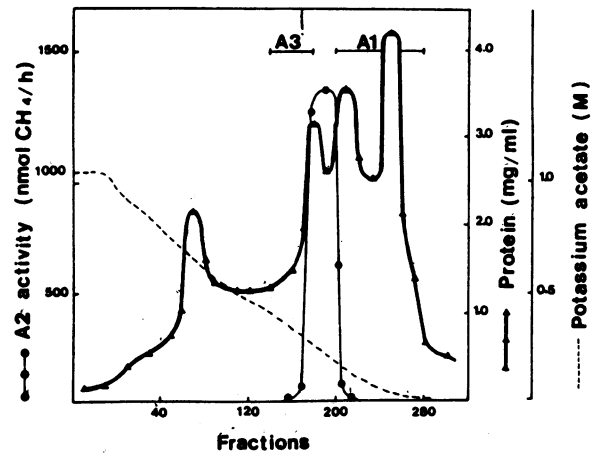

FIG. 2. Phenyl-Sepharose chromatography. The pooled A2 fractions from DEAE-Sephacel chromatography were applied to a phenyl-Sepharose column. Proteins that bound were eluted with a decreasing gradient of potassium acetate. Fractions of 2.5 ml were collected. Component A2 activity was measured as described in the text. The locations of components A1 and A3 were detected with a resolved enzymatic system that required four components (A1, A2, A3, and C).

aerobic preparative tube gel PAGE. Figure 4 shows that homogeneity was reached by this procedure. The relative mobility (R_f) of component A2 on a 10% PAGE is 37%. A total of 0.32 mg of protein for a total activity of 6.3×10^3 U was recovered from the one slice of gel in which A2 was homogenous. This corresponded to 58% of recovery of component A2 itself. Only 15% of the activity loaded on the gel was recovered, implying a loss of 73% of the specific activity of A2.

Subunits and molecular size. When homogenous component A2 from the hydroxylapatite pool was subjected to 10 and 7% SDS-PAGE, only one band was found. The relative molecular mass of the denatured protein was estimated as M_r 59,000. High-performance size exclusion chromatography of the hydroxylapatite A2 pool yielded one active peak,

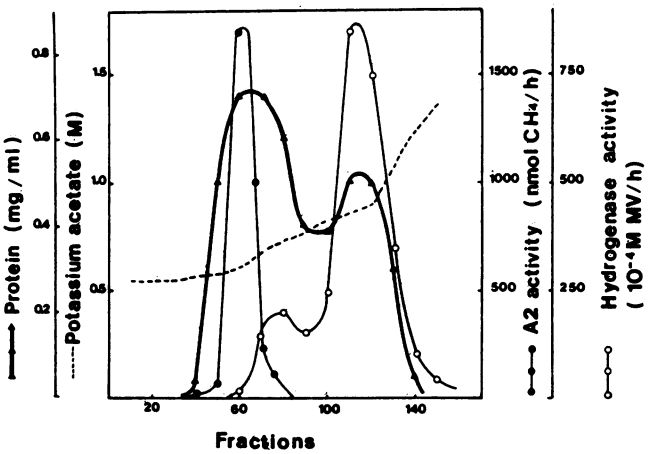

FIG. 3. Separation of A2 activity and hydrogenase by Sephadex A25 sievorptive chromatography. The active pool from TEAE-cellulose chromatography was applied to a Sephadex A25 column loaded with a 50 ml of potassium acetate gradient; 0.5-ml fractions were collected. Hydrogenase activity was assayed by the reduction of methylviologen under an H_2 atmosphere.

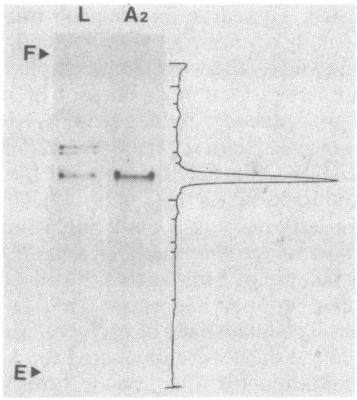

FIG. 4. PAGE of component A2. On the right is shown the densitometer scan of the protein band A2 obtained by preparative PAGE. Lane L shows the A2 preparation as obtained from the hydroxylapatite column.

with an elution time corresponding to a relative molecular mass of M_r 52,000 (Fig. 5).

ATP-agarose affinity chromatography. A DEAE-cellulose eluate (19.5 μg of protein) with complete methylreductase activity was applied onto N^6-ATP-agarose. The pass-through fraction had no methylreductase activity. The missing activity could not be eluted with buffer I containing 0.5 M potassium acetate or 10% ethylene glycol or 3 mM NAD. It eluted with 3 mM ATP in buffer I, yielding 0.18 mg of protein. Other experiments have shown that elution did not take place with 2.5 mM NADP or 2.5 mM FAD, but did with 2.5 mM ADP. The DEAE-cellulose fraction and the ATP eluate were assayed for the missing activity as described above. They contained, respectively, 2,510 and 2,420 U. This latter pool was colorless and maintained the same specific activity after a 48-h exposure to air (13,450 U/mg). When subjected to a nondenaturing PAGE, the ATP eluate presented many protein bands; one of them was highly enriched. Figure 6, lane 3, shows an ATP eluate obtained by the same procedure where the major band accounts for 52% of the protein.

Component A2 eluted from the A25 sieveorptive chromatography was added to the inactive nonbinding fraction from the ATP-agarose in a methanogenic assay and could replace the missing activity. An ATP-agarose binding fraction was further purified by anaerobic preparative PAGE in a small tube gel (0.8 by 13 cm). After PAGE, protein fractions extracted from slices of the gel were assayed to complement the fraction that did not bind the ATP-agarose, and three of them reconstituted the methylreductase activity. These ac-

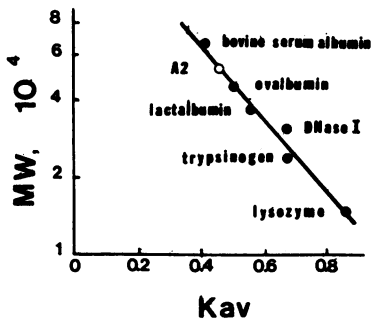

FIG. 5. Molecular size determination of native component A2 by high-performance size exclusion chromatography.

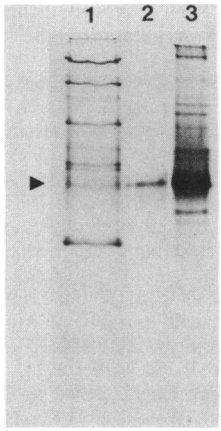

FIG. 6. Comigration of the ATP-agarose binding fraction and of component A2 on nondenaturing PAGE. Lane 1 shows crude component A2 from a Sephadex A25 sievorptive chromatography. The band corresponding to component A2 (▶) lies immediately below the characteristic double band. Lane 2 shows component A2 protein from preparative PAGE of the ATP-agarose binding fraction. Lane 3 shows the ATP-agarose binding fraction.

tive fractions were subjected to analytical nondenaturing PAGE, and they all showed a band comigrating with component A2. For one of those active fractions the only band present was the one comigrating with component A2 (Fig. 6). When this homogeneous "ATP–agarose-binding protein" was assayed for component A2 activity, it reconstituted the methylreductase activity. Although component A2 bound to the ATP-agarose column, we could not detect ATP hydrolysis activity in A2; the reconstituted system (nonbinding fraction plus A2) or the nonbinding fraction alone had the same activity (9×10^{-3} μmol of ATP per min per mg of protein). Other affinity resins were tested. Component A2 also was bound to ATP-agarose attached through the ribose hydroxyls and to N^6-ADP-agarose.

We were unable to show a role of A2 in electron transfer reactions; component A2 was not able to reduce factor F_{420} or methylviologen under H_2, nor could it catalyze the reduction of FAD, NAD, NADP, or methylviologen by reduced F_{420}, even in the presence of added BCE. It also failed to catalyze the reduction of methylene blue from NADH or NADPH.

DISCUSSION

Component A2 of the CH_3-S-CoM methylreductase system of *M. thermoautotrophicum* was purified 368-fold by five liquid chromatography steps to 39% of homogeneity. One should notice that, in contrast to anion-exchange chromatography, where component A2 was present throughout the elution profile, hydrophobic chromatography on phenyl-Sepharose yielded a sharp peak of component A2 activity resolved from component A1 and A3 activities. This could be explained by possible hydrophobic interactions between component A2 and other proteins of *M. thermoautotrophicum*. A fast purification procedure was developed with N^6-ATP-agarose; this chromatographic step extracted component A2 from a crude methylreductase preparation, enriching it more than 100-fold. At a high level of purity, component A2 was stable in solution under anoxic conditions at 4°C over several months without any noticeable loss of activity. However, concentration by ultrafiltration or precipitation by 70% ammonium sulfate caused an irreversible precipitation.

When homogeneity was obtained by anaerobic preparative PAGE, component A2 was a colorless, oxygen-resistant protein consisting of a single polypeptide with an M_r of 52,000 for the native protein. When this protein was denatured in SDS-PAGE, an M_r of 59,000 was estimated. Component A2 was required for the activity of the methylreductase system, affected the lag as well as the rate of the reaction, and saturated the system at very low concentration (1.4 μg of 39% homogenous A2, average M_r of 55,000) compared with component C (250 μg of 90% homogenous A2, M_r of 300,000) which corresponded to a molar ratio of 1/75 of component A2 to component C in an optimized system. It appears highly unlikely that components A2 and C share a common structural unit.

At this point we should emphasize that it is impossible to give an absolute specific activity to any component as long as the only assay available is the reconstitution of the methylreductase system. Many factors have been shown to induce variability in the overall activity of the system, such as batch-to-batch variability of extracts or instability of component B or A3.

The role of component A2 is still unknown. It was not shown to catalyze an electron transfer reaction. We considered the possibility that component A2 could be the methylcobalamin-coenzyme M methyltransferase purified by Taylor and Wolfe in *Methanobacterium* sp. strain MoH (15). This possibility was not confirmed, since the resolved system, in contrast to CFE, would not make methane from methylcobalamin; methyltetrahydromethanopterin-coenzyme M methyltransferase activity was not detected in component A2. The fact that component A2 was bound to ATP-agarose suggests that it may possess a site for an adenosine derivative, and since NAD, NADP, or FAD was unable to elute component A2, these nucleotides appear not to be involved. The specific elution by ATP or ADP suggests that this protein might play a role in the activation of the methylreductase system by ATP. This is in agreement with the effect of component A2 on the lag of the reduction of CH_3-S-CoM and also with the fact that it is required at low concentration compared with component C. However, component A2 alone does not hydrolyze ATP. The activation by ATP is most likely to involve more than one enzyme of the methylreductase system, as is the case for other systems showing an activation by ATP (1, 16).

This work provides an operational definition for component A2, i.e., its characteristic chromatographic behavior on N^6-ATP-agarose. However, its functional definition is its ability to reconstitute methylreductase activity with components A1, A3, and C.

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