Identification of Protein-Arginine \(N\)-Methyltransferase as 10-Formyltetrahydrofolate Dehydrogenase*

(Sangduk Kim‡§, Gil Hong Park‡, Won A. Joo‡, Woon Ki Paik‡, Robert J. Cooki, and Kenneth R. Williams**

From the §Department of Biochemistry, Korea University Medical College, Graduate School of Biotechnology, Korea University, Seoul, 136-701, Korea, the ¶Department of Biochemistry, School of Medicine, Ajou University, Suwon, 442-749, Korea, the **Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, and the §Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut 06510

S-Adenosylmethionine:protein-arginine \(N\)-methyltransferase (EC 2.1.1.23; protein methylase I) transfers the methyl group of S-adenosyl-L-methionine to an arginine residue of a protein substrate. The homogeneous liver protein methylase I was subjected to tryptic digestion followed by reverse phase high performance liquid chromatography (HPLC) separation and either “online” mass spectrometric fragmentation or “off-line” Edman sequencing of selected fractions. Data base searching of both the mass spectrometric and Edman sequencing data from several peptides identified the protein methylase as 10-formyltetrahydrofolate dehydrogenase (EC 1.5.1.6; Cook, R. J., Lloyd, R. S., and Wagner, C. (1991) J. Biol. Chem. 266, 4965–4973; Swiss accession number P28037). This identification was confirmed by comparative HPLC tryptic peptide mapping and affinity chromatography of the methylyase on the 5-formyltetrahydrofolate-Sepharose affinity gel used to purify the dehydrogenase. The purified rat liver methylase had approximately 33% of the 10-formyltetrahydrofolate dehydrogenase and 36% of the aldehyde dehydrogenase activity as compared with the recombinant dehydrogenase, which also had protein methylase I activity. Polyclonal antibodies against recombinant dehydrogenase reacted with protein methylase I purified either by polyacrylamide gel electrophoresis or 5-formyltetrahydrofolate affinity chromatography. In each instance there was only a single immunoreactive band at a molecular weight of ~106,000. Together, these results confirm the co-identity of protein-arginine methyltransferase and 10-formyltetrahydrofolate dehydrogenase.

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‡ To whom all correspondence should be addressed: Dept. of Biochemistry, Korea University Medical College, 126, 5-Ga Anam-Dong, Sung Buk-Gu, Seoul, 136-701, Korea. Tel.: 82-2-920-6409; Fax: 82-2-928-4853; E-mail: sdkim@kucnx.korea.ac.kr.

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**Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut 06510

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kDa monomers (22). The dehydrogenase was purified initially from rat liver and was identified as a folate-binding protein (23) that binds 2 mol of folate per mol of the enzyme (24, 25). The rat liver FDH has been expressed in Baculovirus and purified by 5-FTHF-Sepharose affinity chromatography (26). The amino acid sequence deduced from the nucleotide sequence of the cDNA indicated the monomer to have a molecular mass of 99,015 Da and to consist of 902 amino acid residues. The NH₂-terminal sequence (residues, 1–203) is 24–30% (depending on the species) identical to phosphoribosyl-glycinamide formyltransferase (EC 2.1.2.2.). There is also a 32% identity for residues 1–310 of FDH with Escherichia coli-L-methionyl-tRNA formyltransferase (27, 28), while the COOH-terminal region that spans residues 417–900 of FDH is 48% identical to aldehyde dehydrogenase (NADP⁺) (EC 1.2.1.3.). The middle domain, residues 204–417, does not appear to share significant sequence homology with any known sequence (19, 25). Thus, FDH is a multidomain enzyme that catalyzes at least the following three reactions: 1) the NAD⁺-dependent oxidation of 10-FTHF, 2) the NAD⁺-independent hydrolysis of 10-FTHF, and 3) the NAD⁺-dependent oxidation of propionaldehyde.

In the present article, several independent sets of data demonstrate that rat liver FDH also has protein methyltransferase activity and indeed, is the enzyme that has been named previously as nuclear protein/histone-specific protein methylase (5).

ExPERIMENTal Procedures

Materials—S-Adenosyl-L-[methyl-14C]methionine (specific activity, 50 mCi/mmol) and S-adenosyl-L-[methyl-3H]methionine (specific activity, 78.5 Ci/mmol) were obtained from NEN Life Science Products Inc. AdoMet (chlordiazepoxide, histone (calf thymus, type IIAS), phenylmethylsulfonyl fluoride, pepstatin, 2-mercaptoethanol, and propionaldehyde were from Sigma. DE-52 was obtained from Whatman. 10-FTHF was as prepared by the method of Rabinowitz (29). 10-Formyl-5,8-dideazafolate (10-FDDF) was obtained from Dr. John H. Eynos, Department of Pharmaceutical Chemistry, Medical University of South Carolina. 5-FTHF-affinity gel was prepared by covalently linking 5-FTHF to AH-Sepharose 4B (Pharmacia) as described (24). Recombinant hnRNP protein A1 was purified from E. coli, containing plasmid pEX11 carrying the cDNA coding sequence for protein A1 as described (30).

The Protein Methylation—I—Protein methylase I activity was determined as described (3, 4) in a total incubation mixture of 0.125 ml containing 0.1 m potassium phosphate, pH 7.6, 40 mCi [methyl-3H]AdoMet (diluted with unlabeled AdoMet to ~1,000 dpm/µmol), substrate protein (50 µg of protein A1 or 500 µg of histone) and the enzyme fraction to be assayed. The incubation was carried out at 27 °C for 60 min and was terminated with 15% trichloroacetic acid followed by addition of 4 mg of γ-globulin as a carrier protein. The mixture was treated to remove unreacted, radiolabeled AdoMet by successive treatment with the trichloroacetic acid, and the resulting precipitates were counted for radioactive methyl incorporation into the substrate protein.

Purification of Protein Methylation—I—Protein methylase I was purified from rat liver cytosol as described (3). Briefly, a 100,000 × g rat liver supernatant was chromatographed on DE-52, followed by Sephadex G-200 molecular sieve chromatography. The enzymatically active fractions were then pooled and further purified by nondenaturating (ND) PAGE (see below).

Alternatively, protein methylase I can also be purified by 5-FTHF-Sepharose affinity chromatography. In this instance the Sephadex G-200 purified protein methylase I was loaded onto a column of 5-FTHF-Sepharose (1 × 4 cm) which had been pre-equilibrated in 10 mM potassium phosphate, pH 7.0, 1.0 mM 2-mercaptoethanol and eluted as described (31). The column was first washed with 16 ml of the equilibrating buffer, followed by a salt gradient consisting of 50 ml of the equilibrating buffer and 50 ml of the same buffer containing 1.0 M KCl. Fractions were eluted with equilibrating buffer that contained 10 mM folate and 1.0 M KCl. After removing the folate, an aliquot from each fraction was assayed for both protein methylase I and FDH activities. Fractions containing both enzyme activities were pooled, concentrated, and subjected to Western immunoblot analysis.

Polyacrylamide Gel Electrophoresis—As a part of the enzyme purification, nondenaturing PAGE was carried out in a 7.5% polyacrylamide gel run overnight at 4 °C as described (4). The Sephadex G-200 purified enzyme (about 100 µg/lane) was loaded into several lanes. One of the gel lanes was longitudinally cut into two sections, and one section was stained with Coomassie Blue. The other strip of the unstained lane was sliced into 2-mm sections; the protein was electroeluted from each slice with 1 ml of distilled water. The resulting fractions were assayed for methyltransferase activity. The remaining unstained gel lanes were subsequently sliced, and those sections containing methyltransferase activity were combined, electroeluted, and then concentrated via a small DE-52 column (1 × 3 cm) (4).

SDS-PAGE was carried out according to the method of Laemmli (32). The running gel contained 10% acrylamide, the stacking gel contained 3% acrylamide, and the gel was run overnight at room temperature.

Assay for 10-Formyltetrahydrofolate Dehydrogenase—The dehydrogenase assay was carried out as described (33). Briefly, reaction mixtures contained 50 mM Tris-HCl, pH 7.7, 100 mM 2-mercaptoethanol, 0.1 mM substrate (10-FTHF or 10-FDDF), 0.1 mM NADP, and the enzyme preparation to be assayed in a total volume of 1.0 ml. The reaction rates were followed at 23 °C by the increase in absorbance at either 300 nm (for 10-FTHF) or 295 nm (for 10-FDDF) in a Perkin-Elmer Lambda 4B spectrophotometer. Readings were measured against blanks containing no enzyme or no substrate. Hydrolase activity was measured by omitting the NADP from the reaction mixture.

Assay for Aldehyde Dehydrogenase—Aldehyde dehydrogenase was assayed as described (19, 34). The reaction mixture contained 60 mM potassium phosphate, pH 5.5, 5 mM propionaldehyde, 1 mM NADP, and the enzyme preparation to be assayed. The enzyme activity was estimated from the increase in absorbance at 340 nm.

Preparation of Recombinant FDH—Recombinant rat liver FDH was expressed in insect cells using the pVl 1393 expression vector as described (26, 33, 35) and was purified via Sephacryl S-300 and DE-52 ion-exchange chromatography followed by affinity chromatography on 5-FTHF-Sepharose (24, 31).

Protein Chemistry and Internal Sequencing—Protein concentration was estimated generally either by absorbance at 280 nm or by the Coomassie Blue method of Bradford (36) as modified by Pierce Chemical Co. using bovine serum albumin as the standard. The amount of protein in Coomassie Blue-stained gel bands that were destined for in gel tryptic digestion was estimated by hydrolyzing (8 × HCl, 115 °C, 16 h) an approximately 10% aliquot of the gel band. The extracted digests were then subjected to reverse phase HPLC at a flow rate of 50 µl/min on a 1 × 250-mm Vydac C-18 column as described (37). To facilitate the rapid identification of repeated and trypsin autolysis peaks, blank sections of gel that should not contain protein were brought through the same procedures and were also subjected to reverse phase HPLC. Peak detection in the trypsin digests were collected into “capless” 1.5-ml Eppendorf tubes that were then capped to prevent evaporation of acetonitrile and were stored at 5 °C. Aliquots of fractions selected for amino acid sequencing were subjected first to matrix assisted laser desorption mass spectrometry on a Micromass ToySpec SE as described (38). Amino acid sequencing of selected peptides was carried out on Applied Biosystems Division Model 470 or 494 Protein/Pepptide Sequencers operated according to the manufacturer’s instructions and the protein mass and amino acid sequence (see below) studies were carried out in the W. M. Keck Foundation & HHMI Biopolymer Laboratory at Yale University. More information on these procedures may be found at http://info.med.yale.edu/wmkeck/.

LC-MS/MS Protein Identification—Approximately 10% (5 pmol) of the in-gel trypsin digests of protein methylase I and of the recombinant rat liver FDH that had been isolated by SDS-PAGE were subjected to reverse phase HPLC on-line mass spectrometry as described (39). Briefly, these studies were carried out on a Applied Biosystems Division Model 140 B HPLC that was directly interfaced to a Finnigan Corpora LCQ quadrupole ion trap mass spectrometer. The HPLC separation was carried out at 4 µl/min on a 300 µm × 250 mm Vydac C-18 column that was equilibrated with 0.1% trifluoroacetic acid, 0.09% acetic acid, 2% acetonitrile, 98% water and was eluted with increasing concentrations of acetonitrile. The MS/MS scans were carried out automatically using a relative collision energy of 30–35% with an isolation width of 2.0. An intensity threshold of 13,000 was found to be optimal for triggering the acquisition of these automated scans. A set of products was then selected through the use of the highest quality scans for searching, verify the assigned charge state, and then tabulate the final results. MS/MS scans were searched using the Finnigan Corporation software package Bioworks which 3J. Jones, R. DeAngelis, K. L. Stone, and K. R. Williams, manuscript in preparation.
includes the tandem mass correlation algorithm SEQUEST™ developed in the Yates’ laboratory (40, 41). Relevant parameters used to search the OWL data base included the peptide mass tolerance (2.0 atomic mass units) and the fragment ion tolerance (1.0 atomic mass unit). Average (chemical) molecular weight \( m/z \) values were used. Enzyme cleavage sites were not specified in the search parameters, which increased the confidence of identification when matched peptides had appropriate cleavage sites. The program was set up to take into account cysteines which may or may not have been alkylated. (The \( S \)-methyla-

\[14.0 \text{ Da} \] to the peptide mass.) Those MS/MS scans that had deltCn values (defined in Ref. 41) greater than 0.1 and that matched to peptides that would have been expected to be produced by the enzyme used to effect cleavage (i.e. in the case of trypsin, peptides that would be produced by cleavage after lysine and/or arginine) were considered significant. In our experience (39) two such matches are sufficient to establish the identity of the protein.

**Immunological Studies**—The polyclonal antibodies were raised in rabbits by injection of 200 mg of recombinant FDH with Hunter’s TitreMax adjuvant, and were used for Western immunoblotting analysis as follows. The purified protein was first subjected to SDS-PAGE prior to transferring to a nitrocellulose membrane using a Transblot apparatus (Bio-Rad) overnight in 20 mM Tris-HCl, 150 mM glycine buffer, 20% methanol, pH 8.3, at a constant current of 20 volt. The transferred proteins were then visualized by staining the nitrocellulose with 0.5% Ponceau S in 1% acetic acid and destained with water. After washing, the membrane was incubated at room temperature for 2 h with 3% bovine serum albumin, and with anti-recombinant-FDH containing 3% bovine serum albumin as the first antibody. The blot was washed then with phosphate-buffered saline (10 mM NaH₂PO₄, pH 7.4, 137 mM NaCl, 2 mM KCl) and incubated with rabbit anti-mouse IgG-horseradish per-

oxidase antibody at room temperature for 2 h. Finally, the blotted paper was washed and visualized with 3,3'‐diaminobenzidine, phosphate-

buffered saline, 1% CoCl₂, and water as described (42).

**RESULTS**

**Internal Sequencing of Protein Methylase I**—Protein methylase I was purified by ND-PAGE following DE-52 and Sephadex G-200 chromatography (3). The enzyme activity was localized by extracting the gel slices and then assaying for protein methylase I activity as described under “Experimental Procedures.” The enzymatically active protein band had an apparent size of 450 kDa on ND-PAGE (Fig. 1, A and B) as compared with 110-kDa on SDS-PAGE (Fig. 1C). The corresponding ND-

PAGE band that had been stained with Coomassie Blue was then subjected to in-gel digestion with trypsin and reverse phase HPLC as described under “Experimental Procedures.” As shown in Fig. 2, the digest proceeded well and three of the more symmetrical appearing peaks were subjected to Edman degradation. Although each of these peaks proved to contain two peptides, in the case of peaks 52 and 93 the ratio of the primary to secondary peptide sequence was sufficiently high (i.e. above 5) that the primary sequence could be readily differ-

entiated from the secondary sequence. As depicted in Fig. 3A all 4 of the peptide sequences obtained from peaks 52 and 93 matched exactly to tryptic peptide sequences in the rat FDH sequence (Swiss P28037). In addition, both of the sequences present in peptide 56 also could now be readily matched to predicted FDH tryptic peptides. In toto, six protein methylase I tryptic peptides were sequenced with all 55 of the resulting residues matching exactly to the rat FDH sequence. As shown in Fig. 3B the six protein methylase I sequences are well distributed throughout the FDH sequence.
Affinity Purification of Protein Methylase I by 5-Formyltetrahydrofolate-Sepharose Chromatography—FDH was initially described as a folate-binding protein and the protein can be effectively purified by affinity chromatography using 5-FTHF-Sepharose (31). Hence, a partially purified protein methylase I preparation was subjected to the same affinity purification. As shown in Fig. 4, the affinity gel was very effective in purifying protein methylase I and the methylase and dehydrogenase activities eluted together. The enzyme peaks were pooled and subjected to Western immunoblot analysis using anti-recombinant FDH antibodies. As shown in Fig. 5B this analysis revealed a single protein band at ~106 kDa. Likewise, protein methylase I prepared via the “conventional” scheme (i.e. DE-52 followed by Sephadex G-200 chromatography and ND-PAGE) also revealed a single immunoreactive band at ~106 kDa (Fig. 5A), while control preimmune serum did not show any immunoreactivity (data not shown).

FDH and Methylase Activity in Recombinant FDH and Protein Methylase I—As shown in Table I, protein methylase I purified through ND-PAGE has all three activities that are characteristic of FDH. When the dehydrogenase activity was measured using 10-FTHF as the substrate, the purified methylase was ~33% as active as the recombinant FDH (31.3 versus 94 nmol of THF/min/mg). Similarly, the aldehyde dehydrogenase activity measured in the methylase preparation corresponded to ~36% of the dehydrogenase activity (48.4 versus 135 nmol of NADPH/min/mg). The lower dehydrogenase activities found in the methylase preparation, compared with those in the recombinant dehydrogenase, may be the result of partial inactivation during the relatively lengthy methylase purification. In this regard, while purification (26) of the recombinant FDH involves primarily a single affinity chromatography step (which presumably would remove inactivated FDH), the purification of the methylase involves two chromatographic separations followed by ND-PAGE (see “Experimental Procedures”). As expected, the recombinant FDH also has methyltransferase activity. Interestingly these assays were carried out on a sample of recombinant FDH that had become inactivated by storage without 2-mercaptoethanol, probably via cysteine oxidation as both the FDH and methyltransferase activities are inactivated by sulphydryl reagents (3, 27). As shown in Table II, the 10-FTHF dehydrogenase and methylase activities respond differently to elevated concentrations of 2-mercaptoethanol. While previous studies have shown that optimal dehydrogenase activity requires 15 mM or higher concentrations of 2-mercaptoethanol, prior incubation of recombi-
nant FDH in high concentrations of this reducing agent resulted in loss of methylase activity (Table II). Similarly, while the recombinant FDH is not inhibited by sodium azide, we found that 0.24 mM sodium azide inhibited 70% of the protein methylase activity. Furthermore, 0.5 mM folate also resulted in 50% inhibition of protein methylase I activity. Taken together, these data suggest that FDH and methylase activities may reside in separate domains. Since the methylase activity that was recovered in Table II was about 25% of that reported for the homogenous rat liver protein methylase I (i.e., 9.0 pmol of CH₃ group/min/mg) the activation of the methylase activity in the FDH may not have been complete.

**FIG. 3.** Amino acid sequences of tryptic peptides derived from rat liver protein methylase I and the corresponding FDH sequence. A, comparison of tryptic peptide sequences obtained from the protein methylase I peaks indicated in Fig. 1 with the corresponding sequences predicted from FDH. B, amino acid sequence of FDH deduced from its cDNA with the boxed regions indicating the location of the tryptic peptide sequences that are given in panel A.

Comparative HPLC Tryptic Peptide and LC-MS/MS Analysis of Protein Methylase I and FDH—Two sets of comparative
HPLC tryptic maps were carried out: first, protein methylase I purified by ND-PAGE or SDS-PAGE was compared with the recombinant FDH expressed in insect cells (Fig. 6A); and second, the rat liver and recombinant FDH were compared with the SDS-PAGE purified rat liver methylase (Fig. 6B). Each set of three HPLC profiles are very nearly identical. One exception is that one peak that elutes at about 72 min in the rat liver PM I (ND-PAGE) and recombinant FDH profiles shown in Fig. 6A, which is labeled as peaks 67 and 71, respectively, in these chromatograms, seems to be missing in the rat liver PM I (SDS-PAGE) profile. However, both the mass of this peptide and Edman sequencing indicates that it matches exactly to residues 521–531 of FDH. We suspect the methionine in this peptide may be oxidized in the rat liver PM1 (SDS-PAGE) sample and that this has led to a shift in elution position so this peptide now co-elutes with a neighboring peptide in this chromatogram.

LC-MS/MS analyses followed by SEQUEST data base searching were carried out on the rat liver methylase and (as controls) the rat liver and recombinant FDH. In all cases 18 of the highest quality MS/MS spectra were subjected to SEQUEST data base searching as described under “Experimental Procedures.” This analysis resulted in matching the following number of tryptic peptides to FDH: 5 from rat liver methylase (ND-PAGE); 4 from rat liver FDH; and 8 from recombinant FDH. In no instance was a significant match observed for a predicted tryptic peptide from any protein other than FDH. Since only two SEQUEST matches appear to be required for a
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### DISCUSSION

The present article establishes the co-identity of the rat liver protein-arginine methyltransferase (protein methylase I) and the rat liver FDH. The protein methylase I has been isolated as an ~450-kDa protein tetramer of identical ~100-kDa subunits (3) while the dehydrogenase, which had been isolated initially as the folate-binding protein (23, 24), was reported to exist as various multimers, which had approximate sizes of 410, 350, and 210 kDa and which were all composed of an ~100-kDa monomer (21, 24). Subsequently, the dehydrogenase has been cloned and expressed in *Baculovirus*-infected insect cells (19, 26) as a 99-kDa monomer which forms a tetramer that has an apparent molecular mass of 400–450 kDa (see Ref. 35 as well).

Several independent approaches were taken to verify the identification of protein methylase I as FDH. These included: (i) complete agreement between the amino acid sequences of six tryptic peptides from the methylase with the corresponding tryptic peptides predicted from the FDH sequence; (ii) cross-reactivity of protein methylase with antibodies elicited by recombinant FDH; (iii) demonstration of dehydrogenase activity in the purified protein methylase as well as methylase activity in the recombinant dehydrogenase; (iv) common elution profile of both enzymatic activities during 5-FTHF affinity chromatography; (v) comparative HPLC tryptic peptide mapping studies which demonstrated that protein methylase I purified via either nondenaturing or SDS-PAGE gave an HPLC profile that was nearly identical with that for rat liver or *E. coli* expressed FDH and finally, (vi) LC-MS/MS analysis followed by SEQUEST data base searching which independently identified the protein methylase as FDH.

FDH is an interesting multifunctional enzyme that had already been shown to have three catalytic activities (19); namely, the NADP+-dependent oxidation of 10-FTHF, the NADP+-independent hydrolysis of 10-FTHF hydrolyse for 10-FTHF, and an aldehyde dehydrogenase activity. Since the N$_{H_2}$-terminal domain (residues 1–203) of FDH is 24–30% identical to a group of glycaminide ribonucleotide transformylases (EC 2.1.2.1) (19), while the COOH-terminal domain (residues 417–909) shares 46% identity with a series of NAD+-dependent aldehyde dehydrogenases (EC 1.2.1.3), it seemed reasonable to expect that these regions of sequence homology might correspond to independently folded domains. Indeed, differential scanning microcalorimetry revealed two thermal transitions (25). In addition, a COOH-terminal FDH construct (residues 420–902) retained aldehyde dehydrogenase but not dehydrogenase nor hydrolyase activity (43) while an NH$_2$-terminal construct (residues 1–310) retained 10-FTHF hydrolyse activity and a folate-binding site (28). Since neither construct retained FDH activity, it was hypothesized that this activity results from the action of the COOH-terminal aldehyde dehydrogenase catalytic center acting on the substrate bound in the NH$_2$-terminal domain and that the connecting domain (residues 311–419) is needed to bring the two functional groups into the proper orientation (28). This interpretation follows also from previous limited proteolysis studies. The catalytic domain structure of liver FDH has been studied by treatment with subtilisin (25) and trypsin (44). In both cases, FDH activity was preferentially inhibited while leaving both the aldehyde dehydrogenase and hydrolyse activities intact, suggesting that cleavage of FDH into two domains destroys the ability to oxidize 10-FTHF. Further support for multiple domains derives from a site-directed mutagenesis study which demonstrated that replacement of cysteine 707 with alanine resulted in loss of FDH activity but retention of full hydrolysis activity (35). One intriguing question, of course, is the location of the methyltransferase activity in FDH.

Protein methylase I transfers a methyl group from AdoMet to specific arginine residues on a protein substrate yielding three different isomers of N$^{\text{O}}$-methylated arginine (2, 4). The currently studied protein methylase I from rat liver cytosol has been shown to be highly specific for hnRNP protein A1 (3). In this latter regard the rat liver enzyme appears analogous to one of the two subclasses of methylases present in calf brain cytosol (4, 5). That is, one subclass of calf brain methylase was found to be specific for myelin basic protein, while the other was initially shown to be histone-specific, but later shown to have even higher specificity for recombinant hnRNP protein A1 (5). These two subclasses of methylase have quite different molecular and catalytic properties (4). In view of the fact that liver is not a neuronal organ, it is unlikely that myelin basic protein-specific methylase is present in liver cytosol. Indeed, anion exchange chromatographic fractionation of crude rat liver cytosol extracts revealed only a single, coincident peak of
hnRNP A1 and histone methylase activity (3), which has been shown now to derive from FDH. Taken together, these data suggest that the major protein methyltransferase activity present in rat liver cytosol resides in FDH.

Protein-arginine methyltransferase is one of several post-translational methylation reactions that requires AdoMet as the methyl donor. Several other classes of protein methyltransferase reactions, namely, protein-lysine, protein-histidine, and protein-carboxyl group methyltransferases (1, 2) as well as the enzymes that catalyze methylation of DNA and RNA (45, 46) uniformly require AdoMet as the methyl donor. AdoMet is one of the most important high energy compounds in vivo, next to ATP, and is biosynthesized from methionine, which in turn derives from homocysteine. The conversion of homocysteine to methionine is carried out via introduction of a methyl group that is derived from methyl-cobalamin (vitamin B12) whose methyl group in turn comes from 5-methyltetrahydrofolate. Tetrahydrofolate serves as the principal (and essential) carrier for single carbon units in a number of de novo biosynthetic pathways for purines and thymidine (47–49). The biological

![Comparative HPLC tryptic peptide maps of protein methylase I and FDH](image)

**FIG. 6.** Comparative HPLC tryptic peptide maps of protein methylase I and FDH. The polyacrylamide gel-purified enzymes (50 pmol each) were digested with trypsin and subjected to reverse HPLC as described under “Experimental Procedures.”

A, rat liver protein methylase I was isolated from ND and SDS-PAGE as indicated and was compared with the recombinant FDH purified via SDS-PAGE. B, the SDS-PAGE purified rat liver protein methylase I was compared with the recombinant and rat liver FDH that had been purified by SDS-PAGE as indicated.
role of FDH is not clear, however, since FDH has been suggested to serve as a regulatory mechanism to control the in vivo folate pool size by cleaving the formyl group from FTHF (50, 51), it is tempting to speculate that utilization of the methyl folate pool size by cleaving the formyl group from FTHF (50, 51), it is tempting to speculate that utilization of the methyl group of AdoMet via transmethylation may participate also in maintaining the intracellular concentration of one-carbon units in the cell.

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