Identification of the *S*-Adenosyl-L-methionine Binding Site of Protein-Carboxyl *O*-Methyltransferase Using 8-Azido-*S*-adenosyl-L-methionine†

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ABSTRACT: Protein-carboxyl *O*-methyltransferase (protein methylase II) transfers the methyl group from *S*-adenosyl-L-methionine (AdoMet) to the carboxyl side chains of the amino acids in the proteins. We have used the radiolabeled analogue of AdoMet, 8-azido-*S*-adenosyl-L-[*methyl*-^{3}H]methionine (8-N_3-Ado[*methyl*-^{3}H]Met), to investigate the AdoMet binding site of protein methylase II. The incorporation of the photoaffinity label in the enzyme upon UV irradiation is highly specific. In the absence of UV irradiation or if the photoprobe is irradiated prior to its addition to the reaction mixture, no photoinsertion of the label occurs. Moreover, the presence of a competitive inhibitor of protein methylase II, *S*-adenosyl-L-homocysteine (AdoHcy), or the unlabeled AdoMet itself in the reaction mixture diminished labeling of the enzyme. Sequential digestion of the labeled enzyme with trypsin, chymotrypsin, and endoproteinase Glu-C yielded a modified and radiolabeled decapeptide. When compared with the reported primary amino acid sequence of protein methylase II from rat brain, the amino acid composition of the decapeptide matched residues 113–121. This segment forms the midpoint region of the enzyme (234 amino acid residues). An important characteristic of the sequence is the presence of two adjacent aspartic acid residues (Asp^{117}-Asp^{118}) which most likely provide the negative charge environment for the sulfonium moiety of the AdoMet molecule.

Enzymes which catalyze the posttranslational methylation of proteins are widely distributed in nature (Paik & Kim, 1980, 1985, 1990). All these enzymes utilize *S*-adenosyl-L-methionine (AdoMet)[1] as a methyl donor but have different specificities toward substrate proteins as well as the amino acid residues. Protein-carboxyl *O*-methyltransferase [AdoMet:protein-carboxyl *O*-methyltransferase or protein methylase II; EC 2.1.1.24] methylates the side chain carboxyl groups of a variety of proteins (Kim et al., 1983; Clarke, 1985) and has been shown to exist as two or more isozymes in various tissues (Aswad & Deight, 1983; Gilbert et al., 1988). In brain and erythrocytes, this enzyme appears to recognize and methylate the L-isoaspartyl and D-aspartyl residues (Murray & Clarke, 1984; Aswad, 1984) and is thus believed to play a role in the repair or degradation of the proteins that have suffered spontaneous chemical damage (Aswad & Johnson, 1987). In addition, another form of protein methylase II (Springer & Koshland, 1977) which specifically methylates glutamyl residues has been shown to be involved in chemotaxis in *Escherichia coli* and *Salmonella typhimurium*.

This enzyme has been purified to near-homogeneity from many sources (Kim et al., 1983; Gilbert et al., 1988), and the amino acid sequence of the enzyme from rat brain (Sato et al., 1989) and human and bovine erythrocytes (Gilbert et al., 1988) has been deduced either by cDNA or by sequence analysis of the tryptic peptides of pure enzyme, respectively. However, the assignment of specific amino acids or the peptide sequence of the enzyme essential for AdoMet binding has remained obscure.

Azidopurine nucleotide photoaffinity labels have been used to identify the nucleotide binding sites of many enzymes (Haley & Hoffman, 1974; Bayley, 1983). Recently, Sumner et al. (1986) have demonstrated that 8-azido-*S*-adenosyl-L-methionine (8-N_3-AdoMet) could be used to photoaffinity-label the AdoMet binding site of methylenetetrahydrofolate reductase (EC 1.5.1.20). Here, we report the synthesis and use of 8-azido-*S*-adenosyl-L-[*methyl*-^{3}H]methionine (8-N_3-Ado-[*methyl*-^{3}H]Met) in studying the AdoMet binding site of protein-carboxyl *O*-methyltransferase (protein methylase II).

EXPERIMENTAL PROCEDURES

Materials. L-[*methyl*-^{3}H]Methionine (specific activity, 90 Ci/mmol) was obtained from Amersham Radiochemicals, Arlington Heights, IL. 8-Azido-ATP, TPCK-treated trypsin (chymotrypsin activity inhibited), TLCK-treated chymotrypsin (trypsin activity inhibited), *S*-adenosyl-L-methionine, and *S*-adenosyl-L-homocysteine were purchased from Sigma Chemical Co., St. Louids, MO. Endoproteinase Glu-C (protease V8) was from Boehringer Mannheim Biochemicals, Indianapolis, IN. The HPLC system including various columns was from Waters Associates, and HPLC-grade acetonitrile, methanol, and trifluoroacetic acid (TFA) were from J. T. Baker Co., Phillipsburg, NJ. The remaining chemicals were purchased from various commercial sources and were of the highest purity available.

Partial Purification of S-Adenosylmethionine Synthetase. AdoMet synthetase (ATP:L-methionine *S*-adenosyltransferase; EC 2.5.1.6) was partially purified from rabbit liver according to the method of Cantoni and Durell (1957). Seventy grams of liver freshly obtained from rabbit was homogenized in 175 mL of 0.01 M ice-cold acetic acid and centrifuged at 18000*g* for 30 min. To the supernatant was added half its volume of saturated $(NH_4)_2SO_4$ (saturated at room temperature), stirred gently for 2 h, and centrifuged again at 18000*g* for 30 min. The pellet was discarded, and the supernatant was similarly treated again with saturated

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[1] Abbreviations: AdoMet, *S*-adenosyl-L-methionine; AdoHcy, *S*-adenosyl-L-homocysteine; 8-N_3-Ado[*methyl*-^{3}H]Met, 8-azido-*S*-adenosyl-L-[*methyl*-^{3}H]methionine; TFA, trifluoroacetic acid.

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$(NH_4)_2SO_4$ solution. After 30 min, the mixture was centrifuged, and the precipitate was dissolved in 10 mL of 0.1 M phosphate buffer, pH 6.3. The suspension was then dialyzed for 4 h against a 50 mM aliquot of the same buffer. The protein concentration was adjusted to 8–10 mg/mL with water. Neutralized reduced glutathione was added to the suspension (2 mg/mL of the suspension), and the pH was adjusted to 5.1 with 1.0 M sodium acetate buffer, pH 4.9. The suspension was kept at 0 °C for 15 min and subsequently centrifuged. The precipitate was dissolved in approximately 5.0 mL of 50 mM phosphate buffer (pH 7.0) containing 2.0 mg of reduced glutathione per milliliter. The preparation had a specific activity of 20 units/mg of enzyme protein. The enzyme preparation could be stored at −70 °C for several days without loss of activity.

Synthesis of 8-N_3-Ado[methyl-^{3}H]Met. 8-N_3-Ado[*methyl*-^{3}H]Met was synthesized in the dark by the method of Sumner et al. (1986) with slight modification. The reaction mixture (initially 0.4 mL) contained 100 mM KCl, 30 mM $MgCl_2$, 30 mM HEPES buffer (pH 7.6), 10 mg of 8-N_3-ATP (15 μmol), 500 μCi of L-[*methyl*-^{3}H]methionine, and 400 nmol of nonradiolabeled L-methionine. The pH of the mixture was adjusted to 7.6 by dropwise addition of 2 M KOH. The reaction was initiated by the addition of 0.1 mL (6 mg of enzyme protein) of the above purified AdoMet synthetase. Further addition of reduced glutathione or 2-mercaptoethanol was avoided, because thiol reagents have been shown to degrade the azido compounds (Cartwright et al., 1976). Incubation was carried out in the dark for 3 h at 37 °C and terminated by the addition of 0.5 mL of 15% trichloroacetic acid (TCA). The 8-N_3-Ado[*methyl*-^{3}H]Met formed was purified on a Bio-Rex 70 column [0.7 cm (i.d.) × 5 cm] previously equilibrated with 10 mM phosphate buffer, pH 7.0. After the column was washed with 0.25 M acetic acid, the 8-N_3-Ado[*methyl*-^{3}H]-Met was eluted with 4 M acetic acid. Fractions containing the compound were pooled and lyophilized to remove acetic acid, and the final preparation was dissolved in 0.5 mL of water. Its concentration was estimated from its extinction coefficient of 13 300 at 282 nm (Kaiser et al., 1983). The overall recovery of 8-N_3-Ado[*methyl*-^{3}H]Met was around 90% on the basis of the L-methionine added. The specific radioactivity of the final product was 1.43×10^3 dpm/pmol of 8-N_3-Ado[*methyl*-^{3}H]Met.

Purification of Protein Methylase II. Protein methylase II was purified from calf brain according to the method described by Kim (1984).

Photoaffinity Labeling. All photoaffinity labeling procedures were carried out in the dark (Sumner et al., 1985). Routinely, 80 μg of protein methylase II was incubated with 20–40 μM 8-N_3-Ado-[*methyl*-^{3}H]Met in a total volume of 100 μL containing 125 mM citrate–phosphate buffer (pH 6.0) in a small Eppendorf tube cut to about 1.5–cm size and placed in a small tube stand. After 6 min of incubation at 37 °C, samples were irradiated for 15 min by placing a UV Mineralite (254 nm; Ultraviolet Products, Inc., San Gariel, CA) directly on top of the open tubes. To demonstrate the specificity of 8-N_3-Ado[*methyl*-^{3}H]Met binding, the photoaffinity labeling was also performed in the presence of AdoHcy [a potent competitive inhibitor for protein methyltransferases (Lawrence & Robert-Gero, 1990)] or AdoMet (a natural methyl donor). For quantitation of the amount of 8-N_3-Ado-[*methyl*-^{3}H]Met incorporated, 0.2 mL of 15% TCA was added into the incubation mixture after irradiation; the formed precipitate was washed several times with TCA and then dissolved in scintillation solution for radioactivity counting.

For autofluorographic analysis, the photolabeled enzyme prepared above was separated from the unbound 8-N_3-Ado-[*methyl*-^{3}H]Met by filtration through Centricon microconcentrators (Amicon) without TCA treatment. The enzyme was washed several times with 2 mL of water until the filtrate contained less than 1000 counts/mL. It was then subjected to gel electrophoresis (Laemmli, 1970). After being stained and destained, the gel was treated with Enhancer (NEN Research Product), dried, and exposed to Kodak X-Omat film at −70 °C.

Protein concentration was determined by the method of Lowry et al. (1951), using bovine serum albumin as standard.

Tryptic Digestion of Radiolabeled Enzyme and Subsequent Purification of 8-N_3-Ado[methyl-^{3}H]Met-Labeled Peptides on HPLC. Subsequent to separation of the photoaffinity-labeled enzyme from the unbound 8-N_3-Ado[*methyl*-^{3}H]Met by Centricon microconcentrations, the enzyme was subjected to tryptic digestion as described by DeLange et al. (1969). Briefly described, the enzyme was dissolved in 0.1 M ammonium bicarbonate buffer, pH 8.0. TPCK-treated trypsin dissolved in the same buffer was added (5 μg of trypsin per 50 μg of the enzyme protein), and the digestion was allowed to proceed for 16 h at 37 °C. The reaction was stopped by freezing and lyophilizing the reaction mixture. The samples were washed 4 times by lyophilization with water to remove the buffer and then dissolved in 0.1% TFA. Fifty-microliter aliquots were injected onto an HPLC μBondapak C_{18} column. Various peptide peaks were eluted with a linear gradient of acetonitrile containing 0.1% TFA (0–60% acetonitrile in 60 min of running time) at a flow rate of 1 mL/min. The elution was monitored at 214 nm, and the radioactivity was determined by counting 50-μL aliquots of the elution fraction. The fractions containing the radioactivity were pooled, lyophilized, and washed with water several times by repeated lyophilization. To purify the labeled peptide further, the lyophilized sample was dissolved in 0.1% TFA in water, reinjected into the column, and isocratically eluted with 0.1% TFA (pH adjusted to 3.0 with NaOH), and the pure radioactivity peak was collected.

Digestion of Trypsin-Generated Radiolabeled Peptide with Chymotrypsin. The trypsin-generated pure radiolabeled peptide obtained above was dissolved in 0.2 mL of 0.1 M ammonium bicarbonate buffer (pH 8.0), 5 μg of TLCK-treated chymotrypsin was added to the peptide sample, and the mixture was incubated for 20 h at 30 °C. Subsequently, it was frozen, washed 3 times by repeated lyophilization, and reconstituted with 0.1% TFA. Various peptides were resolved on HPLC by acetonitrile gradient elution; the radioactive peptide was collected and further purified by isocratic elution with 0.1% TFA, as described above for tryptic digestion.

Further Digestion of the Chymotryptic Peptide with Endoproteinase Glu-C. The radioactive peptide obtained from the chymotryptic digest was lyophilized and dissolved in 0.2 mL of 50 mM ammonium bicarbonate buffer, pH 7.8. Twenty-five micrograms of endoproteinase Glu-C dissolved in 20 μL of the above buffer was added, and the mixture was incubated for 16 h at 37 °C (Houmard & Drapeau, 1972). The reaction was terminated by the addition of 0.220 mL of glacial acetic acid, and the sample was injected onto the HPLC system after ammonium bicarbonate was removed by repeated lyophilization. Fractions containing radioactive peptide were collected, and the sample was further purified by isocratic elution on HPLC, as described above.

Analysis of the Amino Acid Composition of Purified Radiolabeled Peptide. The lyophilized and washed radiolabeled peptide generated by sequential digestion by trypsin, chymotrypsin, and endoproteinase Glu-C was hydrolyzed in

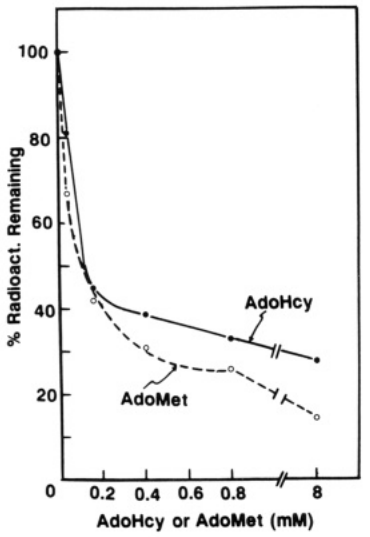

FIGURE 1: Specificity of 8-N_3-Ado[*methyl*-^{3}H]Met photoincorporation into protein methylase II. Eighty micrograms of partially purified protein methylase II was incubated at 37 °C with 74 μM 8-N_3-Ado[*methyl*-^{3}H]Met in 100 μL of reaction mixture containing 125 mM citrate–phosphate buffer (pH 6.0), 4 mM EDTA, and various concentrations of AdoHcy (●) or AdoMet (○) and subsequently irradiated with UV light for 15 min. Two hundred microliters of 15% TCA was added to precipitate the protein. The precipitate was washed 3 times with 15% TCA and once with ethanol and counted for radioactivity.

6 M HCl in a Pierce digestion tube at 110 °C for 22 h. The amino acid analysis was performed on an HPLC Resolve C_{18} column using method 2 analysis (Amino Acid Analysis System, Operator's Manual, 1984; or Park et al., 1986).

RESULTS

Specificity of Photoaffinity Labeling of Protein Methylase II with 8-N_3-Ado[methyl-^{3}H]Met. Preliminary studies showed that protein methylase II was specifically labeled when incubated with 20 μM 8-N_3-Ado[*methyl*-^{3}H]Met followed by irradiation with UV light, maximum labeling occurring with 15 min of irradiation (data not shown). No labeling was observed when UV irradiation was omitted after incubation or when the azido compound was first irradiated and subsequently introduced to the reaction mixture.

As shown in Figure 1, increasing concentrations of AdoHcy or AdoMet in the reaction mixture resulted in diminished labeling of protein methylase II by 8-N_3-Ado[*methyl*-^{3}H]-Met. For further studies on the specificity of 8-N_3-Ado-[*methyl*-^{3}H]Met binding sites, the ligand-bound enzyme was separated from the unbound 8-N_3-Ado[*methyl*-^{3}H]Met by filtration through a Centricon microconcentrator, and the samples were analyzed by polyacrylamide gel electrophoresis (PAGE) in both the presence and absence of sodium dodecyl sulfate (SDS) (Figure 2). It is clear from the inset of the figure (lanes a and b; denaturing condition) that among the several Coomassie-stained bands only a single band corresponding to a molecular weight of 25 000 was found to have radioactivity incorporated [the molecular weight of rat brain protein methylase II has been calculated to be 24 626 from its primary structure deduced from the cDNA sequence (Sato et al., 1989)]. Nondenaturing gel electrophoresis (main figure of Figure 2), however, revealed more than one autofluorographic band (lane A). This is most likely due to the aggregation of the protein methylase II enzyme molecule, since aggregates of various size classes of this enzyme have been noted earlier (Kim, 1984). This radioactivity labeling was inhibited by the simultaneous presence of either AdoHcy (lanes B and C) or AdoMet (lanes D and E).

Therefore, the results shown in Figure 1 and 2 convincingly demonstrate that photoaffinity labeling with 8-N_3-Ado-[*methyl*-^{3}H]Met is highly specific toward protein methylase II, targeting at the AdoMet binding sites. It was observed

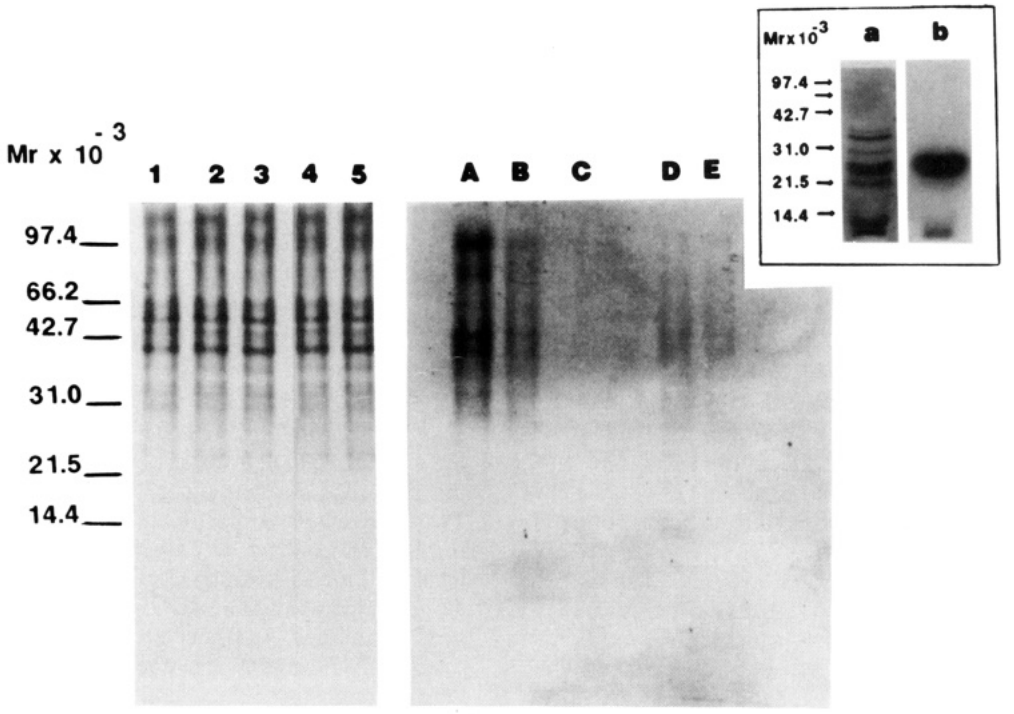

FIGURE 2: Polyacrylamide gel electrophoresis of protein methylase II photoaffinity-labeled with 8-N_3-Ado[*methyl*-^{3}H]Met in the presence of AdoHcy and AdoMet. Reaction conditions were the same as described for Figure 1, except that after irradiation TCA was not added but the unbound 8-N_3-Ado[*methyl*-^{3}H]Met was separated from the bound by Centricon filtration. *Inset*: SDS–PAGE of photolabeled enzyme. Lane a shows the Coomassie-stained bands, and lane b is its autofluorograph showing a single band at $M_r \sim 24\,000$. *Main figure*: Nondenaturing gel electrophoresis of 80 μg of partially purified protein methylase II photolyzed in the presence of 40 μM 8-N_3-Ado[*methyl*-^{3}H]Met plus the following additions. Lane 1, none; lane 2, 0.2 mM AdoHcy; lane 3, 8 mM AdoHcy; lane 4, 0.2 mM AdoMet; lane 5, 8 mM AdoMet. Lanes 1–5 are Coomassie-stained bands, and lanes A–E represent their corresponding autofluorograms.

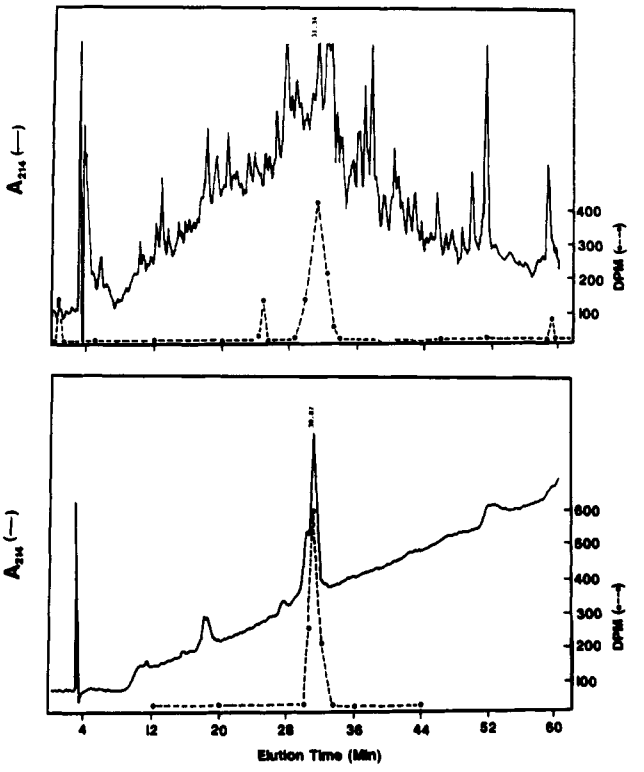

FIGURE 3: Elution of tryptic peptides of 8-N_3-Ado[*methyl*-^{3}H]Met-labeled protein methylase II. 1.2 mg of partially purified protein methylase II was photolabeled according to the procedure described for Figure 1. Unbound label was separated from protein-bound label on a Sephadex G-25 column [0.8 cm (i.d.) × 90 cm] equilibrated with 20 mM phosphate buffer (pH 7.0). Fractions containing the labeled enzyme were pooled, dialyzed against water, and lyophilized. They were dissolved in ammonium bicarbonate buffer. Tryptic digestion and HPLC analysis were carried out by the procedures described under Experimental Procedures. The top panel shows the elution profile for the first analysis and the bottom panel for rechromatography (isocratic elution), showing a radioactive peak at 30.87-min retention time.

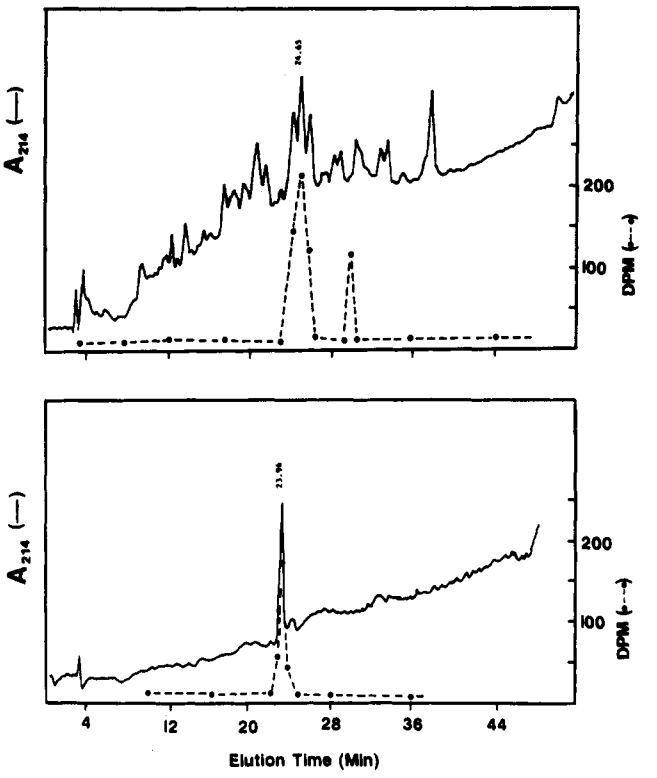

FIGURE 4: HPLC analysis of the chymotryptic digest of the purified tryptic peptide of protein methylase II (shown in Figure 3). Detailed procedures are described under Experimental Procedures and in Figure 1. The top panel shows the first run on HPLC (gradient elution), and the bottom panel shows the second run (isocratic elution) of the peptide which eluted at 24.65 min (top panel). In the bottom panel, the peak has a retention time of 23.96 min.

that 7.6 pmol of 8-N_3-Ado-[*methyl*-^{3}H]Met was incorporated in 18 pmol of the enzyme. Thus, 42% of the enzyme molecules were apparently modified.

Isolation of the 8-N_3-Ado[methyl-^{3}H]Met-Labeled Peptide. In order to investigate the 8-N_3-Ado[*methyl*-^{3}H]Met binding site, the ligand-bound enzyme was separated from the unbound 8-N_3-Ado[*methyl*-^{3}H]Met by Centricon filtration. The radiolabeled enzyme was dissolved in ammonium bicarbonate buffer and subjected to tryptic digestion. HPLC separation of the trypsin-treated enzyme yielded numerous peptides (Figure 3). Practically all of the radioactivity, however, was found along with the peptide eluted at 31 min (top panel of Figure 3). The small radioactive peak at 25 min could be either due to the unbound 8-N_3-Ado[*methyl*-^{3}H]Met remaining even after several washings during Centricon filtration or due to the dissociated radioactivity (more description below). The fractions containing the most radioactivity were pooled, lyophilized, and rechromatographed to further purify the photolabeled peptide. This was achieved by isocratic elution with 0.1% TFA on HPLC (lower panel).

The purified peptide from the tryptic digest obtained after rechromatography was subjected to chymotryptic digestion. Figure 4 shows the HPLC profile of the chymotryptic digest. A single large radioactive peak was obtained whose retention time changed to 24.60 min, indicating a change in peptide size and composition. A small radioactive peak appeared at 28 min. This is most likely due to the dissociation of some radioactive ligand since photolabeling studies with another enzyme, AdoMet:protein-arginine *N*-methyltransferase (EC 2.1.1.23), yielded a similar small peak at this position (data not shown). The radiolabeled peptide was further purified by isocratic elution over an HPLC column (lower panel of Figure 4).

The purified chymotryptic peptide obtained by isocratic elution above was further digested with endoproteinase Glu-C. This enzyme cleaves the peptide bond at the carboxyl side of the glutamyl residue (Houmard & Drapeau, 1972). Endoproteinase Glu-C digestion of the above peptide produced three peptide peaks, and radioactivity was associated with the peptide eluting at 18.00 min (Figure 5). Radioactive fractions were pooled, lyophilized, and isocratically rechromatographed to yield a pure radioactive peptide eluting at 16.55 min (lower panel of Figure 5). This pure peptide was stored for amino acid analysis.

Amino Acid Composition of the 8-N_3-Ado[methyl-^{3}H]Met-Bound Peptide and Its Comparison with the Reported Amino Acid Sequence of Protein Methylase II. HPLC-purified radiolabeled peptide derived from sequential digestions by trypsin, chymotrypsin, and endoproteinase Glu-C was subjected to amino acid analysis on HPLC using a precolumn *o*-phthaldialdehyde derivatization method. To standardize the method, the amino acid composition of bovine serum albumin was also determined and compared with the reported value. As shown in Table I, the values are quite close to the reported values with the maximum variation of ±10% with acidic amino acids. It is obvious in the table that the peptide has 10 amino acid residues (decapeptide).

We have tried to sequence the radiolabeled peptide with our own sequence facility or with the commercial source, but failed to detect the free N-terminus to initiate the sequencing. Alternately, however, comparison of the amino acid composition of the labeled decapeptide with the reported sequence

Table I: Amino Acid Composition of Peptide Derived from Protein Methylase II Photoaffinity-Labeled with 8-N_3-Ado[*methyl*-^{3}H]Met

	bovine serum albumin		photoaffinity-labeled peptide		
amino acid residues	obsd	reported[a]	obsd amount (pmol/sample)	min no. of residues	no. of residues
aspartic + asparagine	52 ± 0.7[b]	48	43.8[c]	2.23	2
glutamic + glutamine	83 ± 1.0	75	20.5	1.05	1
serine	28 ± 0.6	28	22.3	1.14	1
histidine	16 ± 0.6	17	0	0	0
glycine	16 ± 1.0	15	0	0	0
threonine	33 ± 0.5	34	26.7	1.36	1
arginine	23 ± 0.6	23	0	0	0
alanine	42 ± 0.6	46	23.5	1.20	1
tyrosine	18 ± 0.4	19	0	0	0
methionine	4 ± 0.0	4	0	0	0
valine	31 ± 0.0	36	19.6	1.00	1
phenylalanine	26 ± 0.0	26	0	0	0
isoleucine	14 ± 0.0	14	24.0	1.23	1
leucine	58 ± 2.2	61	20.4	1.04	1
lysine	61 ± 3.5	59	22.0	1.12	1
total					10

[a] Hunt et al. (1975). [b] The values are the average of three independent determinations. [c] The values are the average of two independent determinations.

Scheme I: Amino Acid Sequence of the Postulated AdoMet Binding Site of Protein Methylase II

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Human erythrocytes ...   -Ile-Lys-Glu-Leu-Val-Asp-Asp-Ser-Val-Asn-Asn-
    (Gilbert et al.)                    (115)              (120)
Bovine erythrocytes ...  -Ile-Lys-Glu-Leu-Val-Asp-Asp-Ser-Ile-Asn-Asn-
    (Gilbert et al.)
Rat brain          ...   -Ile-Lys-Glu-Leu-Val-Asp-Asp-Ser-Ile-Thr-Asn-
    (Sato et al.)
Calf brain                  Lys-Glu-Leu-Val-Asp-Asp-Ser-Ile-Thr-
    (Ours)
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of rat brain protein methylase II (Sato et al., 1989) revealed the presence of a sequence between residues 113 and 121 (9 residues) that exactly matched the amino acid composition of the above decapeptide (Scheme I). This is the only sequence found to have identical amino acid composition as the decapeptide.

DISCUSSION

Photoaffinity labeling has emerged as a powerful tool for studying enzyme structure–function relationships. Its main advantage over chemical affinity is that the photoaffinity reagents are chemically inert but produce highly reactive intermediates when irradiated with UV light. Aryl azide and diazo compounds are the best examples of such reagents (Bayley, 1983). Upon photolysis, the 8-azido analogues of purine nucleotides are converted to nitrenes, which are highly reactive species. They instantly react and bind covalently to the nucleophilic groups present in the immediate vicinity, releasing nitrogen gas (Czarnecki et al., 1979). Haley and Hoffman (1974) were the first to successfully synthesize and utilize azido-ATP for photolabeling membrane-bound Na^+–K^+-ATPase and Mg^{2+}-ATPase. Kaiser et al. (1983) synthesized and characterized the 8-azido derivative of *S*-adenosyl-L-methionine and demonstrated its usefulness as a photoprobe by specifically photolabeling catechol *O*-methyltransferase (EC 2.1.1.6) in a mixture of proteins as well as in the purified preparation.

Since AdoMet is a methyl donor for all protein methyltransferase reactions and the mechanism of such a reaction is still not well understood, it seemed to be of importance to investigate the AdoMet binding site of one of the protein-specific methyltransferases which was identified in this laboratory. The binding of 8-N_3-Ado[*methyl*-^{3}H]Met to protein methylase II in the present study is highly specific. It can be seen in the inset of Figure 2 that among several Coomassie-stained bands only a single band, was extensively photolabeled with 8-N_3-Ado[*methyl*-^{3}H]Met (lane b). The simultaneous presence of AdoMet or AdoHcy greatly reduced the photolabeling, proving that the protein methylase II is specifically labeled at the AdoMet binding site. This contention is further strengthened by the observation that the tryptic digest of the photolabeled enzyme produced primarily a single radiolabeled peptide. Sequential digestions of this labeled peptide by trypsin, chymotrypsin, and endoproteinase Glu-C produced an 8-N_3-Ado[*methyl*-^{3}H]Met-labeled decapeptide.

The K_i values of AdoMet and AdoHcy, as calculated from the data of Figure 1, are approximately 1.1×10^{-4} M. It is of interest to note that both compounds have almost identical K_i values. This is not unexpected, since they both are very similar compounds, differing only by a methyl group. Moreover, the inhibition of photoincorporation by these two compounds is in accord with their K_i values, since they inhibit the photolabeling only about 50% at 2.0×10^{-4} M as is clear in Figure 2. At higher concentrations, they inhibit this incorporation completely.

Our efforts to sequence the peptide failed presumably due to a blocked N-terminus. Fortunately, however, the complete amino acid sequence of protein methylase II is available from different tissues and species, reported by two independent groups (Gilbert et al., 1988; Sato et al., 1989). When we compared the composition of the decapeptide with the amino acid sequence of the enzyme, we found that 9 out of 10 amino acids matched the sequence of residues 113–121 (Scheme I). Of the 234 amino acid residues of protein methylase II, this segment is the only one with the amino acid composition of the decapeptide. We are at a loss to place alanine residues

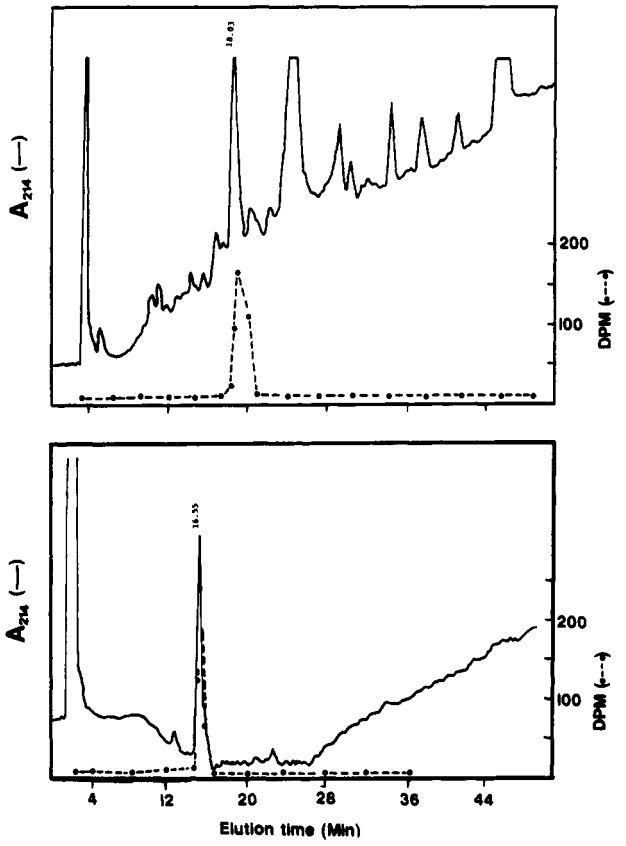

FIGURE 5: HPLC analysis of the endoproteinase Glu-C digest of the radiolabeled peak isolated in Figure 4. Detailed experimental conditions are described under Experimental Procedures. The top panel shows the first run and the bottom panel the second run on HPLC, with the radiolabeled peptide eluting at 16.55 min.

in the sequence of protein methylase II (see Table I and Scheme I). It is quite possible that 8-N_3-Ado[*methyl*-^{3}H]Met might have been bound to the N-terminal residue of the decapeptide, thus interfering with sequence determination. On the other hand, covalently bonded amino acid residues and the ligand complex underwent a characteristic change during acid hydrolysis, and it appeared as an alanine on HPLC analysis.

An interesting characteristic of this peptide segment is the presence of two adjacent aspartyl residues (residues 117 and 118). Since the adenine base and the sulfonium ion of the AdoMet are considered to play important roles in the interaction of AdoMet with its binding site (Lawrence & Robert-Gero, 1990), it is quite possible that the Asp-Asp residues offer the perfect negative charge environment for the methyl sulfonium moiety. A similar diaspartyl residue is also present at positions 126 and 127 of the protein methylase II sequence (Sato et al., 1989). However, the lysine residues present just adjacent to them (Lys-Lys-Asp-Asp) most likely neutralize the negative charge of the di-Asp groups. These observations and the fact that only a single small peptide is labeled help strengthen our contention that this peptide forms part of the AdoMet binding domain. Furthermore, it is of interest to note that this segment (residues 113–121) forms exactly the midpoint of the primary structure of the enzyme (234 amino acid residues). What structural significance, if any, does this offer and how common is the occurrence of this decapeptide in other protein methyltransferases are worth studying further. The only other detailed studies on the AdoMet binding site were reported recently by Reich and Everett (1990) working with *Eco*RI DNA methylase, and by Som and Friedman (1991) with *Eco*RII DNA methylase. However, no discernible common feature was found among the AdoMet binding sequences.

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