REGULATION OF TRANSMETHYLATION BY AN S-ADENOSYLMETHIONINE BINDING PROTEIN*

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SUMMARY. A protein which has a high affinity for S-adenosylmethionine (SAM) has been partially purified from rat liver. This binding protein stimulates both the rate and extent of product formation when added to both a lipid methylating system, phosphatidylethanolamine:SAM-N-methyltransferase, and an RNA methylating system, the t-RNA methylase complex from rat liver. The S-adenosylmethionine binding protein by itself has no enzymatic activity in either transmethylation system.

S-adenosylmethionine† plays a central role in almost all biochemical transmethylations (1). The methyl group can be transferred enzymatically to proteins (2), lipids (3,4) DNA (5), RNA (5,6) and many low-molecular weight metabolites resulting in important modifications in structure and function.

The processes of regulation are important in the study of transmethylation. Transmethylation may be inhibited by S-adenosylhomocysteine, one of the products of all SAM-utilizing enzymes (1), or by other enzyme systems which compete for the substrate SAM (7). Activation of various methylation systems can be achieved by nucleoside triphosphates (8), polyamines (9) or by changes in metabolic state, e.g., developmental (6,7), hormonal (6) or viral (6,10).

Binding proteins have been found for many low-molecular weight compounds

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†Abbreviations: SAM, S-adenosylmethionine; TCA, trichloroacetic acid.

(for example, sterol carrier protein (11)) which are not enzymatically active themselves, yet appear to play a role in transport or in regulation. It was felt that such a binding protein might exist for S-adenosylmethionine. Evidence for such a protein is presented in this paper.

MATERIALS AND METHODS

[^{3}H-CH$_3$]-S-adenosylmethionine (11.9 Ci/mmole) was obtained from New England Nuclear Co. and unlabelled SAM from Calbiochem. Ribonuclease A was obtained from Worthington. Protein was determined by the method of Lowry et al. (12).

RNA methylation: the t-RNA methylase system from rat liver was prepared by the method of Pegg (9) and was assayed by the filter disc method as described by Pegg (9). Sodium chloride insoluble RNA was prepared from crude Torula RNA (Calbiochem) by the method of von Ehrenstein (13).

Lipid Methylation: Rat liver microsomal fraction was the source of phosphatidylethanolamine: SAM-N-methyltransferase which was assayed by the method of Goldfine (14). Phosphatidyldimethylethanolamine was prepared from egg lecithin as described by Stoffel, et al. (15).

Preparation of the SAM Binding Protein: Rat livers from six-week old male Sprague-Dawley rats were homogenized for 30 sec in 9 volumes of 10mM Tris-HCl, pH 7.6, 0.25 M Sucrose in a Waring blender. The homogenate was centrifuged for 15 min at 13,000g in a Sorvall RC-2B centrifuge. The supernatant was then centrifuged for 1 hr at 105,000g in a Spinco L3-50 centrifuge. The 105,000g supernatant was then fractionated with ammonium sulfate. The 15-40% fraction was dissolved in 100mM Tris-HCl, pH 7.6, dialyzed overnight against the same buffer, then separated on a column of Sephadex G100 (4x50 cm).

Assay of the binding protein: A modification of the method of de Plazas and de Robertis (16) was used in which [^{3}H-CH$_3$]-SAM and 100µl of a fraction from the Sephadex column were mixed in a volume of 200µl (final concentrations, 50mM Tris-HCl pH7.6, 0.04µM SAM, 0.1µCi) and separated on columns of Sephadex G-25. The protein and bound SAM eluted in the void volume and were separated from the unbound SAM which was retarded by the column.

RESULTS AND DISCUSSION

The rationale for detection and purification of an SAM binding protein was that such a protein would have a high affinity for SAM. Many enzymes utilize SAM as a substrate and hence bind SAM. By using low levels of SAM (0.04µM) in the assay procedure, it has been possible to partially purify an SAM binding protein with a high affinity for SAM (Kd = 3.8×10^{-7}M)(Fig. 1). Since there is dissociation of the protein-SAM complex on the Sephadex G25 columns, this value for the dissociation constant is probably too large.

The purification of the binding protein shown in Table I represents a 370-fold purification, based on protein. The retained peak (fractions 29-38)

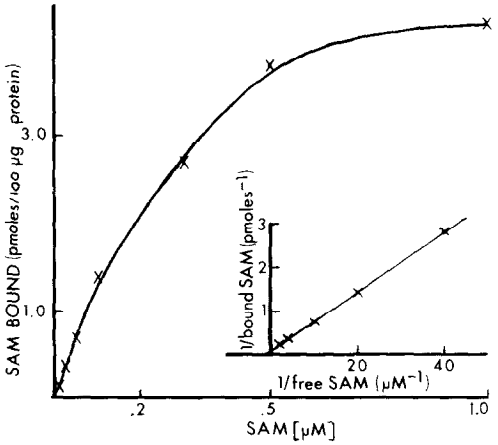

Fig. 1. Binding of SAM by SAM binding protein from the Sephadex column. Amount of SAM bound vs. SAM concentration as assayed on Sephadex G25 columns.

Table I

Purification of SAM Binding Protein

	Protein (mg)	SAM bound/mg protein (cpm)	Purification (fold) by protein	by activity
Homogenate	25,000	--	--	-
100,000g supernatant	2,850	800	9	-
15-40% $(NH_4)_2SO_4$	1,400	1,200	18	1.5
Sephadex fractions 29-38	68	12,900	350	16

on the Sephadex column (Fig. 2) elutes with a molecular weight on the order of 70,000. The binding shown in the figure is given as cpm SAM per 100µl column fraction. On a protein basis the peak fraction of the binding protein binds 14,000 cpm/mg while the void volume peak binds only 850 cpm/mg. The purification as measured by binding activity does not give a true picture because of interference by other proteins, particularly at early stages of purification. The binding protein from the column was lyophilized, dissolved in 10mM Tris-

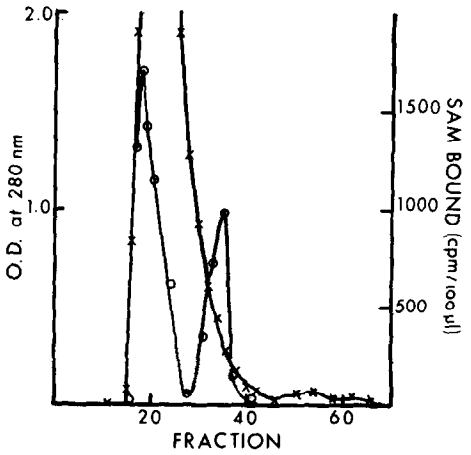

Fig. 2. Separation of SAM Binding protein on the Sephadex G100 column. Protein, X—X; SAM binding, O—O.

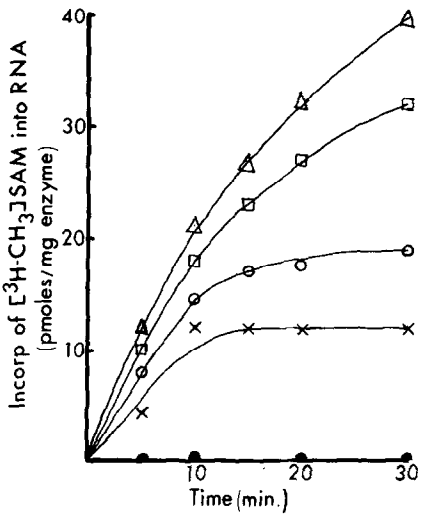

Fig. 3. Incorporation of the CH_3 group from [^{3}H-CH_3] SAM into RNA. The assay mixture contained: 1 mg enzyme protein, 0.1M Tris-HCl pH 8.6, 0.25M ammonium acetate, 0.3mM mercaptoethanol, and 13µM SAM, 0.1µCi in a volume of 150µl. 250µg of RNA and/or 100µg SAM binding protein were added as indicated. The incubation was carried out at 37°C. Basic assay mixture, X—X; binding protein added, O—O; RNA ADDED, □—□ ; RNA and binding protein added, Δ—Δ; RNA and binding protein without enzyme, ●—●.

HCl pH 7.6 and frozen in small aliquots. The binding protein could be stored at -20°C for at least six months with no loss of binding capacity or acti-

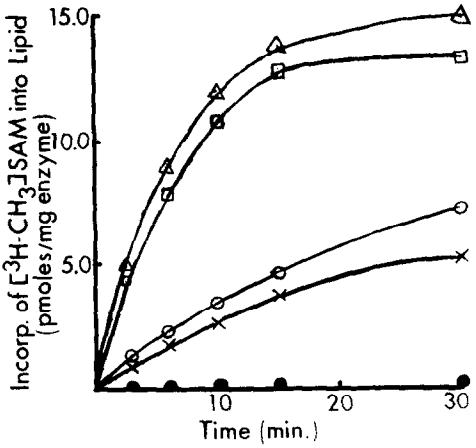

Fig. 4. Incorporation of the CH_3 group from [^{3}H-CH_3] SAM into lipid. The assay mixture contained: 1 mg microsomal protein, 0.1M Tris-HCl pH 8.6 and 0.2µM SAM, 0.1µCi in a volume of 200µl. 100µg of phosphatidyldimethylethanolamine and/or 100µg binding protein were added as indicated. The lipid was dispersed by sonification in the assay mixture before addition of the enzyme. Basic assay mixture, X—X; binding protein added, O—O; lipid added, □—□; binding protein and lipid added, Δ—Δ; lipid and binding protein without enzyme, ●—●.

vating activity. Examination of this fraction by polyacrylamide gel electrophoresis showed that at least 12 proteins were still present at this stage.

The binding protein was tested for its ability to interact with several different methylation systems: t-RNA methyl transferase complex (Fig. 3) and phosphatidylethanolamine: SAM-N-methyltransferase (Fig. 4). In both systems, addition of the SAM-binding protein caused an increase both in the rate and extent of methylation. No enzymatic activity was shown by the binding protein if only the binding protein and either RNA or lipid was present.

The product of the t-RNA methylase complex was characterized as RNA by the following criteria: 1) treatment of the reaction mixture with hot 5% TCA (9) resulted in complete solubilization of the radioactivity; 2) incubation of the reaction mixture for 1 hour with ribonuclease A prevented the cold TCA precipitation of the radioactivity. The specific nucleotides formed have not yet been examined to determine if the stimulatory effect of the binding protein is on the formation of all the methylated nucleotides or only on a few.

The lipid formed in the phosphatidylethanolamine-N-methyl transferase assay was extracted by the procedure of Folch et al. (17), chromatographed on Silica Gel G thin layer plates in a solvent system of $CHCl_3$:CH_3OH:H_2O::65:25:4 and scanned with a Berthold radiochromatogram scanner. All of the radioactive product migrated with authentic phosphatidylcholine.

Because of the number of enzymes which have S-adenosylmethionine as a substrate, it can not be ruled out that the SAM binding protein characterized here may eventually prove to have some enzymatic activity. Nonetheless, the ability to interact with such diverse methylating systems as those which methylate lipids and RNA, makes this protein a candidate for a regulatory protein affecting transmethylation.

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