INHIBITION OF PHOSPHATIDYLETHANOLAMINE BIOSYNTHESIS BY *S*-ADENOSYLMETHIONINE

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1. Introduction

The *N*-methylation of phosphatidylethanolamine represents one of the well-known pathways for the biosynthesis of phosphatidylcholine [1]. It has been reported [2] that this methylation might also occur in vivo on ethanolaminephosphate and CDP-ethanolamine. In studies dealing with the methylation of these precursors [3] we found that the in vitro incorporation of ethanolaminephosphate into rat liver phospholipids was inhibited by *S*-adenosylmethionine (SAM). We thus decided to investigate the action of SAM on one of the enzymes responsible for their biosynthesis: the ethanolaminephosphate cytidylyltransferase (CTP: ethanolaminephosphate cytidylyltransferase, EC 2.7.7.14).

2. Materials and methods

2.1. *Chemicals*

CTP, *S*-adenosyl-L-methionine, *S*-adenosyl-L-ethionine, *S*-adenosyl-L-homocysteine were from Sigma Chemical Company, St-Louis. *S*-adenosyl-L-[*methyl*-^{14}C]methionine and phosphoryl-[2-^{14}C]-ethan-1-ol-2- amine were obtained from Radiochemical Centre, Amersham, England.

2.2. *Preparation of the enzymatic fraction*

The enzyme preparation was enriched in ethanolaminephosphate cytidylyltransferase by precipitation from rat liver post-microsomal supernatant with ammonium sulphate between 25 and 38% of saturation, as described previously [4].

Abbreviation: SAM = *S*-adenosylmethionine

2.3. *Analytical methods*

Proteins were determined by the method of Lowry et al. [5] and phosphorus by the method of Chen et al. [6].

2.4. *Incubations*

2.4.1. Assay of ethanolaminephosphate cytidylyltransferase

The standard incubation mixture contained in a volume of 0.5 ml, 35 mM Tris–maleate buffer (pH 7.7), 10 mM $MgCl_2$, 3 mM CTP, 0.16 mM [^{14}C]-ethanolaminephosphate (AS = 1.12×10^6 dpm/μmol), 0.45 mg protein. SAM and the other effectors were dissolved in the Tris–maleate buffer (pH 7.7) and added at various concentrations as indicated under Results.

Incubations were at 37°C for 15 min and enzymatic activity was determined as reported previously [4].

2.4.2. Study of protein methylation

The incubation mixture contained 30 mM Tris–maleate buffer (pH 7.7), 10 mM $MgCl_2$, 0.036 mM *S*-adenosyl-L-[*methyl*-^{14}C]methionine, 0.8 mg protein, in a final volume of 0.5 ml.

Incubations were performed at 37°C for different times. The reaction was stopped by adding 0.5 ml of 30% trichloracetic acid. The protein residue was then treated according to the method described by Paik et al. [7] to remove any possible contamination due to nucleic acids and phospholipids. The pellet was then dissolved in 1 ml of 0.01 N NaOH and counted

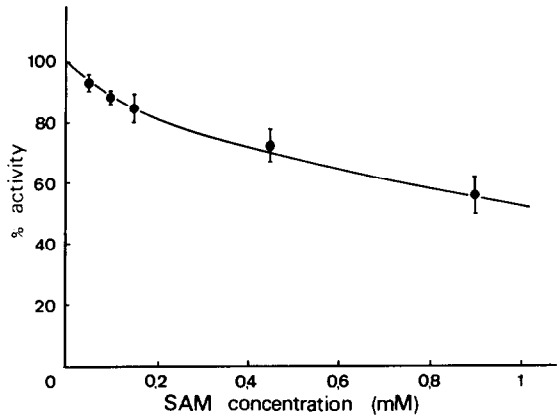

Fig.1. Effect of varying SAM concentration on CDP-ethanolamine synthesis. Assays were performed as described in Materials and methods. The points represent mean values from 4–7 determinations.

in 10 ml of Insta-Gel (Packard) to determine the incorporated radioactivity. The blank was prepared by adding the proteins after the addition of trichloracetic acid.

3. Results

3.1. *Effect of varying SAM concentration on the biosynthesis of CDP-ethanolamine*

In the presence of SAM, the activity of ethanolaminephosphate cytidylyltransferase was decreased (fig.1). The percentage of inhibition was proportional to the concentration and reached about 50% with 1 mM SAM. Total inhibition could be obtained for SAM concentrations varying between 2 and 3 mM (not shown here).

Table 1
Effect of various compounds on ethanolaminephosphate cytidylyltransferase activity

Additions	% Inhibition
SAM 0.15 mM	16 ± 4 (5)
0.90 mM	44 ± 6 (7)
1.20 mM	61 ± 2 (3)
SAH 0.15 mM	3 ± 2 (5)
0.90 mM	4 ± 4 (5)
SAE 0.90 mM	3 ± 2 (3)
1.20 mM	5 ± 3 (3)
ATP 0.90 mM	1 ± 2 (3)
1.20 mM	3 ± 2 (3)

Experimental conditions are described in Materials and methods. Values are means ± S.D. The numbers of determinations are in parentheses. (SAH = *S*-adenosylhomocysteine, SAE = *S*-adenosylethionine.)

3.2. *Comparative effects of SAM and structural analogues on the activity of ethanolaminephosphate cytidylyltransferase*

In order to determine the specificity of this inhibition, different compounds were compared with SAM for their ability to inhibit the synthesis of CDP-ethanolamine (table 1). At the concentra-

Table 2
Effect of *S*-adenosylhomocysteine and *S*-adenosylethionine additions on the inhibition of ethanolaminephosphate cytidylyltransferase by SAM

Additions	CDP-ethanolamine synthesized (nmoles/min/mg protein)	Inhibition (%)
none	7.4	
SAM	4.1	44
SAM + SAH (0.45 mM)	4.3	42
SAM + SAH (0.9 mM)	4.1	44
SAM + SAH (1.5 mM)	4.3	42
SAH (1.5 mM)	7.4	
SAM + SAE (0.45 mM)	4.1	44
SAM + SAE (0.9 mM)	4.1	44
SAM + SAE (1.5 mM)	4.1	44
SAE (1.5 mM)	7.3	2

The assays were carried out as described in Materials and methods. SAM concentration was 0.9 mM. (SAH = *S*-adenosylhomocysteine, SAE = *S*-adenosylethionine.)

tions studied, *S*-adenosylhomocysteine, *S*-adenosylethionine and ATP had no significant action on ethanolaminephosphate cytidylyltransferase. Furthermore, neither *S*-adenosylhomocysteine nor *S*-adenosylethionine influenced the inhibitory effect of SAM (table 2).

3.3. *Time dependence of SAM action*

The inhibition by SAM of ethanolaminephosphate cytidylyltransferase during the course of the reaction is represented in fig.2. While a partial decrease of the enzymatic activity was found immediately after the addition of SAM, the inhibition rate increased progressively and the reaction stopped completely after 20 min.

The effect of the time was then studied in experiments where the proteins were preincubated with SAM before measuring the enzymatic activity. As shown in fig.3, preincubation of the proteins with SAM between 0 and 60 min resulted in an increase of the inhibition from 43–74%, whereas the activity of the non-treated enzyme remained constant after preincubations for the same times. Such an increase did not occur when SAM was preincubated with [^{14}C]ethanolaminephosphate and CTP alone.

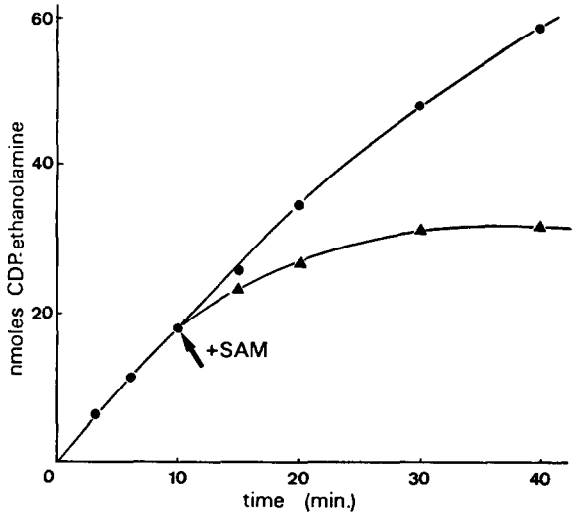

Fig.2. Time course of the inhibition of ethanolaminephosphate cytidylyltransferase by SAM. Assays were carried out as described in Materials and methods except proteins: 0.2 mg. SAM (0.9 mM) was added 10 min after starting the reaction, where indicated. (-●-) no addition, (-▲-)+ 0.9 mM SAM.

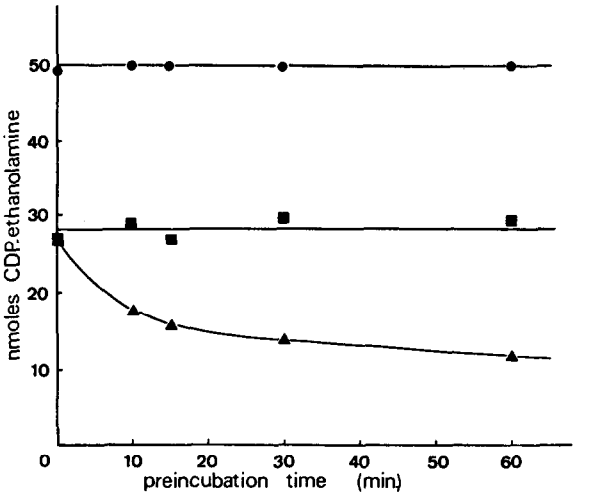

Fig.3. Influence of preincubation time on the inhibition rate of ethanolaminephosphate cytidylyltransferase by SAM. (-●-) Assays performed without SAM; preincubation with proteins prior to addition of [^{14}C]etanolaminephosphate. (-■-) Preincubations of SAM (0.9 mM) with [^{14}C]ethanolaminephosphate prior to addition of proteins. (-▲-) Preincubations of SAM (0.9 mM) with proteins, prior to addition of [^{14}C]ethanolaminephosphate. Incubation time was 15 min. Experimental conditions are described in Materials and methods. Data are representative of three separate experiment.

3.4. *Methylation of the protein fraction*

When [*methyl*-^{14}C]SAM was added to the enzymatic fraction, a time-dependent incorporation of [^{14}C]methyl into the proteins occurred (fig.4).

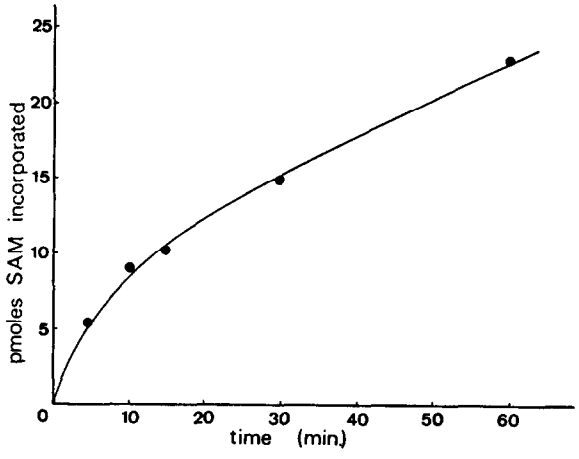

Fig.4. Binding of *S*-adenosyl-L-[*methyl*-^{14}C]methionine with proteins. Experimental procedures are described in Materials and methods.

4. Discussion

SAM is known to modify the activity of several enzymes [8–11]. The present data provide evidence that SAM decreases the activity of ethanolaminephosphate cytidylyltransferase. Although the significance of this finding relative to phospholipid biosynthesis under physiology conditions was not investigated here, we tried to elucidate the mechanism of such an action. Comparison of SAM with some structural analogues (table 1) indicates that the methyl groups might be involved in the observed changes. Noteworthy, the SAM effect goes to completion in a time-dependent way. Taken together, these results suggest the idea that SAM might act through a methylation of ethanolaminephosphate cytidylyltransferase, transforming it into an inactive form. This view is further supported by the fact that the enzymatic fraction can be methylated by incubation in the presence of SAM. In this case, the methylation of ethanolaminephosphate cytidylyltransferase by SAM would occur through a non-enzymatic reaction, since *S*-adenosylhomocysteine, a classical inhibitor of methyltransferases [12,13] does not modify the effect of SAM on this enzyme (table 2). Although this mechanism cannot be unequivocally proved in the present study, it is interesting to notice that non-enzymatic methylation of proteins has been recently reported by Paik et al. [14].

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