the decrease of $d\Phi°/_{dt}$ is of the order: $BrO_3^- > IO_3^- > SO_4^{2-}$. Strong electrostatic solvation (primary solvation) is responsible for the decrease of $d\Phi°/_{dt}$[10,11]. So the ion-solvent interaction is of the order: $BrO_3^- > IO_3^- > SO_4^{2-}$.
Further the plot of $\Phi°$ vs $1/\varepsilon$ (ε, the reciprocal of the dielectric constant) is found to be linear for all the salts. The slope of the lines are of the order $BrO_3^- > IO_3^- > SO_4^{2-}$.

Conductance. The equivalent conductance of all the 6 salts are found to be linear with $C^{1/2}$, which indicates that the Debye-Huckel-Onsager theory of electrolytic conductivity is applicable to these ions. The walden product $\Lambda°\zeta_0$, which can be employed for the study of ion-solvent interaction is recorded in table 3. The plots of $\Lambda°\zeta_0$ vs temperature are found to be linear and negative temperature coefficient is observed. This suggests[12,13] that the ion-solvent interaction is of the order: $BrO_3^- > IO_3^- > SO_4^{2-}$ and is in accordance with that of viscosity and apparent molar volume measurements.

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Protein methylase from calf liver nuclei: Enzyme characterization and stimulation by serum albumins

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Summary. A protein methylase from calf-lifer nuclei was partially purified by sonication of the nuclear pellet at high ionic strength, chromatin removal and ammonium sulphate fractionation of the solubilized activity.

Enzymic methylation is one of the post-synthetic modifications of macromolecules. Methylation of a protein is a common biochemical reaction occurring on different substrates and catalyzed by different enzymes[3]. Utilizing S-adenosyl-L-methionine, a common donor in methylation reactions, it is possible to methylate the guanidino groups of arginine[4], the carboxyl groups of dicarboxylic amino acid residues[5,6] and the ε-amino group of lysine of proteins[7]. The significance of protein methylation is not clear as yet; however, naturally occurring macromolecules are modified differently after their primary structures are established. Acetylation, and phosphorylation of proteins are some other examples of these modification phenomena.
This paper reports the characteristics of a partially purified protein methylase which methylates its endogenous protein. The enzyme activity has a pH optimum of 8.0 and a K_m of 0.95×10^{-5} M for S-adenosyl-L-methionine, and is stimulated by bovine and human serum albumins.
Experimental. Materials and methods. Fresh calf liver: from the local slaughter-house. S-adenosyl-L-[Me^{14}C]methionine, sp.act. 55 mCi/mmole, in dilute sulphuric acid (pH 3.5): from the Radiochemical Centre, Amersham. Crystallized bovine serum albumin and human serum albumin were from Pentex, Kankakee (Illinois, USA). Glutamate dehydrogenase and phosphoglucomutase: from Boehringer, Mannheim (Germany). Carboxypeptidase A: from Worthington (New Jersey, USA). Lysozyme: from Schwarz/Mann, New York (USA). γ-globulins from rabbit, chicken and hog: from NBC, Cleveland (Ohio, USA). All other chemicals used were of analytical grade quality. Sonication was carried out with a Branson model 5-177A sonicator (Branson Sonic Power, USA).
Buffers. Buffer A: 20 mM Tris-HCl pH 7.9, 10 mM $MgCl_2$, 1.66 M sucrose, 10 mM dithiothreitol; buffer B: 50 mM Tris-HCl pH 7.9, 25% (v/v) glycerol, 5 mM $MgCl_2$, 0.10 mM EDTA, 2 mM dithiothreitol; dialysis buffer: 20 mM $(NH_4)_2SO_4$ in buffer B.
Preparation of nuclei. Nuclei were isolated by continuous flow ultracentrifugation in high density sucrose as described elsewhere[8].
Protein determination. Protein was determined by one of the following procedures: the method of Lowry et al.[9] and the turbidimetric method of Layne[10], using crystalline potassium ferricyanide. Nucleic acids were determined spectrophotometrically at 260 nm.
Assay of protein methylase activity. The enzymic activity was expressed as pmoles of the methyl groups incorporated into an acid precipitable product/min/mg of added pro-

Table 1. Partial purification of protein methylase from calf liver

	Protein (mg/ml)	Total protein (mg)	Specific activity*	Total activity	Purification (-fold)	Yield (%)
Intact nuclei	160	26,900	2.82	75,800	1	100
Lysed nuclei	48	5,130	11.2	57,600	4	76
Supernatant I	10	2,900	16.0	46,400	5.7	61
Supernatant II	57	1,250	34.1	42,600	12.1	56

For assay conditions see Materials and methods. Values are given for 1600 g of calf liver. * pmoles of methyl groups incorporated into an acid insoluble product/min/mg of protein

tein. 10 µl of the enzyme preparation, 50 µl of Tris-HCl buffer 0.8 M (pH 8.0) and 20 µl of S-adenosyl-L-[Me-^{14}C]methionine (53 µCi/ml, 19.2 nmoles), in a total volume of 0.2 ml, were incubated at 37 °C for 10 min. The reaction was stopped by adding 10 ml of 10% trichloroacetic acid containing 40 mM sodium pyrophosphate. Removal of nucleic acids and phospholipids was effected by a small modification of the method proposed by Allfrey et al.[11]. After cooling in an ice-bath, the acid-insoluble precipitates were collected on Whatman GF/C glass fibre filters and washed 10 times with 5 ml aliquots of cold 5% trichloroacetic acid containing 40 mM sodium pyrophosphate, and 3 times with 5 ml of ice-cold water. Filters were dried for 30 min under an infrared lamp and counted with a Nuclear Chicago Mark IV Scintillation counter in vials containing 5 ml of a 4% solution of Liquifluor in toluene.

Results and discussion. Preparation of the extract. All the procedures were carried out at 4 °C. Approximately 1600 g of calf liver were normally used for each enzyme preparation. The nuclei, pelletted as described in Methods, were suspended in 55 ml of 0.34 M sucrose and an equal volume of buffer A was added (intact nuclei). 6.3 ml of 4 M $(NH_4)_2SO_4$ (pH 7.9) were added to a final concentration of 0.3 M. The mixture was sonicated in 50 ml fractions at 50% power in an ice-cold beaker until complete lysis of the nuclei (lysed nuclei). 2 volumes of buffer B were added to the mixture and the precipitate removed by centrifugation for 1 h at 105,000 × g in a Spinco No. 30 rotor and discarded (supernatant I).

Enzyme precipitation. Precipitation of the enzyme activity was carried out by adding solid ammonium sulphate, up to 50% saturation (0.33 g/ml), to the cooled supernatant, under constant stirring. The precipitate was collected by centrifugation for 1 h at 105,000 × g in a Spinco No. 30 rotor after which the enzyme activity was resuspended in a minimal volume of buffer B and dialyzed overnight against the same buffer containing 20 mM ammonium sulphate. The dialyzed enzyme was centrifuged for 1 h at 280,000 × g in a Spinco SW65K rotor, and the precipitate discarded (supernatant II). At this purification stage, the enzyme activity, essentially nucleic acid-free, was characterized (table 1). Since our enzymatic preparation methylates its endogenous proteins, it is difficult to deduce whether the 12-fold purification, as shown in table 1, represents enrichment of the transmethylase itself, of the methyl acceptor protein, or both.

Characterization of the partially purified protein methylase. a) Time course of reaction. Time course of reaction is shown in figure 1. Proportionality between activity and incubation time exists only for the first 20 min; a 10-min incubation time was therefore adopted for the standard assay. It should be noted that under the incubation conditions chosen protein methylase II (S-adenosylmethionine: protein carboxyl methyltransferase, EC 2.1.1.24) activity was not measurable. This activity, however, has been found only in cytosol fractions and should not be present in our preparation.

b) Effect of metal ions and reducing agents. EDTA, cysteine, Mn^{++}, Mg^{++}, Co^{++}, Fe^{+++}, at a final concentration of 1×10^{-3} M, did not produce any significant effect on the endogenous enzyme activity, whereas a partial inhibition by Cu^{++} and Zn^{++} was observed at a concentration of 2×10^{-3} M. Partial inhibition by Zn^{++} was also reported for protein methylase I (S-adenosyl methionine: protein-arginine methyltransferase EC 2.1.1.23) from calf thymus[4], whereas for protein methylase III (S-adenosyl methionine: protein-lysine methyltransferase EC 2.1.1.25) the inhibition by Zn^{++} and Cu^{++} was total[7]. Interestingly, β-mercaptoethanol and dithiothreitol at a final concentration of 1×10^{-3} M inhibit the enzymic activity to 60% and 70% of the control, respectively. In all cases, 0.57 mg of methylase supernatant II fraction were used in the standard assay.

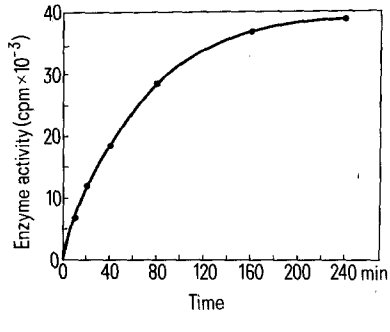

Fig. 1. Time course of reaction. For experimental conditions, see Materials and methods. Assay conditions were as described in the text. 0.57 mg of partially purified enzyme (supernatant II) were used.

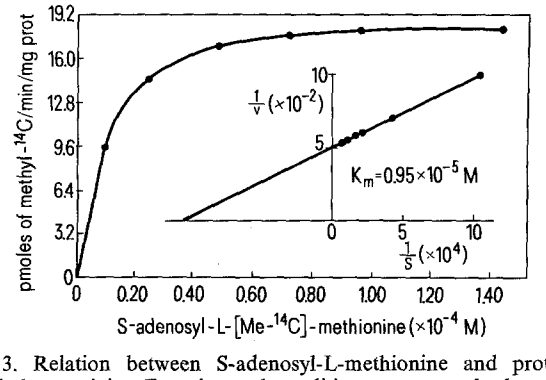

Fig. 3. Relation between S-adenosyl-L-methionine and protein methylase activity. Experimental conditions were standard except that concentration of S-adenosylmethionine varied.

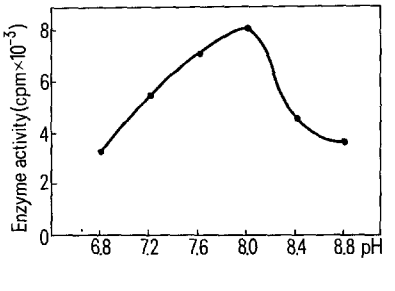

Fig. 2. Effect of pH. Protein methylase assays were standard except that pH was adjusted with Tris-HCl buffer, 0.2 M final concentration. Assays contained 0.57 mg of partially purified enzyme preparation (supernatant II).

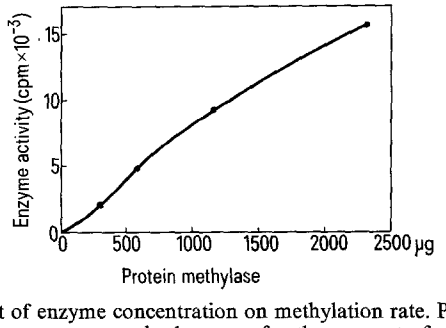

Fig. 4. Effect of enzyme concentration on methylation rate. Protein methylase assays were standard, except for the amount of protein enzyme and S-adenosyl-L[Me-^{14}C]methionine.

c) Effect of pH. As shown in figure 2, the pH optimum was about 8.0 in Tris-HCl buffer, using supernatant II enzyme in the standard assay. Ionic strength up to 0.5 M KCl did not influence the protein methylase activity.

d) S-adenosyl-L-methionine requirement. As shown in figure 3, the relation between S-adenosyl-L-[Me-^{14}C]-methionine and the endogenous protein methylase activity gives a K_m-value of 0.95×10^{-5} M. The kinetic characteristics of the enzyme preparation from calf liver nuclei do not exclude the possibility that the activity could be ascribed to a protein methylase III. At present it is not clear how many enzymes are involved in the methylation of lysine residues[3]. A protein-lysine methyltransferase, highly specific for cytochrome c, has been isolated from the cytosol of *Neurospora crassa*[12]. Recently it has been shown that other nuclear protein methylases exist capable of transferring methyl groups from S-adenosylmethionine to chromosomal non-histone proteins[13].

e) Effect of enzyme concentration. Figure 4 shows the relationship between enzymic activity and protein concentration in standard assay conditions. The curve pattern might suggest the presence of an activator; it is also likely that the enzyme activity, at low concentration, is regulated by other means such as association-dissociation equilibria. Similar sigmoidal curves have been observed with protein methylase III from tadpole liver and *Tetrahymena*[14].

f) Exogenous protein stimulatory effect. The stimulatory effect of various proteins was investigated. The proteins were added to the assay mixture at a final concentration of 1 mg/ml and the reaction started by the addition of the enzyme (0.57 mg methylase supernatant II fraction) without preincubation. Among the proteins tested, only bovine and human serum albumins showed a marked stimulatory effect on the endogenous protein methylase activity. The concentration dependence of the stimulatory effect of bovine serum albumin is shown in figure 5. A plateau is reached at a concentration of 0.5 mg/ml of albumin. No significant effect on enzymic activity was observed in the presence of T4-lysozyme, glutamate dehydrogenase, carboxypeptidase, phosphoglucomutase and rabbit, chicken, hog γ-globulins (fraction II).

Table 2. Fractionation of endogenous protein of precipitated and dialyzed enzyme fraction, labelled with S-adenosyl-L-[Me-^{14}C] methionine

Fraction	A Radioactivity cpm	%	B Radioactivity cpm	%
H_2SO_4-insoluble	53,974	94	101,703	95
H_2SO_4-soluble	3,400	6	5,065	5

A = Standard assay. B = Standard assay in presence of bovine serum albumin (4 mg).

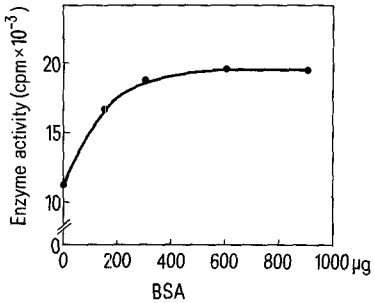

Fig. 5. Effect of bovine serum albumin on protein methylase activity. Assays were standard except for the amount of bovine serum albumin used.

g) Fractionation of the methylated endogenous proteins. Methylated endogenous proteins were fractionated according to Paik and Kim[15] with a minor modification. To obtain a higher incorporation, the volume of the assay mixture was increased to 1 ml and the incubation to 30 min. Incubation was carried out both in the absence and presence of bovine serum albumin; the reaction was stopped by adding an equal volume of 0.4 N H_2SO_4. To obtain the same precipitation conditions, an equal amount of bovine serum albumin was added to the bovine serum albumin-free sample. The results in table 2 show that 94% of the radioactivity is recovered in the H_2SO_4-insoluble fraction. Bovine serum albumin exerts its activating effect to the same extent both in the H_2SO_4-insoluble and H_2SO_4-soluble fractions. In fact, the percentage of total radioactivity incorporated in the 2 fractions is similar, irrespective of whether the reaction was carried out in the absence or presence of bovine serum albumin. In gel electrophoresis experiments, however, no radioactivity was found associated with the bovine serum albumin band, thus excluding that bovine serum albumin activation could be due to its own methylation. Liss et al.[16], in the purification of protein methylase from calf spleen observed, in the absence of endogenous activity, a marked incorporation of ^{14}C-methyl groups in human serum albumin, ovoalbumin and pepsin. In conclusion, partially purified protein methylase activity from calf liver nuclei shows an interesting property not observed so far in other similar enzymes: the stimulatory effect of bovine and human serum albumin. Further studies are necessary, however, in order to clarify the mechanism of activation. This will be possible when an endogenous protein-free methylating activity is available. In fact, the methylation of added proteins described by Liss et al.[16] was found only after the methylase was free from endogenous methylating activity. These proteins, even though exerting their stimulatory effect, were not substrates in the methylation reaction with the partially purified enzyme (i.e. retaining endogenous methylating activity). Probably the enzyme preparation used in the present study exhibits the same characteristics.

The stimulation by serum albumins is observed, even though to a significantly different extent, on 2 completely different classes of substrates (i.e. the H_2SO_4-soluble and insoluble fractions). It is likely that this stimulatory effect is therefore exerted mainly on the methylase system itself with a mechanism as yet unknown.

1 Acknowledgments. The authors thank Ms J. Gilder for editorial assistance.

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