graphy on Silicagel H with chloroform, methanol, water (60:25:4) as developing solvent, dolichol-pyrophosphate-oligosaccharide-mannose [^{14}C] (DPPoligoM) was prepared according to BEHRENS et al.[12,13]. DPM, DPGlu, DPPNAG, DPPoligoM were further purified on a sephadex LH-20 column (1.5 × 115 cm) eluted with chloroform, methanol, water (1:1:0.3) plus 50 m*M* ammonium acetate[14], followed by chromatography through a DEAE-cellulose-acetate column (1 × 20 cm) eluted successively with chloroform, methanol 2:1 (10 vol. column), methanol (10 vol. column), chloroform, methanol, water 1:1:0.3 (5 vol. column) then with a linear gradient of ammonium formate (0, 0.2 *M*) in chloroform, methanol, water (1:1:0.3). All those compounds were recovered as sharp peaks, washed exhaustively with water to eliminate all salt present then hydrolyzed as follows. The hydrolysis was carried out in a water bath at 65 °C and in boiling water. The samples were dryed in the bottom of a glass test tube and 1 ml of 25 m*M* NaCl, 25 m*M* ammonium acetate was added. Heavy metal was added to a final concentration of 10^{-3} or 10^{-2} *M*. Hydrolysis was stopped at desired time by addition of 1 ml of chloroform, methanol, water (60:4:8). The aqueous phase was removed for analysis. The organic phase was washed with chloroform, methanol, water (1:16:16) and the aqueous phase pooled with the first aqueous extraction. The various aqueous phases were counted in a LS-150 Beckman liquid scintillation spectrometer and a duplicate sample chromatographed in a sephadex G-15 or G-50 column (1.5 × 110 cm) eluted with buffer phosphate pH 7.0 μ = 0.1. All the reagents were of analytical grade.

Results and discussion. The incubation of DPM at 65 °C in ammonium acetate 25 m*M* and NaCl 25 m*M* at pH 7.0 was associated with an extremely slow release of a ^{14}C compound that was soluble in water. Addition of Zn^{++} to this mixture increased the rate of release of water soluble radioactivity from lipid. Over 90% of ^{14}C added as DPM [^{14}C] was recovered as water soluble compound after treatment with Zn^{++}, after 15 h at 65 °C or after 30 min at 100 °C. Also, Co^{++} enhanced the release of radioactivity into the water phase but was less efficient than Zn^{++}. Cu^{++}, Mn^{++}, Pb^{++}, Hg^{++}, Ni^{++} have no significant effect on the hydrolysis. The rate of Zn^{++} hydrolysis was not affected significantly by an anaerobic environment indicating the absence of intermediate on oxidative cleavage of ^{14}C from DPM [^{14}C]. The structure of the ^{14}C derivative released by Zn^{++}-catalyzed hydrolysis was examined by chromatography on sephadex G-15 and by paper chromatography (Whatman No.1) using *n*-butanol-pyridine-H_2O (6:4:3). In both experiments, the product had the same Rf as mannose [^{14}C]. The pH was checked before and after hydrolysis to be sure that no difference occurred during such hydrolysis. The reaction catalyzed by Zn^{++} therefore appears to be

$$\text{DPM} \xrightarrow{Zn^{++}} \text{DP} + \text{M}.$$

No presence of mannose-1-phosphate or other compounds was detected in any chromatographic test. The aqueous phase from the hydrolysis DP-Glu[^{14}C] and D-PP-NAG [^{14}C] on sephadex G-15 and paper chromatography give respectively Glu[^{14}C] and N-acetyl-glucosamine [^{14}C]. No other products were present. The aqueous phase from DPP-oligoM[^{14}C] on sephadex G-50 gives an homogeneous compound with 6–8 sugar residues[14]. The solvent was standardized with polysaccharides of different unit numbers between 3 and 7. DPGlu[^{14}C], DPPNAG[^{14}C], DPPoligoM[^{14}C] were submitted at the same Zn^{++} hydrolysis at 100 °C at different times. The kinetics of such hydrolysis are reported in the Figure. The kinetics of the hydrolysis catalyzed by Zn^{++} 100 °C was first order in all cases (Figure). However, the rate of hydrolysis varied with the substituent and followed the order DPM > DPGlu > DPPNAG and DPPoligoM.

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Activation of Particle Associated Rat Liver Guanine Deaminase by Lecithin and Interferences of Lecithin in Protein Precipitation

A. GIRIDHARI SINGH, MEERA TEWARI, V. K. MADAN and C. P. TEWARI[1]

Department of Biochemistry, Lucknow University, Lucknow 226007 (U.P., India), 9 September 1975.

Summary. Guanine deaminase solubilized from the 'light' mitochondrial fraction of rat liver was activated by lecithin. The activation was proportional to the concentration of lecithin taken in the system. A ratio of 1:1 between the two constituents (protein and lecithin) was at least necessary for complete precipitation.

Requirement of various lipids in membrane bound enzymes has been reviewed by COLEMAN[2]. Specific requirement of lecithin in the enzymes β-hydroxybutyrate dehydrogenase (from beef heart mitochondria) and acetyl CoA synthetase (from rat liver mitochondria) has been demonstrated[3–6]. Unspecific requirement for lecithin has been demonstrated in many cases[7–11].

In the present communication, we have demonstrated the activation of the solubilized particle associated rat liver guanine deaminase by lecithin and interference of lecithin in protein precipitation by perchloric acid.

Materials and methods. Albino rats were reared in the departmental animal house. All experiments were performed at 0–4 °C unless otherwise specified. Cell fractionation was according to KUMAR[12], excepting that the residue collected between 5,000 × *g* and 15,000 × *g*, designated as the 'light' mitochondrial fraction was washed twice with the sucrose medium instead of washing only once to get more purified fraction. Solubilization process includes freezing and thawing followed by Vir-Tis disruption and treatment with Triton X-100.

Guanine was procured from E. Merck (Federal Republic of Germany) and lecithin from V.P. Chest Institute (New Delhi). Other reagents used were of analytical grade.

Results and discussion. During the purification of the particle associated guanine deaminase of rat liver, we observed that phospholipid lecithin activated the enzyme proportionally (Table I). After a certain stage, increase in the amount of lecithin suppressed the complete precipitation by perchloric acid. Most of the protein particles remained in a colloidal form and did not settle down by a 10 min centrifugation at 700 × *g*. The spectrophotometric reading at 245 nm was therefore much higher.

From the experiment mentioned above, it looks as if, for complete precipitation from the system, certain specific ratio between lecithin and protein is required. To confirm the fact, a separate experiment with standard bovine serum albumin (BSA) was planned. Assay system for this was the same as that of guanine deaminase described from this laboratory by SREE KUMAR[13], except that enzyme preparation was replaced by BSA. Assay system of 2.0 ml comprized of 1.0 ml of 100 m*M* *Tris*-HCl buffer, pH 8.0, 0.06 μmoles of guanine, pH 11.0 in 0.1 ml, 200 μg lecithin in 0.05 ml ethyl alcohol and various concentrations of BSA in 0.85 ml distilled water. The system was acidified by the addition of 1.0 ml 10% perchloric acid, centrifuged at 700 × *g* for 10 min and spectrophotometric readings were recorded at 245 nm. A series of experiments were also run without lecithin which served as controls (Table II).

The results showed that, without lecithin, the optical density readings were practically the same irrespective of the amount of proteins, thereby indicating a complete precipitation of the protein at least for a certain range of concentration (25–200 μg). In cases where a fixed concentration of 200 μg lecithin was incorporated, the optical density was highest when the amount of protein was lowest (25 μg). When the amount of protein increases, i.e. the ratio of lecithin to protein decreases, the optical density decreases. When the ratio between lecithin to protein is unity, the optical density was almost the same as that of controls. This shows that the ratio between lecithin and protein is very important for a complete precipitation in a lecithin treated sample. As far as the present authors know, there is no such report in literature regarding guanine deaminase.

This interference may be due to the formation of a complex between protein and lipid. Exact mechanism by which the said protein lipid complex interferes in enzymic studies is not yet clear. Experiments are in progress to find out the exact mechanism of interference.

Table I. Activation of the particle associated guanine deaminase of rat liver by lecithin

Lecithin (μg)	Enzyme activity	
	E_{245}	%
Control	0.040	—
50	0.055	37
100	0.060	50
150	0.070	75
200	readings out of range	

The fresh mitochondrial suspension was frozen at −18 °C for 1 night, thawed and then disrupted in a Vir-Tis homogenizer operated at 40,000 rpm for 2 min. The disrupted preparation was finally treated with 1% (v/v) Triton X-100 and was used as the source of the enzyme. The 114 μg protein content of the aliquot was taken. Lecithin concentrations used were 50,100,150 and 200 μg in 0.05 ml ethanol. In the control, 0.05 ml of ethanol without lecithin was added to nullify the effect of ethanol in the system. Percentage activation was calculated on the basis of the activity in the control.

Table II. Effect of protein and lecithin concentration

Protein (μg)	Ratio lecithin/protein	Optical density at 245 nm	
		Without lecithin	With lecithin
25	8.0	0.280	0.400
50	4.0	0.280	0.380
100	2.0	0.280	0.325
150	1.3	0.280	0.305
200	1.0	0.280	0.290
250	0.8	0.280	0.280
300	0.6	0.285	0.285

Assay system consists of 1.0 ml *Tris*-HCl buffer (100 m*M*), pH 8.0, 0.06 μmoles guanine, pH 11.0, in 0.1 ml, 200 μg lecithin in 0.05 ml ethyl alcohol and various concentration of protein (bovine serum albumin). The volume was made up to 2.0 ml and acidified by the addition of 1.0 ml of 10% PCA. In the controls, the lecithin was excluded from the system.

[1] Acknowledgment. The authors are grateful to Professor P. S. KRISHNAN, University of Calicut, Calicut, Kerala, for his interest.

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