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Studies on the Interactions between Phospholipids and Membrane-Bound Enzymes in Microsomes. Effects of Phosphatidylinositol-Specific Phospholipase C on Enzymes of Rat Liver Microsomes

KOHEI SAWAKI,* RYO TAGUCHI, and HIROH IKEZAWA

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan

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The interactions between phosphatidylinositol and membrane-bound enzymes in rat liver microsomes were studied by using phosphatidylinositol (PI)-specific phospholipase C of *Bacillus thuringiensis* and nonionic, anionic and cationic detergents. The dependence of activity of nucleoside diphosphatase on the detergent concentration varied with the detergents used. However, maximal activity with each detergent was attained within 30 min, and the enzyme activity was relatively stable during exposure to each detergent. When phosphatidylinositol in the microsomal membrane was hydrolyzed, the glucose-6-phosphatase activity was decreased in the presence of taurocholate. Nucleoside diphosphatase and 5′-nucleotidase were released by the breakdown of phosphatidylinositol in the membrane, while adenosine triphosphate (ATP)ase, reduced nicotinamide adenine dinucleotide (NADH)-cytochrome b_5 reductase and carboxylesterase were not released. The pattern of release of nucleoside diphosphatase was significantly different from that of 5′-nucleotidase, and the release of the latter enzyme was greater than that of the former. Also, nucleoside diphosphatase was more extensively released from the microsomal membrane by treatment with taurocholate than with PI-specific phospholipase C, while the release of 5′-nucleotidase activity was greater on treatment with this phospholipase C than with taurocholate.

Keywords——rat liver; microsomal membrane; membrane-bound enzyme; phosphatidylinositol; PI-specific phospholipase C; nucleoside diphosphatase

The endoplasmic reticulum of liver is a functionally important organelle which contains numerous enzymes and enzyme systems involved in the synthesis and transport of a number of proteins, the synthesis of lipids and steroids, and the metabolism of drugs. The structural and functional relationships between proteins and lipids within the microsomal membrane have been studied by the use of proteases, phospholipases and detergents.[1-5]

It is well known that phosphatidylinositol is a quantitatively minor constituent of the lipids in animal tissues, being equivalent to 8—10% of microsomal-membrane phospholipids, but phosphatidylinositol may play an important role in transmembrane control through a mechanism such as control of the turnover of phosphatidylinositol by several hormones.[6]

The purpose of the present work was to examine the interactions between membrane-bound enzymes and phosphatidylinositol within the microsomal membrane of rat liver. The binding features of microsomal enzymes with the membrane are discussed on the basis of the results obtained.

Experimental

Materials——The chemicals used were obtained from the following sources: Sodium glucose-6-phosphate and taurocholate were purchased from Boehringer Mannheim and Calbiochem Behring Co., respectively; Nucleoside mono-, di-, triphosphates were from Yamasa Shoyu Co.; NADH from Kyowa Hakko Kogyo Co. Phospholipase A_2

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of porcine pancreas was purchased from Sigma Chemical Co. All other chemicals were of analytical grade, obtained from Katayama Chemical Co. and Nakarai Chemical Co.

Protein Determination——Protein was determined according to the method of Lowry *et al.*[7] with bovine serum albumin as a standard.

Preparation of Phospholipases C——Phosphatidylinositol (PI)-specific phospholipase C was purified from the culture medium of *Bacillus thuringiensis* by the method of Taguchi *et al.*[8] Sphingomyelin and glycerophospholipids other than phosphatidylinositol and lysophosphatidylinositol were not hydrolyzed by this enzyme.[8] Phosphatidylcholine-hydrolyzing phospholipase C was purified from the culture medium of *Bacillus cereus* by the method of Ikezawa *et al.*[21]

Preparation of Microsomes—Male rats of the Wistar strain, weighing 250—350 g, were fasted for over 24 h and killed by decapitation. The livers were perfused with 0.25 M sucrose–0.01 M Tris-HCl buffer (pH 7.4), and a 10% homogenate was prepared with a glass homogenizer in the same buffer. The homogenate was centrifuged at $900 \times g$ for 10 min, then the resulting supernatant was successively centrifuged at $5000 \times g$ for 10 min and $12000 \times g$ for 20 min. Finally, the supernatant was centrifuged at $105000 \times g$ for 60 min, and the final pellet was resuspended in the sucrose–Tris buffer.

Phospholipase Treatment of Micorosomes——Microsomal fraction was incubated with phospholipase for 60—80 min at 30 °C. As a control, microsomal fraction was incubated with the sucrose–Tris buffer (pH 7.4) instead of phospholipase. At the end of incubation, the reaction mixtures were centrifuged at $105000 \times g$ for 60 min at 4 °C. The resulting supernatants and the pellets, as well as uncentrifuged aliquots from the reaction mixtures, were subjected to the determination of enzyme activity. Both the pellets and the aliquots from the reaction mixtures were suspended in, and diluted with the sucrose–Tris buffer to approx. 0.45 mg protein per ml.

Detergent Exposure of Microsomal Preparations——After incubation of rat liver microsomes with or without phospholipases, detergent exposure was carried out by the addition of 1 volume of the concentrated solution of each detergent to 9 volumes of microsomes, in order to determine the total activity in the disrupted microsomes. The mixtures were kept at 4 °C for the indicated time, then the enzyme activities were determined in the presence of 1% bovine serum albumin. The detergent solutions were adjusted to pH 7.4.

Analysis of Microsomal Phospholipids——Aliquots corresponding to 2.3—2.5 mg protein were withdrawn from the incubation mixtures, then microsomal phospholipids were extracted with $CHCl_3$–CH_3OH–HCl (66 : 33 : 1, v/v) according to the method of Folch *et al.*[9] The chloroform layer of each extract was concentrated under N_2 gas, and separated by thin-layer chromatography (TLC) of Thincrod -S-II (crystal rods coated with silica gel) with $CHCl_3$–CH_3OH–CH_3COOH–H_2O (80 : 15 : 10 : 4, v/v). Separated spots on each rod were quantitatively analyzed with an Iatroscan TH-10 TLC analyzer (Iatron Laboratories, Inc., Tokyo). The extent of degradation of phosphatidylinositol was expressed as percent of the amount of phosphatidylinositol in phospholipase-untreated microsomes.

Enzyme Assays——The activity of glucose-6-phosphatase [EC 3.1.3.9] was measured by a modification of the method of Baginski *et al.*,[10] in a reaction mixture containing 25 mM cacodylate buffer (pH 6.4) and 20 mM glucose-6-phosphate in a final volume of 0.4 ml. The assay of nucleoside diphosphatase [EC 3.6.1.6] was carried out in a reaction mixture containing 50 mM Tris-HCl buffer (pH 7.5), 4 mM $MgCl_2$ and 3 mM inosine diphosphate in a final volume of 0.4 ml, by a modification of the methods of Kuriyama.[11] The assay of ATPase [EC 3.6.1.3] was carried out as described by Schwartz *et al.*[12] with some modifications, using the following reaction medium: 50 mM Tris-HCl buffer (pH 7.5), 3 mM $MgCl_2$ and 3 mM ATP in a final volume of 0.4 ml. The activity of 5′-nucleotidase [EC 3.1.3.5] was measured according to the method of Emmelot and Bos[13] with some modifications. In a total volume of 0.4 ml, the assay system contained 50 mM glycine–NaOH buffer (pH 8.5), 4 mM $MgCl_2$ and 10 mM adenosine monophosphate (AMP). The reactions of these four enzymes in the assays were carried out for 5—15 min at 37 °C, and were terminated by the addition of 2 ml of 10% trichloroacetic acid containing 2% ascorbic acid. Then, liberated inorganic phosphate was measured by the method of Baginski *et al.*[10] The activity of alkaline phosphatase [EC 3.1.3.1] was determined according to the method of Engström,[14] by incubation of the reaction mixture containing *p*-nitrophenyl phosphate as a substrate for 10 min at 37 °C. Reduced nicotinamide adenine dinucleotide (NADH)-cytochrome b_5 reductase [EC 1.6.2.2] was determined at 25 °C, as described by Strittmatter.[15] The activity of carboxylesterase [EC 3.1.1.1] was determined titrimetrically with a pH-Stat (Radiometer, Copenhagen) by using *n*-propyl *n*-butyrate emulsion as a substrate, according to the method of Ishihara *et al.*[16]

Results

Effects of Various Detergents on Microsomal Nucleoside Diphosphatase

Arion *et al.*[17] reported the effects of various detergents on microsomal glucose-6-phosphatase. As shown in Fig. 1, we also examined the effects of various detergents on the activity of nucleoside diphosphatase, which is localized on the luminal side of the microsomal membrane, like glucose-6-phosphatase.

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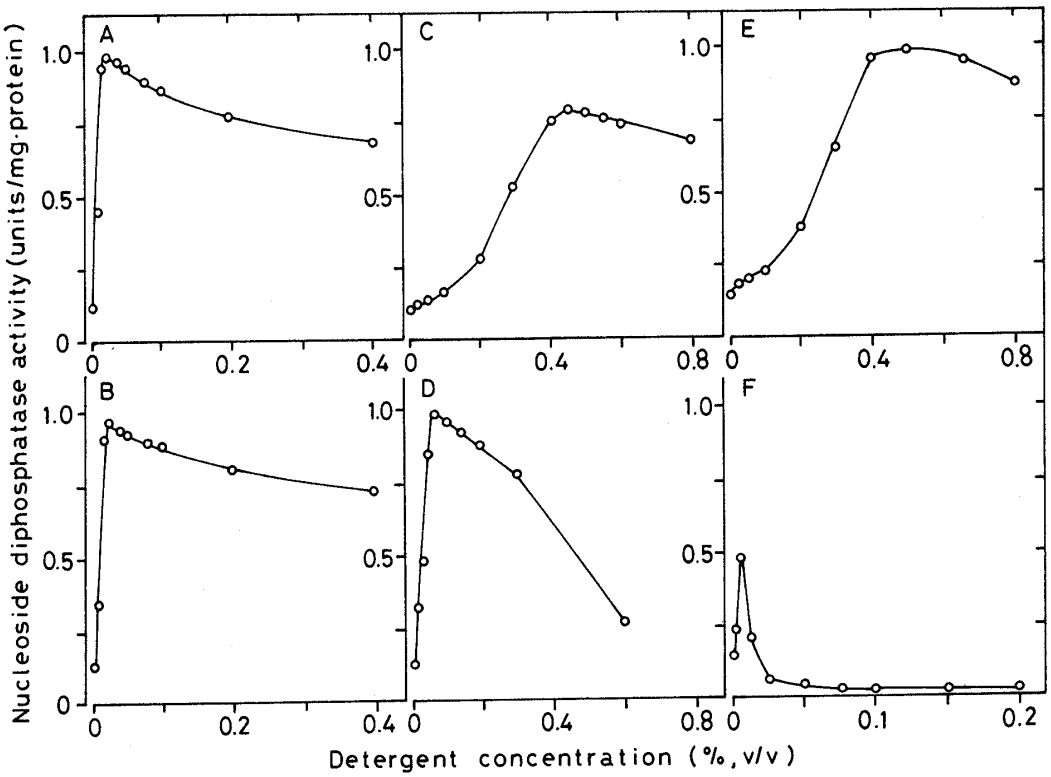

Fig. 1. Effects of Various Detergents on Nucleoside Diphosphatase Activity in Rat Liver Microsomes

Microsomal fractions (0.4 mg protein) were exposed to several detergents as described in the text. The mixtures were kept at 4 °C for 30 min, and the enzyme activities were determined.

A, Triton X-100; B, Triton X-114; C, sodium taurocholate; D, sodium deoxycholate; E, sodium cholate; F, cetyltrimethylammonium bromide.

In the treatments of microsomal fraction with nonionic detergents such as Tritons X-100 and X-114, the maximal values of nucleoside diphosphatase activity were obtained at low concentration, 0.03% (v/v), and the enzyme activity gradually decreased with increasing concentration of these detergents. The enzyme activity in the microsomes treated with anionic detergents such as sodium taurocholate and cholate increased with increasing concentration of detergents, and maximal activity was exhibited at the concentration of 0.4 to 0.5%. Maximal activity in the sodium deoxycholate-treated microsomes was obtained at the concentration of 0.1%, but the inhibition of enzyme activity was observed at higher concentration of this detergent. Maximal activity in the microsomes treated with cetyltrimethylammonium bromide was about half that in the microsomes treated with other detergents, and was obtained at a very low concentration of this cationic detergent, 0.006%. Furthermore, the treatment of microsomes with higher concentrations of this detergent resulted in enzyme inactivation.

Time Course of the Changes in the Activity of Nucleoside Diphosphatase during Exposure to Various Detergents

We reported recently[4] that the stability of glucose-6-phosphatase activity in phospholipase C-treated and untreated microsomes varied depending on the detergent used. As shown in Fig. 2, we also examined the time course of the activation of nucleoside diphosphatase after exposure to various detergents at the optimal concentrations shown in Fig. 1.

With all six detergents, the activity of nucleoside diphosphatase in microsomes reached the maximal value within 30 min, and thereafter remained relatively stable during exposure to

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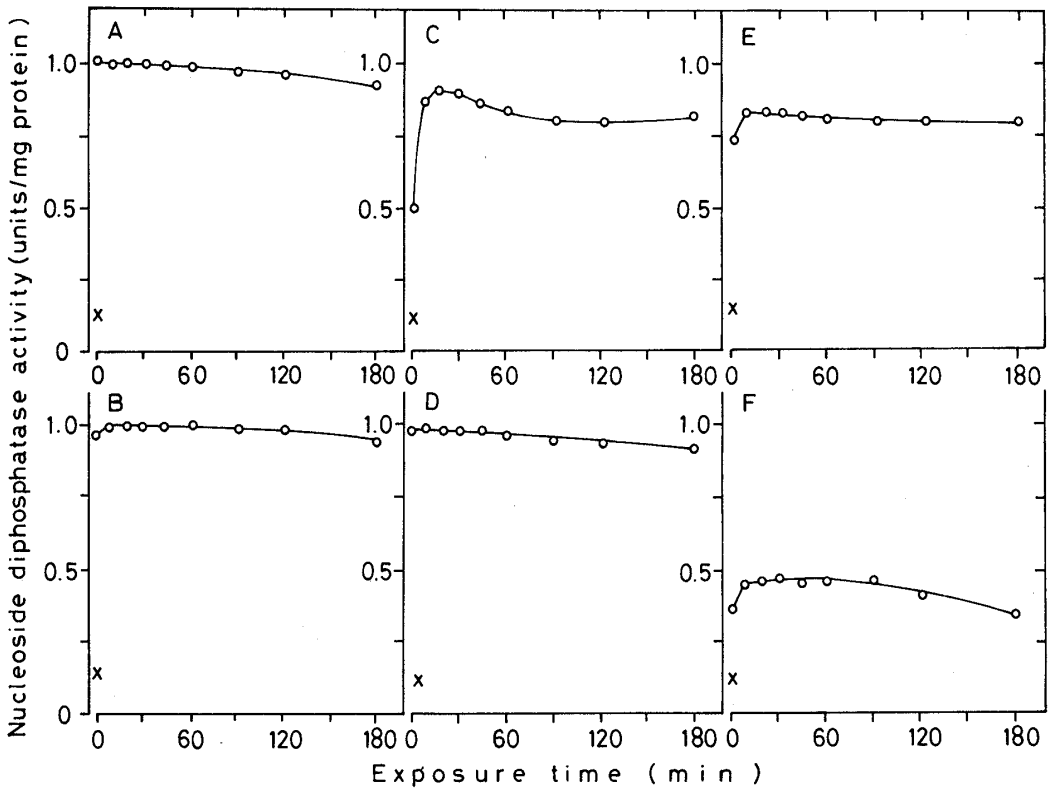

Fig. 2. Time Course of the Changes in Nucleoside Diphosphatase Activity on Exposure of Microsomes to Various Detergents

The mixtures containing microsomal fraction (0.4 mg protein) and several detergents were kept at 4 °C for the indicated time, then the enzyme activities were determined.
×, without detergent; A, 0.03% Triton X-100; B, 0.03% Triton X-114; C, 0.5% sodium taurocholate; D, 0.1% sodium deoxycholate; E, 0.5% sodium cholate; F, 0.006% cetyltrimethylammonium bromide.

detergents for at least 3 h. The enzyme activity determined immediately after the addition of these detergents was always higher than that determined without detergents. Apparently, the activation or solubilization of nucleoside diphosphatase must occur readily through disruption of the membrane by the detergents.

The Effects of Hydrolysis of Phosphatidylinositol on the Activities of Membrane-Bound Enzymes in Rat Liver Microsomes

The effects of enzymatic hydrolysis of phosphatidylinositol on the activities of membrane-bound enzymes in rat liver microsomes are shown in Table I.

When phosphatidylinositol in the microsomal membrane was hydrolyzed by PI-specific phospholipase C of *B. thuringiensis*, the activity of glucose-6-phosphatase in the detergent-exposed microsomes was apparently decreased. The decrease of enzyme activity in the detergent-unexposed microsomes was smaller than that in the detergent-exposed microsomes. Furthermore, no solubilization of this enzyme was observed. Under the same conditions, the latency of mannose-6-P phosphohydrolase, an index of microsomal integrity,[18] was more than 80%. In this context, we concluded in our recent reports[4,5] that the breakdown of phosphatidylinositol impaired glucose-6-P phosphohydrolase activity, while the activity of glucose-6-P translocase, which transports glucose-6-P from the cytoplasmic to the luminal side of the microsomal membrane, was stimulated by the breakdown of phosphatidylinositol, and consequently the stimulation of this translocase partly compensated for the decrease in the activity of glucose-6-P phosphohydrolase.

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TABLE I. Effects of Enzymatic Hydrolysis of Phosphatidylinositol on the Activities of Membrane-Bound Enzymes in Rat Liver Microsomes

Microsomes	Enzyme activity[a]											
	Glucose-6-phosphatase detergent		Nucleoside diphosphatase detergent		Carboxyl-esterase detergent		ATPase detergent		NADH-Cytochrome b_5 reductase detergent		5′-Nucleotidase detergent	
	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)
Control												
ppt+sup	100 (4.0)[b]	100 (5.8)	100 (2.7)	100 (10.7)	100 (7.0)	100 (7.1)	100 (1.1)	100 (1.1)	100 (0.58)	100 (0.58)	100 (1.15)	100 (1.15)
ppt	94.8	92.0	73.4	72.7	82.0	82.2	90.7	85.4	90.0	89.4	81.0	66.1
sup	0.5		16.1		4.6		3.0		2.0		3.1	
PIase[c]-treated microsomes[d]												
ppt+sup	93.0	77.7	102	97.9	102	98.8	103	96.7	92.0	98.0	102	97.4
ppt	82.8	66.3	63.8	68.6	83.6	81.6	88.7	87.5	85.6	85.6	68.7	62.0
sup	0.6		22.0		4.1		5.4		1.1		16.3	

Microsomal fraction (19.7 mg protein) was incubated with 480 munits of PI-specific phospholipase C. After the reaction with the phospholipase, the incubation mixtures were centrifuged and the supernatant and particulate fractions were obtained. Then, a part of the incubation mixtures and the resulting particulate fractions were exposed to 0.4% taurocholate at 4°C for more than 30 min, and several enzyme activities of the uncentrifuged mixtures (ppt+sup), particulate fractions (ppt) and supernatant fractions (sup) were determined.

a) The enzyme activities are expressed in terms of relative activity, based on the activities of uncentrifuged mixtures in the control taken as 100.
b) The values in parentheses represent total units of enzymes.
c) PI-specific phospholipase C.
d) The extent of degradation of phosphatidylinositol was 58%.

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In contrast, the total activities (ppt+sup) of nucleoside diphosphatase and carboxylesterase in the detergent-unexposed and -exposed microsomes were not affected by the breakdown of phosphatidylinositol, although these enzymes are localized on the luminal side, like glucose-6-phosphatase. However, it seemed that the release of nucleoside diphosphatase from the membrane was enhanced by treatment with PI-specific phospholipase C, although a small but significant leakage of this enzyme was observed in the phospholipase-untreated microsomes.

The treatment of microsomes with PI-specific phospholipase C did not affect the activities of adenosine triphosphate (ATP)ase and NADH-cytochrome b_5 reductase in the detergent-unexposed and -exposed microsomes. These enzymes were not solubilized, whereas the release of 5′-nucleotidase was observed after the breakdown of phosphatidylinositol.

The Release of Nucleoside Diphosphatase from Rat Liver Microsomes by Several Phospholipases

As shown in Table II, nucleoside diphosphatase was not released from microsomes by treatment with heat-inactivated PI-specific phospholipase C, but was released by treatment with phospholipase C of *B. cereus* or phospholipase A_2 of porcine pancreas. Furthermore, the disruption of microsomes by these phospholipases was apparently responsible for the increase in total activity (ppt+sup). The results indicate that the release of this enzyme generally take place as a consequence of the hydrolysis of the microsomal phospholipids. Table II also shows that the released enzyme was not activated by treatment with taurocholate.

The Hydrolysis of Nucleotides by the Enzymes in the Supernatants from Phospholipase C- or Taurocholate-Treated Microsomes

Table III shows the relative enzyme activities toward various nucleotide substrates in the supernatant obtained by the treatment of microsomes with PI-specific phospholipase C or

TABLE II. The Release of Nucleoside Diphosphatase from Rat Liver Microsomes by Several Phospholipases

Treatment	Enzyme activity (units)			
	ppt+sup	ppt	sup	
Control	598 (100)[a]	328 (54.8)	138 (23.1)	131[b]
1.2 unit PIase[c]	608 (102)	284 (47.5)	207 (34.6)	191[b]
Heat-inactivated PIase	567 (94.8)	327 (54.7)	137 (22.9)	
0.1 unit phospholipase C (*B. cereus*)	2327 (389)	722 (121)	1254 (209)	
0.69 unit phospholipase A_2 (Porcine pancreas)	3026 (506)	433 (72.4)	1893 (317)	

Microsomal fractions (5.1 mg protein) were incubated with several phospholipases or heat-inactivated PI-specific phospholipase C. After the reaction, the incubation mixtures were centrifuged and the enzyme activity of the uncentrifuged mixtures (ppt+sup), particulate fractions (ppt) and supernatant fractions (sup) were determined.

a) The values in parentheses are expressed in terms of relative activity, on the basis of the activity of the uncentrifuged mixture in the control run taken as 100.

b) The activity of supernatant fraction in the presence of 0.4% taurocholate.

c) The same as in Table I.

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TABLE III. The Hydrolysis of Nucleotides by the Supernatant from PI-Specific Phospholipase C- or Taurocholate-Treated Microsomes

Substrate	Enzyme activity[a] released in 105000 *g* supernatant		
	Control	PIase[b]-treated microsomes[c]	0.4% taurocholate-treated microsomes[d]
AMP	100 (39)[e]	794	146
IMP	100 (28)	393	121
UMP	100 (26)	769	138
GMP	100 (29)	372	143
ADP	100 (39)	100	305
IDP	100 (459)	185	2614
UDP	100 (307)	189	4463
GDP	100 (357)	189	3697
CDP	100 (49)	108	1020

Microsomal fraction (16.5 mg protein) was incubated with 300 munits of PI-specific phospholipase C. Then 0.4% taurocholate-treated microsomes was prepared by exposing the microsomal fraction (16.0 mg protein) to taurocholate solution at 4 °C for 80 min. The mixtures were centrifuged and the activity in each supernatant was determined by the assay method described for nucleoside diphosphatase.

a) The enzyme activities were expressed in terms of relative activity, on the basis of the activities of the control taken as 100.
b) The same as in Table I[c].
c) The extent of degradation of phosphatidylinositol was 46%.
d) When the nucleoside diphosphatase activity in the uncentrifuged fraction was taken as 100, the recoveries of this enzyme activity in the particulate fraction and supernatant fraction were 9.8% and 75.5%, respectively.
e) The values in parentheses represent the total amounts of hydrolyzed substrates in terms of nmol/min.

0.4% taurocholate.

At least two activities of microsomal nucleotidases were released into the supernatant; the activity of nucleoside monophosphate phosphohydrolase released from phospholipase C-treated microsomes was 4 to 8 times that from the control, whereas only a small amount of this enzyme activity was liberated by the treatment with the detergent. Further, the supernatant from phospholipase C- or taurocholate-treated microsomes contained enzyme activity that mainly hydrolyzed inosine diphosphate (IDP), uridine diphosphate (UDP) and guanosine diphosphate (GDP) but had little activity towards adenosine diphosphate (ADP) and cytidine diphosphate (CDP), suggesting that nucleoside diphosphatase activity was released from the microsomes.

Thus, these results indicate that nucleoside mono- and diphosphates are hydrolyzed by liberated 5′-nucleotidase and nucleoside diphosphatase, respectively, judging from their substrate specificities.[19,20]

Enzyme Release from Slices and Microsomes of Rat Liver by Treatment with PI-Specific Phospholipase C

As shown in Table IV, the release of nucleoside diphosphatase, alkaline phosphatase and 5′-nucleotidase from rat liver slices and microsomes induced by treatment with PI-specific phospholipase C was examined.

The release of alkaline phosphatase and 5′-nucleotidase from plasma membrane of liver slices was greatly stimulated by treatment with PI-specific phospholipase C, as reported previously.[21-23] Furthermore, stimulation of the release of these enzymes was observed not only in slices but also in microsomes. The release of alkaline phosphatase and 5′-nucleotidase from microsomal fraction may be mainly due to plasma membrane contamination. On the

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Table IV. Enzyme Release from Slices and Microsomes of Rat Liver by PI-Specific Phospholipase C

	Enzyme activity[a] in 105000 *g* supernatant		
	Nucleoside diphosphatase	Alkaline phosphatase	5′-Nucleotidase
Liver slices			
Minus PIase[c]	100 (0.794)[b]	100 (0.013)	100 (0.358)
Plus PIase	97	573	156
Microsomes			
Minus PIase	100 (0.316)	100 (0.003)	100 (0.03)
Plus PIase	163	520	968

Rat liver slices (wet weight 0.7 g) were incubated with 78 munits of PI-specific phospholipase C for 90 min at 30 °C. The total volume of reaction mixture was 3.7 ml. Microsomal fraction (16.8 mg protein) was incubated with 390 munits of PI-specific phospholipase C. Then each mixture was centrifuged and the enzyme activities in the supernatant were determined.

a) The enzyme activities were expressed in terms of relative activity, on the basis of the activities of the control taken as 100.

b), *c*) The same as in Table I.

other hand, the stimulation of release of nucleoside diphosphatase by treatment with PI-specific phospholipase C was observed only in microsomes, but not in liver slices. These results indicate that the stimulation of release of nucleoside diphosphatase is specifically caused by the enzymatic breakdown of phosphatidylinositol on endoplasmic reticulum.

These results with phospholipases were reproducible, although the extents of degradation of phospholipids varied slightly.

Discussion

The activating effects of several detergents on nucleoside diphosphatase and the optimal concentration of each detergent for activation were similar to those observed in the activation of glucose-6-phosphatase which was reported by Arion *et al.*,[17] with the exceptions of sodium deoxycholate and cetyltrimethylammonium bromide (Fig. 1). Furthermore, the activity of nucleoside diphosphatase remained fairly stable during the exposure of microsomes to these detergents for at least 3 h, in contrast with our recent report[4] that the stability of glucose-6-phosphatase activity varied with the detergents used for the treatment of microsomes (Fig. 2). These results suggest that the stability or sensitivity of enzymes localized on the luminal side of the microsomal membrane depends on the detergents used for exposure.

The release of nucleoside diphosphatase from the microsomal membrane was observed even on incubation without PI-specific phospholipase C at 30 °C, while other microsomal enzymes such as glucose-6-phosphatase, NADH-cytochrome b_5 reductase, ATPase, carboxylesterase and 5′-nucleotidase were not solubilized under the same conditions (Table I). Yamazaki and Hayaishi[24] and Kuriyama[11] reported that a clear difference in solubilization of nucleoside diphosphatase and other microsomal enzymes such as NADH-cytochrome c reductase and cytochrome b_5 was observed upon alkaline or sodium deoxycholate treatment of microsomes. In our experiments, the release of nucleoside diphosphatase from the microsomal membrane was enhanced by the breakdown of phospholipids (Table II). The identity of this enzyme released by treatment with PI-specific phospholipase C was confirmed by its specificity against IDP, UDP and GDP (Table III). Also, the stimulation of release of nucleoside diphosphatase was specifically observed with the microsomal membrane, but not with the liver slices (Table IV).

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The activities of ATPase, NADH-cytochrome b_5 reductase and carboxylesterase were not affected by the removal of 58% of membranous phosphatidylinositol (Table I). Thus, the interaction between each of these enzymes and phosphatidylinositol within the microsomal membrane is very weak, if any. However, Mandersloot *et al.*[25] reported that phosphatidylinositol served as the lipidic activator of the (Na+K)-ATPase present in the microsomal membranes prepared from rabbit kidney, by using phospholipase A_2, phospholipase C of *B. cereus* and sphingomyelinase of *Staphylococcus aureus*. Roelofsen and Van Linde–Sibenius Trip[26] also pointed out a relationship between the (Na+K)-ATPase activity and the non-degraded fraction of membranous phosphatidylinositol after the treatment of rabbit kidney microsomes with PI-specific phospholipase C of *B. cereus*. Therefore, non-degraded phosphatidylinositol after treatment of microsomes with PI-specific phospholipase C of *B. thuringiensis*, may affect the activities of ATPase, NADH-cytochrome b_5 reductase and carboxylesterase. In order to confirm this, further detailed study will be necessary.

5′-Nucleotidase was released from microsomes and liver slices by treatment with PI-specific phospholipase C (Table IV). However, the proportion of this enzyme released from microsomes was higher than that from liver slices, or than the proportion of nucleoside diphosphatase released from the microsomal membrane by treatment with PI-specific phospholipase C. Furthermore, the release of 5′-nucleotidase from microsomes by treatment with taurocholate was very low, whereas the proportion of nucleoside diphosphatase released from microsomes by treatment with this detergent was exceedingly high (Table III).

Therefore, these results reflect various binding properties of microsomal enzymes to the membrane and various activated states of enzymes within the membrane, and suggest the close association of 5′-nucleotidase and alkaline phosphatase with phosphatidylinositol in the membrane. Further, a weak interaction might exist between nucleoside diphosphatase and membranous phosphatidylinositol, as with other phospholipids, suggesting that this enzyme is loosely bound to the microsomal membrane, as pointed out by Kuriyama,[11] and that other microsomal enzymes such as glucose-6-phosphatase, NADH-cytochrome b_5 reductase, ATPase, carboxylesterase and 5′-nucleotidase are more tightly bound to the membrane. This would account for the release of nucleoside diphosphatase upon slight disruption of the microsomal membrane by treatment with phospholipases or detergents.

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