Regulation of neutral cholesterol esterase activity by phospholipids containing negative charges in substrate liposome

Itsuko Ishii,[1],* Rie Onozaki,* Eriko Takahashi,* Saori Takahashi,* Nami Fujio,* Tomoko Harada,* Nobuhiro Morisaki,† Kohji Shirai, Yasushi Saito,† and Seiyu Hirose***

Department of Biochemistry, Faculty of Pharmaceutical Sciences,* and the Second Department of Internal Medicine,† Chiba University, Japan, and Department of Clinical Functional Physiology, Faculty of Medicine,** University of Toho, Japan

Abstract The effect of phospholipids on cholesteryl ester hydrolysis by neutral cholesterol esterase in alveolar macrophages was studied. Among the phospholipids used as emulsifiers, those with a negative charge, such as phosphatidylserine, phosphatidic acid, phosphatidylinositol, and cardiolipin, gave a higher level of hydrolysis by neutral cholesterol esterase than other less negatively charged phospholipids, such as phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, and sphingomyelin. Phospholipase D treatment of liposomes emulsified with phosphatidylcholine produced phosphatidic acid and enhanced cholesteryl ester hydrolysis. Phospholipase A_2 treatment produced lysophosphatidylcholine and decreased the hydrolysis. The hydrolysis of cholesteryl ester in lipid droplets obtained from cholesterol-laden macrophages elicited by thioglycollate in the rat peritoneal cavity was low compared to artificial liposomes emulsified with phosphatidylcholine. The reason for this was speculated to be that lipid droplets were low in total phospholipids and poor in phospholipids with strong negative charges but rich in phosphatidylethanolamine and sphingomyelin. These results suggest that the polar heads of phospholipids may play an important role in cholesteryl ester hydrolysis by neutral cholesterolesterase.–**Ishii, I., R. Onozaki, E. Takahashi, S. Takahashi, N. Fujio, T. Harada, N. Morisaki, K. Shirai, Y. Saito, and S. Hirose.** Regulation of neutral cholesterol esterase activity by phospholipids containing negative charges in substrate liposome. *J. Lipid Res.* 1995. **36:** 2303–2310.

Supplementary key words phospholipase A_2 • phospholipase D • cholesteryl esters

In atherosclerotic lesions, foam cells with large amounts of lipid droplets are found. The major component of lipid droplets is cholesteryl ester, but these droplets also contain other lipids (1, 2). Hata, Hower, and Insull (1) reported that human atherosclerotic lesions contain many types of neutral lipids and phospholipids, and that the composition of lipids changed depending on the development of atherosclerosis from fatty streaks to fibrous plaques.

Foam cells in early atherosclerotic lesions are believed to be derived mainly from macrophages. Macrophage cholesterol metabolism takes place as follows (3, 4). Macrophages take up denatured low density lipoproteins (LDL) (5–7) and β-very low density lipoproteins (βVLDL) (8, 9) through specific receptors (10–13). These lipoproteins are rapidly delivered to lysosomes where their cholesteryl ester is hydrolyzed by acid cholesterol esterase (4, 14–16). A small portion of free cholesterol released is excreted from the cells (17) and the rest is re-esterified by acyl-CoA:cholesterol acyltransferase (ACAT) in microsomes (18, 19). Then, the re-esterified cholesterol is stored in lipid droplets together with other lipids (1). Furthermore, cholesteryl ester stored in lipid droplets is also hydrolyzed by neutral cholesterol esterase (20–25).

Macrophages are converted into foam cells in cultured systems. Inflammatory macrophages, phorbol ester-treated monocyte-like cells (24, 26), thioglycollate (23, 27), and lipopolysaccharide-stimulated macrophages (28) take up βVLDL and accumulate cholesteryl ester intracellularly as lipid droplets. However, the reason why cholesteryl ester in lipid droplets is hardly hydrolyzed by neutral cholesterol esterase is unclear. On the other hand, alveolar macrophages have been shown to catabolize exogenous cholesterol derived from βVLDL, i.e., these cells take up βVLDL, release cholesterol as a result of the high activity of neutral cholesterol

Abbreviations: VLDL, very low density lipoprotein; PC, phosphatidylcholine; PA, phosphatidic acid.

[1]To whom correspondence should be addressed at: Department of Biochemistry, Faculty of Pharmaceutical Sciences, Chiba University. 1-33, Yayoi-cho, Inage-ku, Chiba, 263, Japan.

esterase, and, as a result, accumulate less cholesteryl ester (23). However, the mechanism of the intracellular regulation of this enzyme activity is also unclear. Lipids other than cholesteryl ester in lipid droplets may be important for the regulation of this enzyme activity. In this study, we investigated the role of the lipid composition of intracellular lipid droplets in the regulation of this enzyme activity, using several substrate conditions.

MATERIALS AND METHODS

Chemicals

Cholesteryl [^{14}C]oleate (2.1 GBq/mmol) and [^{3}H]cholesteryl oleate (3.0 GBq/mmol) were purchased from New England Nuclear (Boston, MA). Phospholipids, phospholipase A_2 (*Naja naja* venom) and phospholipase D (cabbage) were obtained from Sigma (St. Louis, MO).

Preparation of lipid droplets from rat thioglycollate-elicited macrophages

Thioglycollate-elicited peritoneal macrophages were obtained from Wistar-King strain rats (180–200 g) 4 days after the intraperitoneal injection of 3% thioglycollate medium (DIFCO). Macrophages were obtained by peritoneal lavage with 50 ml phosphate-buffered saline (PBS) as described previously (29). These cells were plated at a concentration of 2×10^6 cells/ml in Dulbecco's modified Eagle's medium (DMEM, Nissui Pharmaceutical Co., Tokyo) containing 10% fetal bovine serum (DMEM/10% FBS). The cells were cultured overnight and unattached cells were washed off. Then, 1 mg cholesterol of βVLDL or [^{3}H]cholesteryl oleate-βVLDL was added to the medium and incubated for 48 h. The cells were washed twice with DMEM/10% FBS and twice with PBS, and then harvested.

Lipid droplets were prepared by the method of Okuda, Morimoto, and Tsujita (30). One packed volume of cells was suspended in 4 ml of 5 mM Tris-HCl buffer (pH 7.4). The suspension was mixed by slowly inverting the centrifuge tube three times and then centrifuged at 200 *g* for 3 min at room temperature. The fat layer was removed and mixed with 4 ml of 5 mM Tris-HCl buffer (pH 7.4) containing 0.0025% Triton X-100 by slowly swinging the tube three times, and the mixture was centrifuged at 200 *g* for 3 min at room temperature. The fat layer was washed once with buffer A (25 mM TES, pH 7.4, containing 135 mM NaCl, 5 mM KCl, and 1 M $MgCl_2$), incubated with buffer A at 37°C for 10 min, and centrifuged at 200 *g* for 3 min. It was then washed again with buffer A. Approximately 95% of protein was removed from the fat layer by these procedures. This fat layer was used as lipid droplets in our study.

Electron microscopy

Lipid droplets and artificial liposomes were negatively stained and analyzed by electron microscopy (SRL, Tokyo).

Preparation of enzyme source

Alveolar macrophages were obtained by alveolar lavage after perfusing the lung three times with 15 ml PBS. The collected cells (1×10^8 cells) were homogenized in 1 ml of 0.25 M sucrose, 10 mM Tris-HCl buffer (pH 7.4) and centrifuged (15,000 *g*, 25 min). The supernatant was used as enzyme solution.

Preparation of reconstituted [^{3}H]cholesteryl oleate in βVLDL

βVLDL (d < 1.006 g/ml) was isolated from the serum of cholesterol-fed rabbits by ultracentrifugation for 16 h (8). Incorporation of [^{3}H]cholesteryl oleate into βVLDL was measured essentially by the method of Brown, Dana, and Goldstein (31). One GBq of [^{3}H]cholesteryl oleate was added with 1 ml of dimethylsulfoxide. The mixture was sonicated for 30 sec. Then, 2 ml of plasma density buffer (0.154 M NaCl, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4, 0.01% NaN_3) was added and the mixture was resonicated for 30 sec. It was then added dropwise to 6 ml of βVLDL (10 mg total cholesterol/ml) in 3 min. The solution was incubated for 8 h at 37°C and then dialyzed against 3 L plasma density buffer for 10 h. After dialysis, the solution was centrifuged for 16 h at 105,000 *g*. The top layer was used as [^{3}H]cholesteryl oleate-βVLDL. The specific activity was about 8×10^6 dpm/mg total cholesterol.

Assay of neutral cholesterol esterase activity

Hydrolysis of cholesterol [^{14}C]oleate (23). Preparation of substrate emulsion particles was as follows: 10 mM cholesteryl oleate, 18.5 kBq of cholesteryl [^{14}C]oleate, and various concentrations of phospholipids were mixed well and dried under N_2 gas. Two ml of 0.9% NaCl was added and sonicated for 90 min in an ice bath. This emulsion was used for neutral cholesterol esterase activity assay as substrate emulsion particles. The reaction mixture of neutral cholesterol esterase activity contained 40 µl of substrate emulsion particles, enzyme (100–150 µg protein), and 100 mM Tris-HCl (pH 7.4) in a total volume of 200 µl. Incubation was carried out at 37°C for 1 h. The [^{14}C]oleate release was stopped by the addition of 3.25 ml of chloroform–methanol–heptane 1.42:1.25:1.00, and 1 ml 0.1 N NaOH was added. The water phase was then counted.

Hydrolysis of [^{3}H]cholesteryl ester in lipid droplets. Lipid droplets containing [^{3}H]cholesteryl ester and enzyme were mixed in 400 µl of reaction mixture (about 100,000 dpm/tube) containing 100 mM Tris-HCl, pH 7.4. The

reaction mixture was incubated at 37°C for 3 h. Four ml chloroform–methanol 2:1 (v/v) was added to the reaction mixture, and the extracted lipids were separated by thin-layer chromatography (32). The radioactivity in free cholesterol and cholesteryl ester was counted. Liposomes containing [^{3}H]cholesteryl oleate and phosphatidylcholine (40% dry weight) were prepared and used as a control substrate.

Analysis of neutral lipids and phospholipids of βVLDL and lipid droplets

The lipid extraction (33) and determination of lipid composition (34) of βVLDL and lipid droplets were carried out according to the methods of previous reports by using HPTLC plates (silica gel 60, 0.20-mm layer thickness, 10 × 10 cm, Merck).

Protein concentration

Protein concentration was determined by Bradford's method with a kit (Bio-Rad, protein assay).

RESULTS

Effect of taurocholate on neutral cholesterol esterase activity

It is known that pancreatic neutral cholesterol esterase has especially high activity for hydrolysis of cholesteryl ester compared with other esterases, and that bile acid is the essential factor for this enzyme activity. To clarify whether the characteristics of neutral cholesterol esterase in macrophages are similar to the pancreatic enzyme, we examined the effect of sodium taurocholate on enzyme activity (**Fig. 1**). The activity in pancreas, adrenal gland, and liver was increased 3- to 12-times by taurocholate, in agreement with previous reports (35, 36). However, the activity in alveolar macrophages and thioglycollate-elicited macrophages was not increased. The activity in lung without alveolar macrophages, after washing with PBS, was very low both with and without taurocholate. These results suggested that the regulation of neutral cholesterol esterase activity in macrophages is probably different from the pancreatic one.

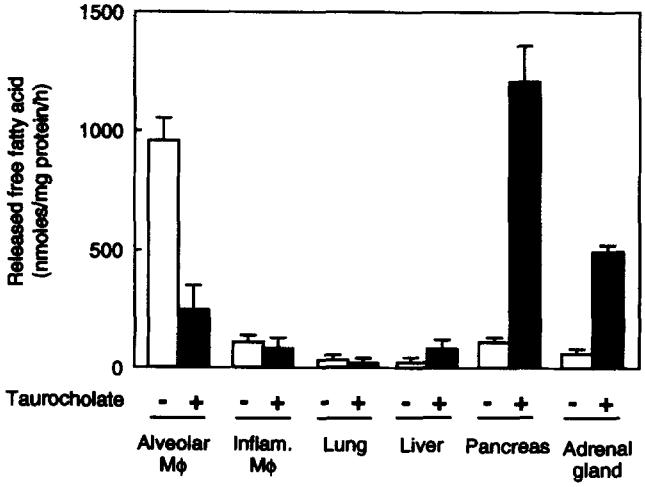

Fig. 1. Effect of sodium taurocholate on neutral cholesterol esterase activity in various organs. The substrate used was cholesteryl [^{14}C]oleate emulsified with 40% (dry weight) phosphatidylcholine. The mixture with or without 5 mM sodium taurocholate was assayed as described in Methods. Alveolar Mϕ, alveolar macrophages; Inflam. Mϕ, inflammatory macrophages (thioglycollate-elicited macrophages). Values are mean ± SD for triplicate experiments.

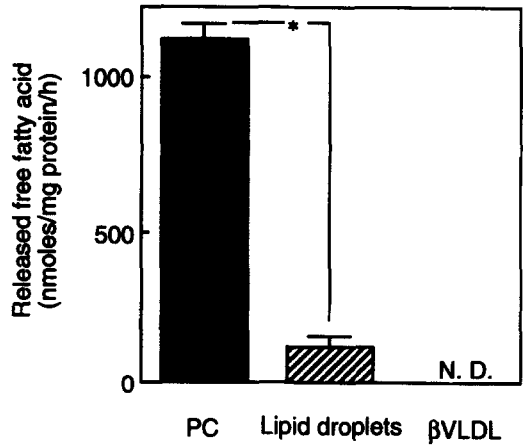

Fig. 2. [^{3}H]cholesteryl ester hydrolysis in lipid droplets by neutral cholesterol esterase. Hydrolysis of [^{3}H]cholesteryl oleate emulsified with phosphatidylcholine (PC), [^{3}H]cholesteryl ester in lipid droplets, and [^{3}H]cholesteryl oleate-βVLDL was measured as described in Methods. Values are mean ± SD for triplicate experiments; N.D., not detected; $*P < 0.001$.

Hydrolysis of cholesteryl ester in lipid droplets

Liposomes containing cholesteryl ester emulsified with phosphatidylcholine were used as substrate in the general assay of neutral cholesterol esterase, because phosphatidylcholine is the major phospholipid in intracellular lipid droplets. However, the lipid droplets also contain other phospholipids (1), and their structure may be important. Therefore, we examined the hydrolytic activity of cholesteryl ester by neutral cholesterol esterase from alveolar macrophages in lipid droplets compared with that in the liposome, using phosphatidylcholine as emulsifier. **Figure 2** shows that hydrolysis of [^{3}H]cholesteryl ester in lipid droplets was about one-ninth that in the artificial liposome. [^{3}H]cholesteryl ester in βVLDL was not hydrolyzed by macrophage neutral cholesterol esterase under these conditions.

The size of lipid droplets and the artificial liposome was measured using electron microscopy. The diameter of 84% of the lipid droplets was less than 10 nm (**Fig. 3**), and that of the rest was 10–1580 nm. The diameter of

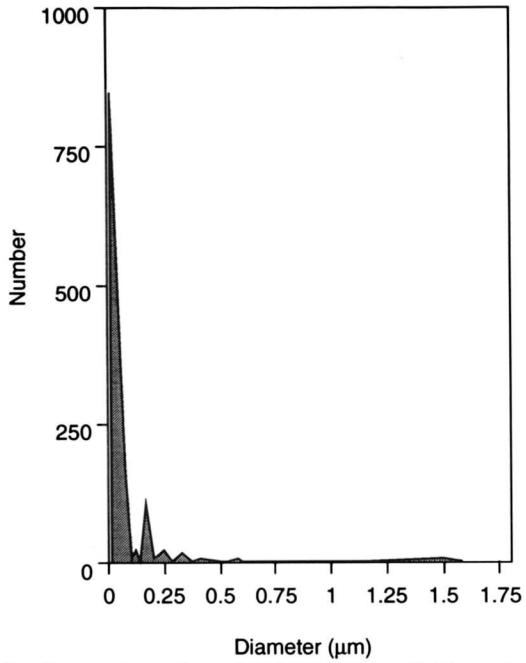

Fig. 3. Size and number of lipid droplets. Lipid droplets were prepared from thioglycollate-elicited, βVLDL-loaded macrophages. Diameter of lipid droplets was measured by electron microscopy as described in Methods (n = 1,207).

almost all the artificial liposomes was also less than 10 nm. Therefore, generally speaking, the diameters of both substrates were almost the same in our study. These data suggested that there was little possibility that the size of the substrate, or the surface area of the substrate, was the major factor regulating lipolytic activity in lipid droplets and the artificial liposomes.

Lipid composition of βVLDL and lipid droplets

Next we analyzed the lipid composition of βVLDL (**Table 1**) and of lipid droplets (**Table 2**). Phospholipids accounted for about 44% of the lipids in βVLDL and phosphatidylcholine was the major class. The amounts of cholesteryl ester and triglyceride were almost the same. In lipid droplets, the major lipid was cholesteryl ester. The percentage of total phospholipids was only 8.5%. The three major phospholipids were phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin, which are known to be mainly produced in macrophages (37).

Effect of phospholipids on neutral cholesterol esterase activity

As shown in Table 2, lipid droplets contained various classes of phospholipids. To clarify the effect of these phospholipids on cholesterol esterase activity, we prepared artificial substrate particles using these phospholipids. Phospholipids affected the hydrolysis of cholesteryl ester by neutral cholesterol esterase in different ways (**Fig. 4**). The optimal concentration of phosphatidic acid and phosphatidylinositol was 40% of dry weight in substrate particles and increased neutral cholesterol esterase activity 1.5- to 3.8-times compared with phosphatidylcholine. That of phosphatidylserine and cardiolipin was 80% or more and increased the activity 2.3-times. Phosphatidylethanolamine- or sphingomyelin-emulsified substrate showed a lower enzyme activity compared with other phospholipid-emulsified substrates at any concentration, and the specific activity at 40% was one-seventh compared with the activity at 40% phosphatidylcholine. At 10% of phospholipids, neutral cholesterol esterase activity was very low in all substrates.

The neutral cholesterol esterase was not changed by the addition of triglyceride the concentration range, 0–40% triglyceride, covers the triglyceride content of βVLDL as shown in Tables 1 and 2 in substrates emulsified with phosphatidylcholine or phosphatidic acid (data not shown). These results suggested that triglyceride is a negligible factor in the regulation of the activity of neutral cholesterol esterase.

TABLE 1. Lipid composition of βVLDL from rabbits fed high cholesterol diet

Component	Content
	%
Total lipids[a]	100
Neutral lipids	
Free cholesterol	7.94 ± 3.96
Cholesteryl ester	27.5 ± 5.35
Triglyceride	20.5 ± 2.84
Phospholipids	
Phosphatidylcholine	23.8 ± 5.45
Sphingomyelin	11.7 ± 3.98
Lysophosphatidylcholine	8.5 ± 1.30
Phosphatidylinositol	0.0028 ± 0.0008
Cardiolipin	0.0031 ± 0.0025

[a]Seven to eight mg of total lipids was used.

TABLE 2. Lipid composition of lipid droplets from βVLDL-loaded macrophages

Component	Content
	%
Neutral lipids	
Free cholesterol	6.77 ± 2.17
Cholesteryl ester	57.6 ± 4.35
Triglyceride	27.1 ± 2.56
Phospholipids	
Phosphatidylcholine	4.42 ± 1.59
Phosphatidylethanolamine	2.23 ± 0.78
Sphingomyelin	1.81 ± 0.89
Phosphatidylinositol	0.0142 ± 0.0023
Cardiolipin	0.0046 ± 0.0003

Lipid droplets were prepared from about 10^8 cells. Mean of total lipids was 405.7 ± 71.8 μg.

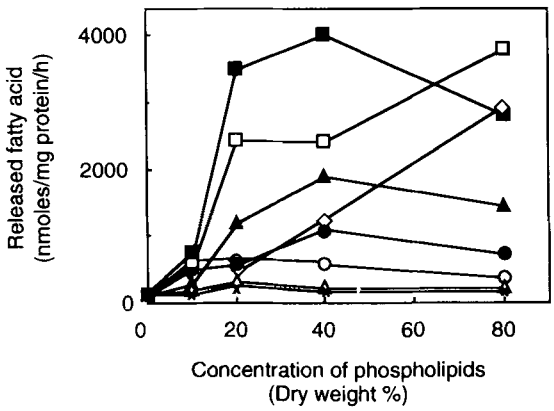

Fig. 4. Effect of various phospholipids on neutral cholesterol esterase activity. Cholesteryl [^{14}C]oleate was prepared with various phospholipids at indicated concentrations (dry weight %). Hydrolysis of cholesteryl [^{14}C]oleate was measured as described in Methods. (■) Phosphatidic acid; (□) phosphatidylserine; (◇) cardiolipin; (▲) phosphatidylinositol; (●) phosphatidylcholine; (○) lysophosphatidylcholine; (△) phosphatidylethanolamine; (x) sphingomyelin. Values are mean for triplicate experiments and standard deviation was within a range of 5% for each point.

Effect of phospholipases on neutral cholesterol esterase

Phospholipase treatment can change the structure and characteristics of phospholipids. To determine the possibility of the regulation of neutral cholesterol esterase activity by treatment of the substrate with phospholipase as chemical modifier, we investigated the effect of phospholipases A_2 and D on the enzyme activity. Phosphatidylcholine is converted to lysophosphatidylcholine by phospholipase A_2 and to phosphatidic acid by phospholipase D. Phospholipase A_2-treated substrate decreased neutral cholesterol esterase activity to one-fourth even at a low level (1 unit/tube) of phospholipase A_2. On the other hand, phospholipase D-treated substrate increased cholesterol esterase activity in a dose-dependent manner, with the activity being 2-fold at 20 units of phospholipase D (**Fig. 5**). Not all, but part of phosphatidylcholine was expected to convert to each respective product by treatment with the phospholipases in this experiment. Therefore, the lipid composition of substrates treated with phospholipase A_2 after Sephacryl S-400 gel filtration was analyzed; cholesteryl ester–phosphatidylcholine–lysophosphatidylcholine was 59.0%–37.1%–3.1% at 10 units per tube and 56.0%–22.5%–21.5% at 20 units per tube, respectively. Further, phospholipase D treatment (20 units per tube) converted 17.2% of phosphatidylcholine to phosphatidic acid. These results indicated that modification of the polar heads in phospholipids with phospholipases affected the hydrolysis of cholesteryl ester by neutral cholesterol esterase.

Furthermore, to confirm the results shown in Fig. 4, we prepared reconstituted liposomes using two kinds of phospholipids and measured the cholesteryl ester hydrolysis activity (**Fig. 6A**). When lysophosphatidylcholine was increased in the substrate liposome, the neutral cholesterol esterase activity decreased up to 53% in a dose-dependent manner. On the other hand, when phosphatidic acid was increased in the substrate, the enzyme activity increased in a dose-dependent manner up to 3.4-times (Fig. 6B). These results are consistent with those using substrates modified with phospholipases (Fig. 5), suggesting that phospholipids affect the neutral cholesterol esterase activity.

DISCUSSION

Cholesterol esterase derived from pancreas has been described in terms of protein structure, gene expression, function, and regulation (35, 36, 38–41). It is secreted from pancreatic cells into food in the small intestine for the digestion of cholesteryl ester in order to absorb free cholesterol through the intestinal cell membrane. Bile acid is an essential factor for the enzyme activity. The increase of neutral cholesterol esterase activity observed in pancreas, adrenal gland, and liver by the addition of sodium taurocholate (Fig. 1) is in accord with the previous report that the enzyme in liver is the same as the pancreatic enzyme (35). As far as activation by bile acid is concerned, it is of interest and deserves greater attention in future studies: the enzyme in adrenal gland may be the same as that in liver and pancreas. However, neutral cholesterol esterase in

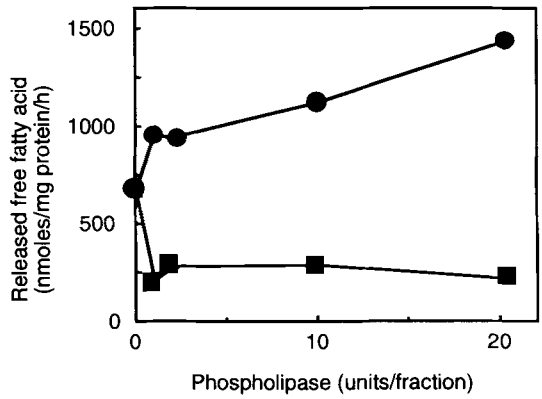

Fig. 5. Effect of treatment of phosphatidylcholine-emulsified cholesteryl [^{14}C]oleate with phospholipase A_2 or D on neutral cholesterol esterase activity. Cholesteryl [^{14}C]oleate was prepared with phosphatidylcholine and pre-incubated with phospholipase A_2 (30 mM Tris-HCl, pH 8.4) at 25°C (■) or phospholipase D (30 mM acetate buffer, pH 5.6) at 30°C (●) for 1 h. One unit will hydrolyze 1.0 μmol of phosphatidylcholine to lysophosphatidylcholine and a fatty acid per min at each condition. The neutral cholesterol esterase activity was measured as described in Methods. Values are mean for triplicate experiments and the standard deviation was within a range of 5% for each point.

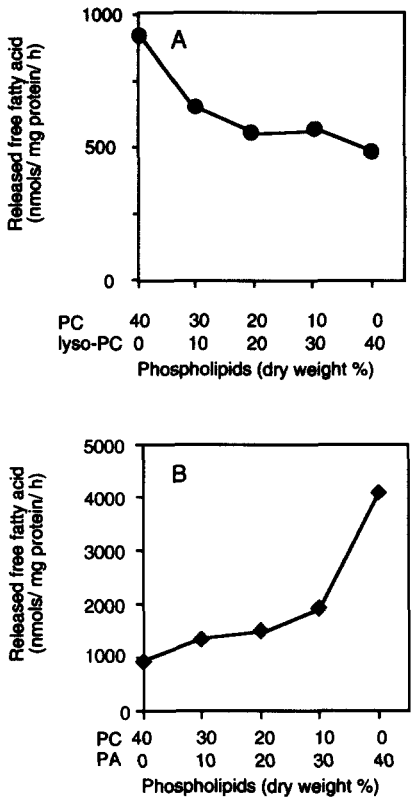

Fig. 6. Effect of lysophosphatidylcholine and phosphatidic acid on neutral cholesteryl esterase activity. Cholesteryl [^{14}C]oleate was emulsified with various concentrations of phosphatidylcholine and lysophosphatidylcholine (A) or phosphatidic acid (B) for use as substrates. The neutral cholesterol esterase activity was measured as described in Methods. Values are mean for triplicate experiments and the standard deviation was within a range of 5% for each point.

macrophages acts on intracellular cholesteryl ester derived from ACAT reaction. The addition of sodium taurocholate did not increase neutral cholesterol esterase activity in resident alveolar and thioglycollate-elicited peritoneal macrophages, suggesting that this enzyme is different from the other enzymes mentioned above (Fig. 1). Furthermore, the neutral cholesterol esterase was not stimulated by cAMP-dependent protein kinase in our study (data not shown), thus being inconsistent with the data of Khoo, Mahoney, and Steinberg (20). We then investigated other regulatory factor(s) in the cell for hydrolysis of intracellular cholesteryl ester stored in lipid droplets.

The structure of the artificial liposome substrate used in this study was spherical, with cholesteryl ester in the center surrounded by phospholipids. In the assay of neutral cholesterol esterase using these liposomes as substrate, the enzyme would first recognize phospholipids on the surface of the liposome, and then bind to cholesteryl ester at the active site to hydrolyze. It is speculated from the data in Fig. 4 that the stronger the negative charge of phospholipids is, the higher the affinity between liposomes and the enzyme. To confirm these results, we investigated the effect of phospholipase D (Fig. 5) and phosphatidic acid (Fig. 6B) on the artificial substrate emulsified with phosphatidylcholine. Because part of phosphatidylcholine in liposomes is converted into negatively charged phosphatidic acid by treatment with phospholipase D, the affinity between the substrate and the enzyme would increase and, as a result, more cholesteryl ester would be hydrolyzed, as observed in Fig. 5. Further, the fact that an increase in phosphatidic acid in reconstituted liposomes enhanced the neutral cholesterol esterase activity strongly supports this speculation. These results suggested that neutral cholesterol esterase might interact with negative charges of substrate liposomes.

The concentration of total phospholipids in lipid droplets was about 8.5% (Table 2), and they contained relatively large amounts of phosphatidylethanolamine and sphingomyelin. Low concentrations of phospholipids (less that 10%) resulted in a low activity of neutral cholesterol esterase. Phosphatidylethanolamine and sphingomyelin in lipid droplets are weakly negatively charged at neutral conditions and have less neutral cholesterol esterase activity than other phospholipids with a stronger negative charge (Fig. 4). This phospholipid composition and the relatively low concentration of phospholipids (Fig. 2) may in part explain why cholesteryl ester in lipid droplets is hydrolyzed with difficulty. As the incorporation of cholesteryl ester in atherogenic lipoproteins is not decreased by down-regulation of their receptors, macrophages would inevitably be converted into foam cells with the low hydrolyzing activity of cholesteryl ester.

Phospholipids that elicit a low activity of neutral cholesterol esterase result not only in an imbalance between hydrophobic and hydrophilic sites but also in a disturbance of the molecule (42). Substrates without a uniform structure would change affinity between substrate and enzyme. The substrate emulsified with lysophosphatidylcholine is known to make a micelle, and the negative charge of lysophosphatidylcholine is about the same as that of phosphatidylcholine at a neutral condition. Nevertheless, the neutral cholesterol esterase activity was low with lysophosphatidylcholine compared with phosphatidylcholine (Figs. 5 and 6A). These data suggest that the regulatory factor for neutral cholesterol esterase is not only the negative polar heads of phospholipids but also their physical structure.

Cholesteryl ester in βVLDL was not hydrolyzed by neutral cholesterol esterase (Fig. 2) in spite of the presence of about 40% of total phospholipids (Table 1). One

reason for this is that the lysophosphatidylcholine concentration was high compared with other lipoproteins (Table 1). As for lipid droplets, we could not detect lysophosphatidylcholine in this experiment, but Hata et al. (1) determined that lysophosphatidylcholine increased in fibrous plaques compared with fatty streaks. This suggests that lysophosphatidylcholine may inhibit the hydrolysis of cholesteryl ester in highly developed atheromatous lesions (fibrous plaques). Furthermore, there have been many reports about lysophosphatidylcholine in relation to atherogenesis; it is a chemotactic factor for blood monocytes and T-lymphocytes (43, 44); it can induce the specific expression of adhesion molecules ICAM-1 and VCAM-1 (45, 46); and it is a mediator of the expression of the scavenger pathway in medial smooth muscle cells (47).

The present findings have demonstrated another atherogenic feature of lysophosphatidylcholine. Considering the result that only a slight increase of lysophosphatidylcholine in liposomes decreased the enzyme activity (Figs. 4 and 5) and the report that activated macrophages retained high activity of phospholipase A_2 (48), it is possible that phospholipase A_2 may act on lipid droplets, release lysophosphatidylcholine, and induce cholesteryl ester accumulation in macrophages by decreasing neutral cholesterol esterase activity for lipid droplets with abnormal structure. This suggests that lysophosphatidylcholine is an important regulator in atherosclerotic lesions. Studies about whether inhibition of the production of lysophosphatidylcholine by phospholipase A_2 inhibitor prevents atherogenesis need to be carried out. ■

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