Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1758 (2006) 499–508
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Preferable stimulation of PON1 arylesterase activity by phosphatidylcholines with unsaturated acyl chains or oxidized acyl chains at *sn-2* position

Su Duy Nguyen, Dai-Eun Sok *

College of Pharmacy, Chungnam National University, Yuseong Ku, Gung-Dong, Taejon 305-764, Korea

Received 20 December 2005; received in revised form 3 March 2006; accepted 16 March 2006
Available online 17 April 2006

Abstract

To examine the effect of phospholipids on PON1 activities, purified PON1 was exposed to phospholipids prior to the determination of arylesterase and paraoxonase activities. Phosphatidylcholines with saturated acyl chains (C10–C16) showed a stimulation of both activities, chain length-dependent, with a greater stimulation of arylesterase activity, suggesting the implication of lipid bilayer in the stimulatory action. Such a preferable stimulation of arylesterase activity was more remarkable with phosphatidylcholines with polyunsaturated acyl chains or oxidized chains at *sn-2* position, implying that the packing degree of acyl chain may be also important for the preferable stimulation of arylesterase activity. Separately, 1-palmitoyl-lysoPC also stimulated arylesterase activity preferably, indicating that the micellar formation of lipids around PON1 also contributes to the stimulatory action. Additionally, phosphatidylglycerols slightly enhanced arylesterase activity, but not paraoxonase activity. In contrast, phosphatidylserine and phosphatidic acid ($\geq$0.1 mM) inhibited both activities Further, such a preferable stimulation of arylesterase activity by phosphatidylcholines was also reproduced with VLDL-bound PON1, although to a less extent. These data indicate that phosphatidylcholines with polyunsaturated acyl chains or oxidized chain, or lysophosphatidylcholine cause a preferable stimulation of arylesterase activity, thereby contributing to the decrease in the ratio of paraoxonase activity to arylesterase activity.

Keywords: PON1; Arylesterase; Paraoxonase; Preferable stimulation; Phospholipid; Acyl chain; Unsaturation; Oxidized

1. Introduction

Paraoxonase1 (PON1), exclusively associated with HDL, has the ability to hydrolyze organophosphate compounds, aromatic carboxylic acid esters, and some lactone compounds [1–4]. In addition to the potential role of PON1 in detoxification of toxic organophosphates [5–7], another interest in the enzyme is that PON1 may exert antiatherogenic action [3,7,8]. The role of PON1 in atherosclerosis development was demonstrated in studies using mice lacking PON1 [7] or PON1-knock-out mice [9], or overexpressing PON1 [10,11]. Antiatherogenic actions of PON1 include protection of HDL-associated PON1 against oxidative stress [8,9,11–15]; attenuation of macrophage oxidative stress [11], inhibition of oxidized LDL-induced MCP-1 production in endothelial cells [14], and stimulation of HDL-mediated cholesterol efflux from macrophages [15]. Noteworthy, among HDL subfractions, HDL_3, which carries the high PON1 activity, is efficient in reverse cholesterol transport [16]. Further, PON1 is a more potent antioxidant and stimulant of macrophage cholesterol efflux in HDL-bound state than in lipid-deficient state [17], suggesting that antiatherogenic function of PON1 may be expressed in lipid-bound state. In addition, the composition of HDL may be also important for the function of PON1 as suggested from the change of PON1 arylesterase activity during the transition from HDL to pre beta HDL [18]. Noteworthy, there was a remarkable reduction of arylesterase activity in lipid-deficient PON1, whereas a negligible change

Abbreviations: PON1, paraoxonase1; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositiol; PG, phosphatidylglycerol; PS, phosphatidylserine; PA, phosphatidic acid; PAF, 1-hexadecyl-2-acetyl-phosphatidylcholine; LPC, palmitoyl-lysophosphatidylcholine; HDL, high density lipoprotein; VLDL, very low density lipoprotein; Trp, tryptophan

* Corresponding author. Tel.: +82 42 821 5940; fax: +82 42 823 6566.
E-mail address: daesok@cnu.ac.kr (D.-E. Sok).

0005-2736/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2006.03.022

of paraoxonase activity was observed [17]. In this respect, the interaction between PON1 activities and lipids needs further clarification.

Previous studies showed that in contrast to a negligible change of arylesterase activity, there was frequently the reduction of serum paraoxonase activity in some oxidative stress-associated disease patients [19,20], as well as with smoking and advancing age [21,22], indicating that the preferential decrease of paraoxonase activity was associated with oxidative stress.

One explanation for preferential decrease of paraoxonase activity may be suggested from the differential effect of some negatively-charged oleoylated lipids on PON1 activities [23]. Alternatively, it is quite possible that the preferable stimulation of arylesterase activity by HDL lipids, susceptible to the redox state in vivo [22], may result in the preferential reduction of paraoxonase activity of PON1. There have been reports [22,24] that the level of oxidized phospholipids was enhanced in lipoproteins of oxidative stress-associated state, where the preferential reduction of paraoxonase activity have been frequently observed [19–22]. Previously, it had been found that PON1 arylesterase activity was stimulated by some phospholipids including phosphatidylcholine [25,26]. Meanwhile, during a lengthy exposure to oxidized phosphatidylcholine, PON1 arylesterase activity was reported to be inactivated [27].

Thus, the contrasting effect between phosphatidylcholine and oxidized phosphatidylcholine remains to be clarified. Therefore, an extensive study, employing phospholipids differing in the headgroup as well as the size of fatty acyl chain [28,29], is to be explored to elucidate the mode by which phospholipids interact with PON1 molecule. Furthermore, it would be worthwhile to find out the type of phospholipids responsible for the preferential stimulation of PON1 arylesterase activity, which may contribute to the reduction in the ratio of paraoxonase activity to arylesterase activity [19–22].

In this study, we examined the effect of various phospholipids on two activities of PON1, soluble or lipid-bound, and the properties of phospholipids expressing the preferable stimulation of PON1 arylesterase activity.

2. Materials and methods

2.1. Materials

All materials were purchased from Sigma Chemical Co. (St. Louis. MO, USA) unless otherwise noted. Various phospholipids including 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycerophosphorylcholine, 1-palmitoyl-2-glutaroyl-*sn*-glycerophosphorylcholine, 1-palmitoyl-2-azelaoyl-*sn*-glycerophosphocholine or 1-alkyl-2-acetoyl-*sn*-glycero-phosphocholine as well as lysophosphatidylcholine were provided by Avanti Polar Lipids, Inc. (Alabaster, AL, USA).

2.2. Purification of PON1 from human plasma

PON1 was purified from human plasma by chromatographic procedures according to a slight modification of the published procedures [6,26,30], and kept in 25 mM Tris buffer containing 1 mM Ca^{2+} at 4 °C. The purified enzyme possessed a specific activity of approximately 1018 and 0.552 μmole/min/mg protein in the hydrolysis of phenyl acetate and paraoxon, respectively. The purified PON1, used mainly for this study, was found to belong to phenotype AB group, based on the dual substrate method [31].

2.3. Assay of paraoxonase1 (PON1)

Arylesterase activity of PON1 was measured by adding enzyme solution to 0.5 ml of 50 mM Tris buffer, pH 7.4 containing 1 mM $CaCl_2$ and 1 mM phenyl acetate as described before [6,26]. Paraoxonase activity was determined in 50 mM Tris buffer (pH 7.4) containing 1 mM $CaCl_2$ and 1 mM paraoxon as described before [6]. One unit of arylesterase activity is expressed as 1 μmol of phenol produced per min, and one unit of paraoxonase activity, as 1 nmol of p-nitrophenol produced per min.

2.4. Isolation of lipoproteins

Lipoprotein fractions, VLDL (d<1.006 g/ml) and HDL (d=1.063–1.21 g/ml), were isolated from human serum by density-gradient ultracentrifugation [32]. Lipoproteins, after dialysis against phosphate-buffered saline (PBS), pH 7.4 containing 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 137 mM NaCl and 2.0 mM KC1, were stored at 4 °C.

2.5. Preparation of liposomes

Liposome preparation was carried out according to previous publication [33] with some modification. Briefly, phospholipids, dissolved in chloroform/methanol, was subjected to vacuum drying, and the lipid film was sonicated vigorously for 5 min under N_2 in 50 mM Tris buffer (pH 7.4) using dismembrator (Branson sonifier 250) to give a stock solution of phospholipids (final concentration, 1 mM).

2.6. Effect of lipids on PON1 activities

Purified PON1 (5 arylesterase unit/ml) was preincubated with various concentrations (1–400 μM) of lipids in 0.1 ml of 50 mM Tris buffer pH, 7.4 containing 1 mM $CaCl_2$ at 25 °C for 30 min, and then 20 μl was taken for assay of hydrolysis of phenyl acetate [30]. Similarly, PON1 (5 arylesterase unit/ml) was exposed to various concentrations (1–400 μM) of phospholipids in 0.1 ml of 50 mM Tris buffer, pH 7.4 containing 1 mM $CaCl_2$ at 25 °C for 30 min and then 50 μl was taken for assay of hydrolysis of paraoxon. Separately, the effect of oxidized phosphatidylcholine, lysophosphatidylcholine or PAF on PON1 activities was determined as described above.

2.7. Effect of phospholipids on VLDL-bound PON1

VLDL-bound PON1 (0.2 unit arylesterase/ml) was preincubated with phospholipids in 0.1 ml of 50 mM Tris buffer, pH 7.4 containing 1 mM $CaCl_2$ at 25 °C, and 30 min later, aliquots (20 μl or 50 μl) were taken for assay of arylesterase and paraoxonase activities.

2.8. Molecular sieve chromatography of liposomes

The size distribution of liposome was determined according to published procedures [34,35] with minor modifications. In brief, 1 ml of liposomes (1 mM) was applied to a Sepharose 4B column (1.5×20 cm), which was eluted with 50 mM Tris (pH 7.4) containing 1 mM $CaCl_2$ and 100 mM NaCl at a flow rate of 0.2 ml/min. Each fraction (0.5 ml) was collected, and monitored for the absorbance at 208 nm or 300 nm [34,35].

2.9. Effect of phospholipids on PON1 Trp fluorescence

PON1 (5 unit arylesterase/ml, 0.1 μM) was preincubated with various concentrations (100–400 μM) of DMPC or DPPS in 1 ml of 50 mM Tris (pH 7.4) containing 1 mM $CaCl_2$ at 25 °C. After 30 min preincubation, the fluorescence emission spectra of PON1 Trp residue in the presence or absence of phospholipid were monitored [36] using Perkin-Elmer 550-55 Fluorimeter at 25

°C, with the excitation wavelength of 280 nm and excitation and emission slit widths of 10 nm.

2.10. Determination of E_{max}, E_{med} or EC_{50} values

The potency of each phospholipid in stimulating PON1 activity was evaluated on the basis of E_{max}, E_{med} or EC_{50} values. E_{max} represents the maximal stimulation % (relative to control) over the range of concentrations used, and E_{med} indicates the stimulation % achieved in the presence of each phospholipid of 30 μM. EC_{50}, the concentration of phospholipids required for 50% maximal stimulation, was determined by fitting the concentration–stimulation % curve of each phospholipid to hyperbolic function using Sigma Plot 2000 for Windows as follows; $Y=A\ X/B+X$, where X represents the concentration of phospholipids, Y is the stimulation %, and A and B are hyperbolic coefficients.

2.11. Statistics

All statistical analyses were performed using a SPSS program for window. Statistical assessments were performed using ANOVA for the initial demonstration of significance at $P<0.05$, followed by post hoc Duncan's multiple-range test [37].

3. Results

3.1. Effect of various phospholipids on PON1 activities

Previously, it was observed that some negatively-charged lysophospholipids or monounsaturated fatty acids caused the reduction in ratio of paraoxonase activity to arylesterase activity by inhibiting PON1 paraoxonase activity preferentially [23]. Separately, the preferable stimulation of PON1 arylesterase activity may lead to the reduction in ratio of paraoxonase activity to arylesterase activity. To see the preferable stimulation of PON1 arylesterase by phospholipids, purified PON1 was preincubated with various phospholipids for 30 min, and then PON1 activities, after 25-fold dilution, was determined using paraoxon or phenyl acetate as substrate. First, when PON1 was preincubated with 1-palmitoyl-2-myristoyl-PC (PMPC) at various concentrations (1–400 μM), both arylesterase activity and paraoxonase activity were enhanced with arylesterase activity being more responsive to PMPC (Fig. 1A and B). Similarly, the exposure of PON1 to sphingomyelin also resulted in some increase of arylesterase activity but not paraoxonase activity (data not shown). In contrast, neither dimyristoylphosphatidylethanolamine (DMPE) nor dioleoylphosphatidylethanolamine (DOPE) up to 400 μM had a significant effect on both activities. Subsequently, when the effect of negatively-charged phospholipids on PON1 activities was examined, dimyristoylphosphatidylglycerol (DMPG) was found to cause an apparent increase of arylesterase activity dose-dependently while having an inhibitory effect on paraoxonase activity. Likewise, soy bean phosphatidylinositol (PI) enhanced arylesterase activity, but slightly decreased paraoxonase activity. Meanwhile, dimyristoylphosphatidylethanol (DMPEt), negatively-charged, which showed a significant inhibition (~20%) of paraoxonase activity at 0.2 mM, had no effect on arylesterase activity (data not shown). Thus, soybean PI and DMPG expressed a selective stimulation of arylesterase activity.

In contrast, dimyristoylphosphatidylserine (DMPS), which had no significant inhibition of both activities up to 30 μM, showed a remarkable inhibition of both activities at higher concentration (0.1–0.4 mM) with a greater inhibition of arylesterase activity. Likewise, dimyristoylphosphatidic acid (DMPA) also inhibited both activities remarkably with a greater

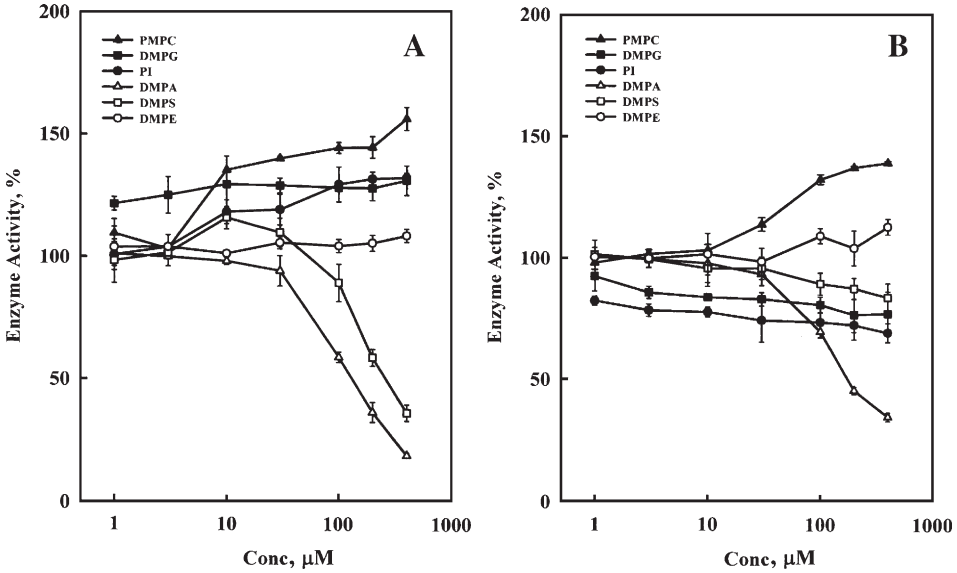

Fig. 1. Effect of phospholipids on arylesterase activity of PON1. A, PON1 (5 arylesterase units/ml) was preincubated with each phospholipid (1–400 μM) for 30 min in 0.1 ml of 50 mM Tris buffer (pH 7.4) containing 1 mM Ca^{2+} at 25 °C, and then the aliquot (20 μl) was taken for the assay of arylesterase activity in 0.5 ml of 50 mM Tris buffer, pH 7.4 containing 1 mM $CaCl_2$ and 1 mM phenyl acetate as described in Materials and methods. Enzyme activity was expressed as a percentile value (%) of arylesterase activity of PON1 (control), which was preincubated in the absence of phospholipid. Data are expressed as a mean±S.D. value of triplicate assays. B, PON1 (5 arylesterase units/ml) was preincubated with various phospholipids (1–400 μM) as described above, and then the aliquot (50 μl) was taken for the assay of paraoxonase activity in 50 mM Tris buffer (pH 7.4) containing 1 mM $CaCl_2$ and 1 mM paraoxon. Enzyme activity was expressed as a percentile value (%) of paraoxonase activity of PON1 (control), which was preincubated in the absence of phospholipids. Data are expressed as a mean±S.D. value of triplicate assays.

inhibition of arylesterase activity; 81% inhibition of arylesterase activity vs. 64% inhibition of paraoxonase activity by DMPA (400 μM).

3.2. Preferable stimulation of PON1 arylesterase activity by phosphatidylcholines

Compared to paraoxonase activity, arylesterase activity was more responsive to phosphatidylcholine stimulation. Therefore, a preferable stimulation of arylesterase activity was further studied using various types of phosphatidylcholine. As shown in Fig. 2A and B, both arylesterase activity and paraoxonase activity of PON1 were enhanced after preincubation with didecanoylphosphatidylcholine (DDPC) with arylesterase activity being more responsive to DDPC; arylesterase activity was remarkably (E_{max}, 45%) augmented by DDPC, in contrast to a modest stimulation (E_{max}, 33%) of paraoxonase activity (Table 1). A greater stimulation (E_{max}, 55–63%) of arylesterase activity was observed after the exposure of PON1 to dilauroylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC) or dipalmitoylphosphatidylcholine (DPPC). Among PC series with saturated acyl chains (C10–C16), the values of E_{med} values (stimulation % achieved with 0.03 mM lipid) seem to depend on the length of acyl chain as shown in Table 1. Noteworthy, the efficiency of PC series, expressed as the ratio of E_{med} value to EC_{50} value, in stimulating arylesterase activity was enhanced in accordance with the increase of fatty acyl chain length; C10<C12<C14<C16. However, the extension of acyl chain length over C16 failed to further enhance the stimulatory action, based on the E_{max} values; DPPC (56%) vs. DSPC (29%). Furthermore, such a stimulation of arylesterase activity was more remarkable with dilinoleoylphosphatidylcholine (DLnPC), which showed Emax of 78% and E_{med} of 58%. Similarly, 1-palmitoyl-2-linoleoylphosphatidylcholine (PLnPC) and 1-palmitoyl-2-arachidonoylphosphatidylcholine (PAPC) also displayed a remarkable stimulation of arylesterase activity with E_{max} values of 74–80% and E_{med} values of 57–58%. Thus, the stimulatory effect of phosphatidylcholines on arylesterase activity differed according to property of acyl chain. Nonetheless, there was no remarkable difference of E_{max} values (30 to 42%) in the stimulation of paraoxonase activity by phosphatidylcholines. Overall, the preferable stimulation of arylesterase activity was more prominent with phosphatidylcholines containing polyunsaturated fatty acyl chains than those with saturated ones.

3.3. Preferable stimulation of PON1 arylesterase activity by oxidized phosphatidylcholine, lysophosphatidylcholine or PAF

Subsequently, we examined the stimulatory effect of oxidized phosphatidylcholines with aldehyde or acid at the *sn-2* position [38]. As shown in Fig. 3A, arylesterase activity was more remarkably enhanced after the exposure of PON1 to 1-palmitoyl-2-(5′-oxo-valeroyl)-phosphatidylcholine (POVPC), compared to its precursor, 1-palmitoyl-2-arachidonyl-phosphatidylcholine (PAPC); the E_{max} and the EC_{50} value were 102% and 3.4 μM for POVPC vs. 74% and 3.0 μM for PAPC (Table 1). Meanwhile (Fig. 3B), the stimulation of paraoxonase activity was observed to be similar between POVPC (E_{max}, 31.1% and EC_{50}, 15.9 μM) and PAPC (E_{max}, 30.2% and EC_{50}, 28.9 μM). Thus, POVPC was more effective than PAPC in exerting a preferable stimulation of arylesterase activity. Additionally, 1-palmitoyl-2-glutaroyl-phosphatidylcholine (PGPC), another oxidation product of PAPC, exhibited E_{max} value of 97.9% and

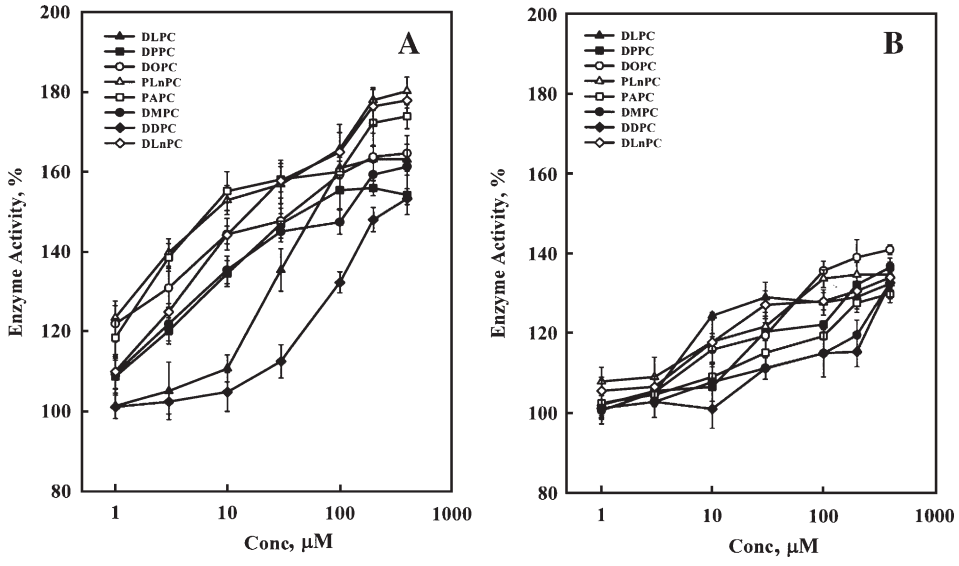

Fig. 2. Stimulatory effect of phosphatidylcholine on PON activities. A, PON1 (5 arylesterase units/ml) was preincubated with each PC (1–400 μM) for 30 min in 0.1 ml of 50 mM Tris buffer (pH 7.4) containing 1 mM Ca^{2+} at 25 °C, and then the aliquot (20 μl) was taken for the assay of arylesterase activity as depicted in Fig. 1A. Enzyme activity was expressed as a percentile value of arylesterase activity of PON1 (control), which was preincubated in the absence of PC. Data are expressed as a mean±S.D. value of triplicate assays. B, PON1 (5 arylesterase units/ml) was preincubated with each phosphatidylcholine (1–400 μM) as described above, and then the aliquot (50 μl) was taken for paraoxonase activity assay as described in Fig. 1B. Enzyme activity was expressed as a percentile value of paraoxonase activity of control. Data are expressed as a mean±S.D. (bar) value of triplicate assays.

Table 1
E_{max}, E_{med} and EC_{50} of each lipid in the stimulation of purified PON1 activities

Phospholipids	E_{max} (%)		E_{med} (%)	EC_{50} (μM)	
Substrate	Phenyl acetate	Paraoxon	Phenyl acetate	Phenyl acetate	Paraoxon
Control	0	0	0		
+DDPC	44.6±1.5[a]	33.0±2.1	12.5±8.2[a]	71.08±4.6[a]	128.24±6.2
+DLPC	63.4±3.4[b]	32.8±4.8	35.5±5.4[b]	25.36±2.9[b]	–
+DMPC	62.6±5.5[b]	39.6±0.7	45.8±2.6[c]	7.60±0.5[c]	87.56±5.8
+DPPC	56.2±1.9[bc]	36.9±1.3	47.1±2.5[c]	5.47±1.4[c]	34.28±1.2
+DSPC	28.6±0.9[d]	–	18.3±2.6[a]	12.3±1.2[d]	–
+DOPC	64.9±4.4[ce]	41.5±1.2	47.9±4.3[c]	3.27±0.3e	22.96±2.8
+PLnPC	80.1±1.7[e]	32.8±10.8	57.1±3.0[d]	3.41±0.9[e]	22.03±1.3
+PAPC	74.1±5.1[e]	30.2±1.2	58.3±4.2[d]	3.01±0.8[e]	28.92±2.1
+DLnPC	78.1±1.9[ce]	34.4±4.9	57.9±3.3[d]	4.52±1.1[e]	23.56±3.2
+POVPC	102.0±3.5[f]	31.1±1.6	76.1±7.6[f]	3.41±0.9[e]	15.93±2.6
+PGPC	97.9±2.2[f]	26.5±3.5	68.6±2.2[f]	4.14±0.8[e]	14.60±1.5
+AzPC	91.8±10.4[f]	7.6±4.9	69.4±3.4[f]	5.29±0.9[e]	–
+PAF	87.8±10.0	29.2±3.1	49.8±4.4	17.54±2.1	19.93±1.7
+LPC	134.1±18.2	29.8±3.6	38.9±3.8	52.40±4.0	59.00±6.4

E_{max}, E_{med} and EC_{50} values were determined as described in Materials and methods.
–, not measured; control, without phospholipids; E_{max}, maximal stimulation %; E_{med}, stimulation % in the presence of 30 μM lipid; EC_{50}, concentration required for 50% maximal stimulation. Means with the same letter are not significantly different ($P<0.05$, $n=3$).

EC_{50} value of 4.1 μM in the hydrolysis of phenyl acetate, and the E_{max} value of 27% and EC_{50} value of 14.6 μM in the hydrolysis of paraoxon. A similar result was also observed with 1-palmitoyl-2-azelaoyl-phosphatidylcholine (AzPC), showing E_{max} value of 91% and 7.6% in the hydrolysis of phenyl acetate and paraoxon, respectively, compared to its precursor (PLnPC), which showed E_{max} value of 80% in the hydrolysis of phenyl acetate, and E_{max} value of 33% in the hydrolysis of paraoxon. Thus, AzPC was also more efficient than its precursor, PLnPC, in exerting a preferable activation of arylesterase activity. Overall, oxidized phosphatidylcholines with shorter acyl chains at the *sn-2* position seemed to be slightly more effective than corresponding precursor PCs in expressing a preferential stimulation of arylesterase activity. However, there was no significant difference of EC_{50} values between oxidized PCs and corresponding precursor PCs. In related study, 1-hexadecyl-2-acetyl-phosphatidylcholine (PAF) or palmitoyl-lysophosphatidylcholine (LPC) was tested for their stimulation of PON1 activities. PAF (E_{max}, 88%; EC_{50}, 17.5 μM) enhanced arylesterase activity in a concentration-dependent manner, but it was less efficacious than oxidized PCs in stimulating arylesterase activity below 30 μM (Fig. 3A). Paraoxonase

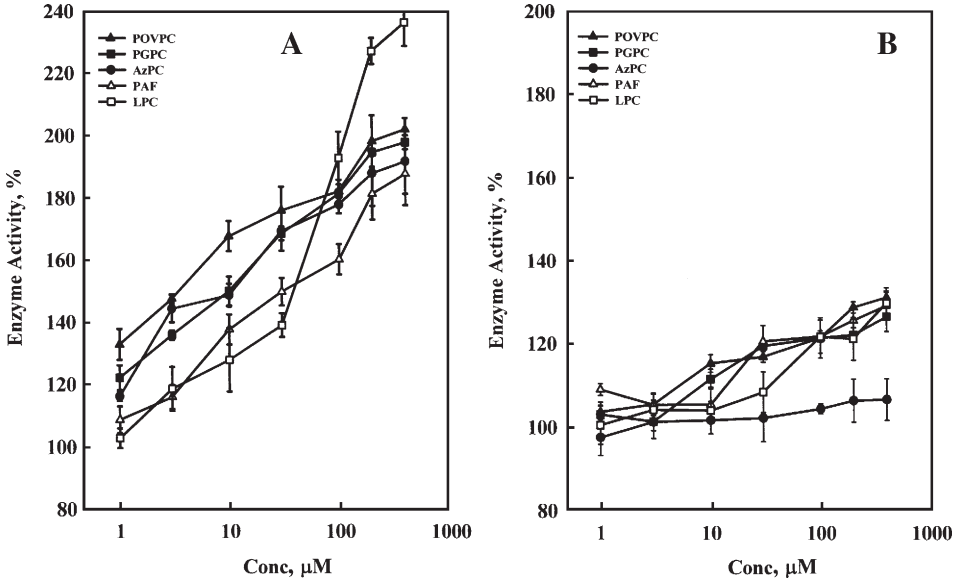

Fig. 3. Stimulatory effect of oxidized phosphatidylcholine, LPC or PAF on PON1 activities. A, PON1 (5 arylesterase units/ml) was preincubated with oxidized phosphatidylcholines, PAF or LPC (1–400 μM) for 30 min in 0.1 ml of 50 mM Tris buffer (pH 7.4) containing 1 mM Ca^{2+} at 25 °C and then the aliquot (20 μl) was taken for arylesterase activity assay. Enzyme activity was expressed as a percentile value of arylesterase activity of control. Data are expressed as a mean±S.D. (bar) value of triplicate assays. B, PON1 (5 arylesterase units/ml) was preincubated with various oxidized phosphatidylcholines, PAF or C16:0 Lyso PC (1–400 μM) as described above, and then the aliquot (50 μl) was taken for paraoxonase activity. Enzyme activity was expressed as the percentile value of control paraoxonase activity. Data are expressed as a mean±S.D. (bar) value of triplicate assays.

activity was also stimulated by PAF (E_{max}, 29%; EC_{50}, 20 μM), although to a less extent than arylesterase activity (Fig. 3B). Likewise, LPC (E_{max}, 134%; EC_{50}, 52.4 μM) also stimulated arylesterase activity remarkably, but it was less stimulatory than oxidized phosphatidylcholines below 30 μM. Again, paraoxonase activity was also stimulated by LPC (E_{max}, 29.8% and EC_{50}, 59 μM), although to a lower extent than arylesterase activity.

3.4. Effect of phospholipids on VLDL-bound PON1 activity

Subsequently, we investigated the effect of phospholipids on VLDL-bound PON1. As seen in Fig. 4 and Table 2, phosphatidylcholines such as DLPC, PAPC or PLnPC (1–400 μM) stimulated arylesterase activity (E_{max}, 25–34% and EC_{50}, 11.4–29.8 μM), but not paraoxonase activity (E_{max}, at ≤6.7%), demonstrating a preferable stimulation of VLDL-associated arylesterase activity. Similarly, POVPC, PGPC or AzPC (1–400 μM) also expressed a substantial stimulation of arylesterase activity (E_{max}, 28–38% and EC_{50}, 6.6–9.7 μM), but not paraoxonase activity. Although there was no remarkable difference of E_{max} values among PCs, the EC_{50} value of DLPC was higher than that of the other PCs. Further, the analysis of E_{med} values indicates that DLPC is less stimulatory than the other PCs. Separately, LPC expresses a remarkable stimulation of VLDL-associated arylesterase activity (E_{max}, 47%). Thus, phosphatidylcholines with polyunsaturated acyl chains or oxidized acyl chains are more efficient than DLPC in expressing a preferable stimulation of VLDL-bound arylesterase activity, similar to the finding with purified PON1. Nonetheless, such a stimulatory effect of phosphatidylcholines was not significant with HDL-bound arylesterase activity, where PON1 might be tightly associated with phosphatidylcholines in HDL particle [23].

Table 2
E_{max}, E_{med} and EC_{50} of each lipid in the stimulation of VLDL-bound PON1 activities

Phospholipids	E_{max} (%)		E_{med} (%)	EC_{50} (μM)	
Substrate	Phenyl acetate	Paraoxon	Phenyl acetate	Phenyl acetate	Paraoxon
Control	0	0	0		
+DLPC	25.0±3.3	−2.2±4.9	9.9±3.3	29.8±5.8	–
+PLnPC	32.8±3.8	4.3±3.9	21.0±5.3	11.4±3.6	–
+PAPC	34.2±3.8	4.3±4.1	20.2±1.9	17.2±3.1	–
+POVPC	37.7±2.1	6.7±5.2	34.2±1.6	6.6±0.8	–
+PGPC	27.8±4.3	−1.5±7.9	18.5±3.1	9.7±2.6	–
+AzPC	30.2±6.4	7.4±4.5	20.0±3.9	8.8±1.7	–
+LPC	46.6±6.4	8.7±5.8	20.6±2.5	26.7±3.8	–

E_{max}, E_{med} and EC_{50} values were determined as described in Materials and methods.

–, not measured, control, without phospholipids. Means with the same letter are not significantly different ($P<0.05$, $n=3$).

3.5. Molecular sieve chromatography of phospholipids vesicle

In order to examine which forms of phosphatidylcholines account for the stimulation of PON1 activity, various phosphatidylcholine vesicle preparations were subjected to Sepharose 4B gel chromatography in the elution buffer containing 1 mM

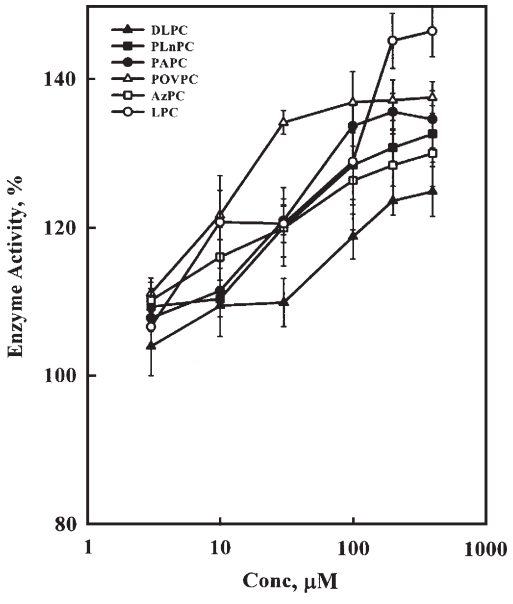

Fig. 4. Effect of phospholipids on VLDL-bound PON1. PON1 (5 arylesterase units/ml) was preincubated with various phospholipids (1–400 μM) for 30 min in 0.1 ml of 50 mM Tris buffer (pH 7.4) containing 1 mM Ca^{2+} at 25 °C, and then the aliquot (20 μl) was taken for arylesterase activity assay. Data are expressed as a mean±S.D. (bar) value of triplicate assays, presented as a percentage of control activity.

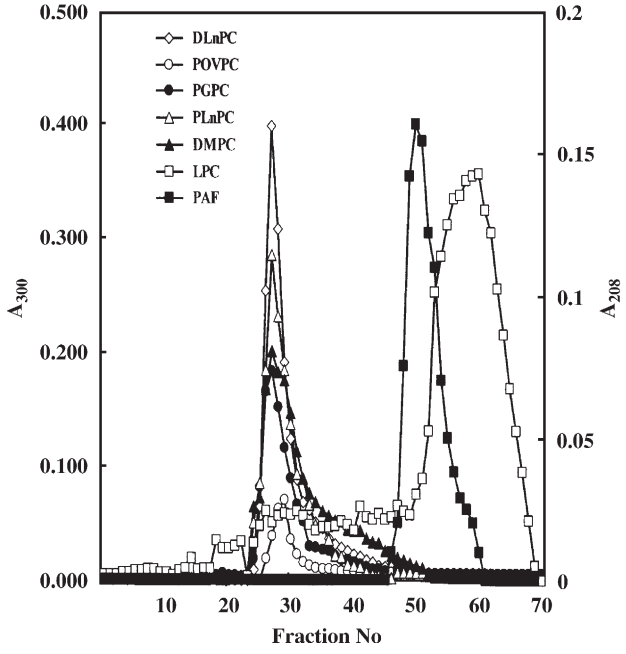

Fig. 5. Molecular sieve chromatography of phospholipids vesicle. Aliquot (1 ml) of each phospholipid stock solution (1 mM) was applied to Sepharose 4B column (1.5×20 cm), which was eluted with 50 mM Tris (pH 7.4) containing 1 mM $CaCl_2$ and 100 mM NaCl at a flow rate of 0.2 ml/min. Each fraction (0.5 ml) was monitored for absorbance at 300 nm (micelle-free vesicle) or at 208 nm (micelle).

Ca^{2+}, and the elution fractions were monitored at 300 nm for the lipid turbidity, or at 208 nm for PAF.

As shown in Fig. 5, DMPC was found to be eluted in the front peak corresponding to micelle-free unilamellar vesicles, in agreement with previous observations [34,35]. Similar results were also obtained with PLnPC, DLnPC or oxidized PCs (PGPC or POVPC). In contrast, palmitoyl-lysophosphatidylcholine (LPC) and PAF, when the elution was monitored at 208 nm [34], appeared much later in chromatogram, indicating that LPC and PAF behaved as micellar forms.

3.6. Effect of phospholipids on PON1 Trp fluorescence

Previously [36], the hydrophobic character of Trp residues in or close to Ca^{2+}-binding sites of PON1 had been demonstrated to be very important for the enzyme activity. In a related study to examine the relationship between the effect of phospholipids on PON1 activities and their effect on PON1 Trp fluorescence by lipids, it was found DMPC enhanced the Trp fluorescence of PON1 in a concentration-dependent pattern (0.01–0.4 mM) at a range of concentrations showing the stimulatory effect on arylesterase activity (Fig. 6A). Conversely, DMPS caused a remarkable decrease of PON1 Trp fluorescence (Fig. 6B) at concentrations (0.1–0.4 mM) showing the inhibitory effect on arylesterase activity, indicating that there may be a direct link between the loss of PON1 activities and reduction of PON1 Trp fluorescence. Similarly, DMPG enhanced PON1 Trp fluorescence, but DMPA reduced it (data not shown). Thus, it seems that stimulatory phospholipids caused the increase in Trp fluorescence, while the inhibitory phospholipids decrease the PON1 Trp fluorescence.

4. Discussion

There have been reports [19–22] on the preferential decrease of PON1 paraoxonase activity in serum of persons with oxidative stress-associated pathological signs. Our previous report [23] demonstrated that monoenoic acids or negatively-charged lysophospholipids displayed a preferential inhibition of paraoxonase activity. Here, we demonstrate that the preferable stimulation of PON1 arylesterase activity by some phosphatidylcholines may contribute to the reduction of the ratio of paraoxonase activity to arylesterase activity.

In present study, the effect of phospholipids on PON1 activities varied greatly according to the type of phospholipids, with which PON1 was associated. Most of phosphatidylcholines expressed a preferable stimulation of PON1 arylesterase activity. Among PC series with saturated acyl chains, the stimulatory action of PC was enhanced in accordance with the increase of fatty acyl chain length ($C10 < C12 < C14 \leq 16$). Thus, the stimulatory action of PC series seems to be ascribed to their ability in stabilizing bilayers over micelles [39]. Noteworthy, the greatest stimulation of arylesterase activity was observed with a chain length of C16, corresponding to a bilayer of a hydrophobic thickness of ca. 2.4 nm [40], suggesting that the hydrophobic match between PON1 N-terminal sequence and phosphatidylcholine bilayer is important for the maximal stimulation. Alternatively, the greater stimulation by phosphatidylcholine series (C10–C16) with higher T_m values may imply that the stimulatory action of PC series may be related to the relative stability of acyl chain in the gel state. However, the greater stimulation by DOPC (T_m, −21 °C), compared to DLPC (T_m, −2.5 °C), and no effect of temperature (15–37 °C) on the stimulatory action of DMPC excludes the

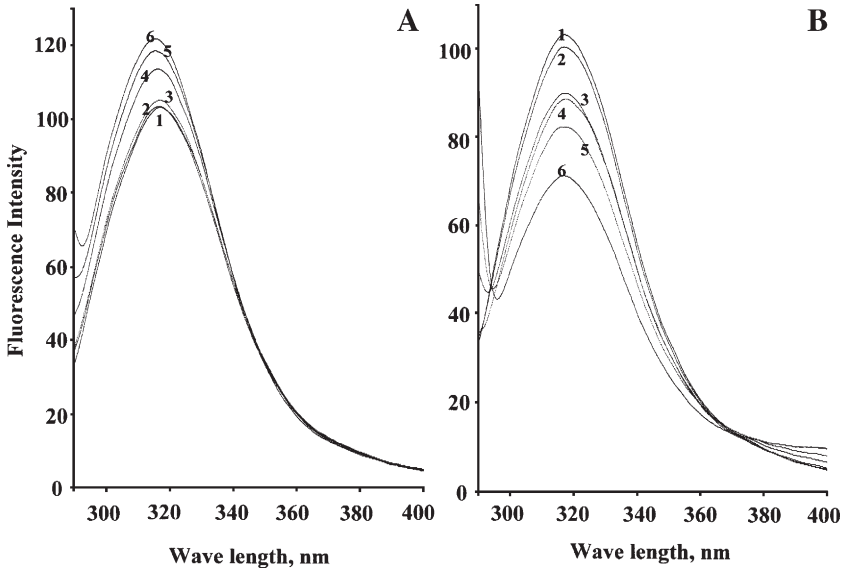

Fig. 6. Effect of phospholipids on PON1 Trp fluorescence. PON1 (5 unit arylesterase/ml, 0.1 μM) was preincubated with DMPC (A) or DPPS (B) at various concentrations (10–400 μM) in 1 ml of 50 mM Tris (pH 7.4) containing 1 mM $CaCl_2$ at 25 °C. After 30 min incubation, fluorescence emission spectra of PON1 were monitored using Perkin-Elmer 550-55 Fluorimeter at 25 °C, with the excitation wavelength of 280 nm and excitation and emission slit widths of 10 nm. (A) 1, control: 2, 10 μM DMPC; 3, 30 μM DMPC; 4, 100 μM DMPC; 5, 200 μM DMPC; 6, 400 μM DMPC; (B) 1, control: 2, 10 μM DMPS; 3, 30 μM DMPS; 4, 100 μM DMPS; 5, 200 μM DMPS; 6, 400 μM DMPS.

possibility that the stimulatory action is remarkable in gel phase [41,42]. Instead, it is suggested that the size of acyl chain region in PC bilayer may be important for the stimulation of arylesterase activity. A support for this is from the greater stimulation by phosphatidylcholines with unsaturated fatty acyl groups, compared to those with saturated acyl group, suggesting that the diameter of acyl chain relative to that of polar head group may be an important factor; the presence of unsaturation in PC acyl chain is known to disturb the ordered packing of acyl groups [43,44], and thereby cause a loose packing of phospholipid bilayer [45], which may facilitate the incorporation of PON1 N-terminal into phospholipid bilayer. Although the head group spacing effect was proposed to explain the activation of PKC activity by polyunsaturated PC series [41], this is less applicable to the stimulation of arylesterase activity by DMPC vesicles, which showed a similar degree of stimulation, irrespective of preparation methods, sonication or extrusion. Thus, the disorder of acyl chains seems to be more important for the stimulation of PON1 activity than the head group spacing. A further support is provided by another finding that oxidized PC (POVPC, PGPC or AzPC) with shorter and less non-polar chain at the sn-2 position were no less stimulatory than PC with unsaturated fatty acyl chains. Alternatively, the negative curvature effect of oxidized PCs (POVPC, PGPC or AzPC) could be implicated in their stimulatory action as had been observed in the activation of CTP:phosphocholine cytidyltransferase activity by 1-palmitoyl, 2-(11,15-dihydroxy) eicosatrienoyl PC, exerting a more negative curvature effect than its precursor, PAPC [45]. However, the negative curvature effect of oxidized PCs (POVPC, PGPC or AzPC) on PON1 activity may not be remarkable, since the stimulatory action of oxidized PCs used here is only slightly greater than that of unsaturated PCs, and the EC_{50} values, used as a rough measure of the relative binding affinity, are similar between oxidized PCs and unsaturated PCs. In addition, oxidized PCs may be different from palmitoyl-lysoPC, possessing a positive curvature [46], in that they are more stimulatory than palmitoyl-lysoPC on the basis of E_{med} and EC_{50} values. Moreover, in chromatographic analyses, oxidized PC series (POVPC or PGPC) behave like bilayer of DMPC or PLnPC, in contrast to micellar behavior of palmitoyl-lysoPC [47] or PAF [48]. In this regard, it is supposed that the maximal stimulation of aryesterase activity is achieved primarily by the anchoring of PON1 N-terminal to PC bilayer [49]. Additional mode by which arylesterase activity is stimulated may be the micellar association of lysophospholipids around N-terminal sequence or the interface of PON1 [49], which had been proposed to interact with lipids in HDL particle. In support of the latter, our unpublished data indicate that DMPC liposome-bound PON1 aryesterase activity was further stimulated by palmitoyl-lysoPC.

Separately, phosphatidylglycerol, despite the negative charge in phosphodiester linkage, significantly stimulated arylesterase activity as observed with zwitterionic PC series. This might be explained by the previous reports [50,51] that DMPG, in combination with Ca^{2+}, exhibited bilayers of crystalline-like gel phase with highly ordered acyl chains. Thus, DMPG and PI may behave like bilayer lipids, associable with PON1, in the incubation condition containing 1 mM calcium ion. Nonetheless, DMPG was less stimulatory than PC series, indicating that the presence of negatively-charged phosphate group at the bilayer surface of PG may affect the property of the bilayer interface.

In contrast, DMPE or DOPE, characterized by pyramid type and negative intrinsic curvature effect [52,53], showed no effect on arylesterase activity. Additionally, DMPS or DMPA), more likely to form hexagonal type of micelle in the acidic condition [54,55], had no remarkable effect on arylesterase activity at low concentrations (1–30 μM). Meanwhile, at higher concentrations (0.2–0.4 mM), DMPS or DMPA expressed a strong inhibition of arylesterase and paraoxonase activities in the presence of Ca^{2+}, which had been reported to form a stable multilamellar complex with PS at neutral pH [56]. From this, it is supposed that the inhibition of PON1 activity by DMPS/Ca^{2+} may be due to the structural constraint of PON1 during Ca^{2+}-induced adhesion of PS vesicles [56].

Probably in support of this, the inclusion of DMPC in DMPS vesicle suppressed the inhibitory action of DMPS. Additionally, apolipoprotein AI, an amphipathic protein [18], restored the suppressed activity of PON1 in PS vesicle. Meanwhile, no effect of NaCl (1 M) on the inhibitory action of PS excludes the implication of electrostatic attraction the inhibition of PON 1 by DMPS. Such an inhibitory action of DMPS or DMPA at ≥100 μM is in a contrast to the stimulatory action of DMPC or DMPG at the same concentrations, reflecting a structural change of PON1 by phospholipids. A support for this may be from the opposite effects of phospholipids on fluorescence spectra of PON1 Trp residue [36]. DMPC or DMPG enhanced PON1 Trp fluorescence with a blue shift, consistent with the conformational change of Trp residue-containing region in non-polar lipid [40]. Meanwhile, DMPS or PMPA reduced Trp fluorescence with no change of emission wavelength, implying that Trp fluorescence was quenched in polar environment induced by PS.

Overall, compared to paraoxonase activity, arylesterase activity is more sensitive to the lipid environment, whatever type of phospholipids, in agreement with the notion that the binding site for phenyl acetate may be distinguished from paraoxon-binding site in PON1 molecule [48]. However, the inhibitory role of negatively-charged phospholipids is less likely in in vivo system, since the amount of those lipids is limited in lipoproteins. Therefore, the ratio of PC series with unsaturated acyl chain or oxidized chain to those with saturated chains may be a crucial factor to determine the preferable stimulation of arylesterase activity. For this purpose, oxidized PC, expressing a preferable stimulation of arylesterase activity, may be more efficient than unsaturated PC. Noteworthy, such a preferable stimulation of PON1 arylesterase activity by PC series was also observed with VLDL-associated PON1, but not HDL-associated PON1. This might be explained by the notion that PON1 in HDL particle, rich in phosphatidylcholine, is already activated by lipids in HDL, while PON1 is not fully activated in VLDL particle, rich in triacylglycerol [57]. Additionally, apolipoprotein AI, in combination with phosphatidylcholines, is also important for the maximal activation of

PON1 arylesterase activity in HDL particle [58]. However, such a beneficial effect of apolipoprotein-A1 was not announced significantly in combination with AzPC (oxidized PC) or lysoPC (data not shown), suggesting that the stimulation by oxidized PC with loosely-packed acyl chains or micellar lysoPC may not require the lipid-dispersing action of apolipoprotein A-I [59].

Taken together, the present data demonstrate that some phosphatidylcholine derivatives in HDL particle indirectly contribute to the reduction in the ratio of paraoxonase activity to arylesterase activity by exerting a preferable stimulation of arylesterase activity. In addition to the previous finding [23] that monounsaturated fatty acids or negatively-charged lysophospholipids directly cause the preferential inhibition of paraoxonase activity, present data may provide another explanation for the cause for the relative reduction of paraoxonase activity in sera of oxidative stress-related disease patients. Therefore, the level of PC derivatives with unsaturated acyl chain or oxidized chains, responsible for the preferable stimulation of arylesterase activity, in HDL particle as well as the amount of preferential inhibitors (monoenoic acid, lysoPI or lysoPG etc.) of paraoxonase activity in serum may be responsible for the relative decrease of PON1 paraoxonase activity in vivo. According to previous reports [38,60], the oxidation of PC derivatives leads to the formation of PC containing acid or aldehyde group at sn-2 position, belonging to preferential stimulator of arylesterase activity. In turn, the hydrolysis of oxidized phosphatidylcholine by PAF acetylhydrolase or PLA2 can generate lysophosphatidylcholine, another stimulator of arylesterase activity [61,62]. Finally, the preferable stimulation of arylesterase activity by oxidized PC and lysoPC would contribute to the decrease in the ratio of paraoxonase activity to arylesterase activity, which could be magnified in the presence of monoenoic acids or negatively-charged lysophospholipids [23]. Further study on the relationship between the PON1 activity and the composition of phospholipids in HDL particles of different oxidative status could reveal the physiological significance of preferential stimulation of PON1 arylesterase activity.

Acknowledgement

This work was financially supported by the Korean Research Foundation (KRF-2003-042-C20050), Korea.

References

[1] B.N. La Du, Structural and functional diversity of paraoxonase, Nat. Med. 2 (1996) 1186–1187.
[2] J.A. Berliner, M. Navab, A.M. Fogelman, J.S. Frank, L.L. Demer, P.A. Edwards, A.D. Watson, A.J. Lusis, Atherosclerosis: basic mechanisms. Oxidation, inflammation, and genetics, Circulation 91 (1995) 2488–2496.
[3] M.I. Mackness, B. Mackness, P.N. Durrington, P.W. Connelly, R.A. Hegele, Paraoxonase: biochemistry, genetics and relationship to plasma lipoproteins, Curr. Opin. Lipidol. 7 (1996) 69–76.
[4] H. Jakubowski, Calcium-dependent human serum homocysteine thiolactone hydrolase. A protective mechanism against protein N-homocysteinylation, J. Biol. Chem. 275 (2000) 3957–3962.
[5] H.G. Davies, R.J. Richter, M. Keifer, C.A. Broomfield, J. Sowalla, C.E. Furlong, The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin, Nat. Genet. 14 (1996) 334–336.
[6] K.N. Gan, A. Smolen, H.W. Eckerson, B.N. La Du, Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities, Drug Metab. Dispos. 19 (1991) 100–106.
[7] D.M. Shih, L. Gu, Y.R. Xia, M. Navab, W.F. Li, S. Hama, L.W. Castellani, C.E. Furlong, L.G. Costa, A.M. Fogelman, A.J. Lusis, Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis, Nature 394 (1998) 284–287.
[8] A.D. Watson, M. Navab, S.Y. Hama, A. Sevanian, S.M. Prescott, D.M. Stafforini, T.M. McIntyre, B.N. Du, A.M. Fogelman, J.A. Berliner, Effect of platelet activating factor-acetylhydrolase on the formation and action of minimally oxidized low density lipoprotein, J. Clin. Invest. 95 (1995) 774–782.
[9] O. Rozenberg, M. Rosenblat, R. Coleman, D.M. Shih, M. Aviram, Paraoxonase (PON1) deficiency is associated with increased macrophage oxidative stress: studies in PON1-knockout mice, Free Radic. Biol. Med. 34 (2003) 774–784.
[10] A. Tward, Y.R. Xia, X.P. Wang, Y.S. Shi, C. Park, L.W. Castellani, A.J. Lusis, D.M. Shih, Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice, Circulation 106 (2002) 484–490.
[11] O. Rozenberg, D.M. Shih, M. Aviram, Paraoxonase 1 (PON1) attenuates macrophage oxidative status: studies in PON1 transfected cells and in PON1 transgenic mice, Atherosclerosis 181 (2005) 9–18.
[12] M. Aviram, M. Rosenblat, C.L. Bisgaier, R.S. Newton, S.L. Primo-Parmo, B.N. La Du, Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase, J. Clin. Invest. 101 (1998) 1581–1590.
[13] M. Aviram, M. Rosenblat, Paraoxonases 1, 2, and 3, oxidative stress, and macrophage foam cell formation during atherosclerosis development, Free Radic. Biol. Med. 37 (2004) 1304–1316.
[14] B. Mackness, D. Hine, Y. Liu, M. Mastorikou, M. Mackness, Paraoxonase-1 inhibits oxidised LDL-induced MCP-1 production by endothelial cells, Biochem. Biophys. Res. Commun. 318 (2004) 680–683.
[15] M. Rosenblat, J. Vaya, D. Shih, M. Aviram, Paraoxonase 1 (PON1) enhances HDL-mediated macrophage cholesterol efflux via the ABCA1 transporter in association with increased HDL binding to the cells: a possible role for lysophosphatidylcholine, Atherosclerosis 179 (2005) 69–77.
[16] C. Bergmeier, R. Siekmeier, W. Gross, Distribution spectrum of paraoxonase activity in HDL fractions, Clin. Chem. 50 (2004) 2309–2315.
[17] M. Rosenblat, R. Karry, M. Aviram, Paraoxonase 1 (PON1) is a more potent antioxidant and stimulant of macrophage cholesterol efflux, when present in HDL than in lipoprotein-deficient serum: relevance to diabetes, Atherosclerosis (in press).
[18] M. Navab, G.M. Anantharamaiah, S.T. Reddy, B.J. Van Lenten, G. Datta, D. Garber, A.M. Fogelman, Human apolipoprotein A-I and A-I mimetic peptides: potential for atherosclerosis reversal, Curr. Opin. Lipidol. 15 (2004) 645–649.
[19] B. Mackness, P. Durrington, P. McElduff, J. Yarnell, N. Azam, M. Watt, M. Mackness, Low paraoxonase activity predicts coronary events in the Caerphilly Prospective Study, Circulation 107 (2003) 2775–2779.
[20] G. Paragh, P. Balla, E. Katona, I. Seres, A. Egerhazi, I. Degrell, Serum paraoxonase activity changes in patients with Alzheimer's disease and vascular dementia, Eur. Arch. Psychiatry Clin. Neurosci. 252 (2002) 63–67.
[21] R.W. James, I. Leviev, A. Righetti, Smoking is associated with reduced serum paraoxonase activity and concentration in patients with coronary artery disease, Circulation 101 (2000) 2252–2257.
[22] I. Seres, G. Paragh, E. Deschene, T. Fulop Jr., A. Khalil, Study of factors influencing the decreased HDL associated PON1 activity with aging, Exp. Gerontol. 39 (2004) 59–66.
[23] S.D. Nguyen, D.E. Sok, Preferential inhibition of paraoxonase activity of human paraoxonase 1 by negatively charged lipids, J. Lipid Res. 45 (2004) 2211–2220.
[24] L. Jaouad, C. de Guise, H. Berrougui, M. Cloutier, M. Isabelle, T. Fulop, H. Payette, A. Khalil, Age-related decrease in high-density lipoproteins

antioxidant activity is due to an alteration in the PON1's free sulfhydryl groups, Atherosclerosis (in press).
[25] B.N. La Du, S. Adkins, C.L. Kuo, D. Lipsig, Studies on human serum paraoxonase/arylesterase, Chem. Biol. Interact. 87 (1993) 25–34.
[26] C.L. Kuo, B.N. La Du, Comparison of purified human and rabbit serum paraoxonases, Drug Metab. Dispos. 23 (1995) 935–944.
[27] M. Aviram, M. Rosenblat, S. Billecke, J. Erogul, R. Sorenson, C.L. Bisgaier, R.S. Newton, B. La Du, Human serum paraoxonase (PON 1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants, Free Radic. Biol. Med. 26 (1999) 892–904.
[28] R.B. Gennis, Biomembranes: Molecular Structure and Function, Springer-Verlag, New York, 1989, pp. 1–84.
[29] R.M. Epand, Lipid polymorphism and protein–lipid interactions, Biochim. Biophys. Acta 1376 (1998) 353–368.
[30] S.D. Nguyen, D.E. Sok, Beneficial effect of oleoylated lipids on paraoxonase1: protection against oxidative inactivation and stabilization, Biochem. J. 375 (2003) 275–285.
[31] H.W. Eckerson, C.M. Wyle, B.N. La Du, The human serum paraoxonase/arylesterase polymorphism, Am. J. Hum. Genet. 35 (1983) 1126–1138.
[32] T.G. Redgrave, D.C. Roberts, C.E. West, Separation of plasma lipoproteins by density-gradient ultracentrifugation, Anal. Biochem. 65 (1975) 42–49.
[33] S. Chatterjee, D.K. Banerjee, Preparation, isolation, and characterization of liposomes containing natural and synthetic lipids, Methods Mol. Biol. 199 (2002) 3–16.
[34] B.J. Litman, Lipid model membranes. Characterization of mixed phospholipid vesicles, Biochemistry 12 (1973) 2545–2554.
[35] F.M. Megli, L. Russo, K. Sabatini, Oxidized phospholipids induce phase separation in lipid vesicles, FEBS Lett. 579 (2005) 4577–4584.
[36] D. Josse, W. Xie, F. Renault, D. Rochu, L.M. Schopfer, P. Masson, O. Lockridge, Identification of residues essential for human paraoxonase (PON1) arylesterase/organophosphatase activities, Biochemistry 38 (1999) 2816–2825.
[37] R.G.D. Steel, J.H. Torrie, Analysis of variance I: The one-way classification, Principles and Procedures of Statistics, McGraw-Hill, New York, 1960, pp. 99–132.
[38] N. Leitinger, Oxidized phospholipids as modulators of inflammation in atherosclerosis, Curr. Opin. Lipidol. 14 (2003) 421–430.
[39] J.M. Walker, J.J. Sando, Activation of protein kinase C by short chain phosphatidylcholines, J. Biol. Chem. 263 (1988) 4537–4540.
[40] A.M. Powl, J.M. East, A.G. Lee, Lipid–protein interactions studied by introduction of a tryptophan residue: the mechanosensitive channel MscL, Biochemistry 42 (2003) 14306–14317.
[41] S.J. Slater, M.B. Kelly, F.J. Taddeo, C. Ho, E. Rubin, C.D. Stubbs, The modulation of protein kinase C activity by membrane lipid bilayer structure, J. Biol. Chem. 269 (1994) 4866–4871.
[42] J.J. Chicano, A. Ortiz, J.A. Teruel, F.J. Aranda, Organotin compounds alter the physical organization of phosphatidylcholine membranes, Biochim. Biophys. Acta 1510 (2001) 330–341.
[43] B.J. Litman, E.N. Lewis, I.W. Levin, Packing characteristics of highly unsaturated bilayer lipids: raman spectroscopic studies of multilamellar phosphatidylcholine dispersions, Biochemistry 30 (1991) 313–319.
[44] C.D. Niebylski, N. Salem Jr., A calorimetric investigation of a series of mixed-chain polyunsaturated phosphatidylcholines: effect of sn-2 chain length and degree of unsaturation, Biophys. J. 67 (1994) 2387–2393.
[45] A.E. Drobnies, B. van Der Ende, J.L. Thewalt, R.B. Cornell, CTP: phosphocholine cytidylyltransferase activation by oxidized phosphatidylcholines correlates with a decrease in lipid order: a 2H NMR analysis, Biochemistry 38 (1999) 15606–15614.
[46] M.E. Haque, B.R. Lentz, Roles of curvature and hydrophobic interstice energy in fusion: studies of lipid perturbant effects, Biochemistry 43 (2004) 3507–3517.
[47] H. Heerklotz, R.M. Epand, The enthalpy of acyl chain packing and the apparent water-accessible apolar surface area of phospholipids, Biophys. J. 80 (2001) 271–279.
[48] C. Huang, J.T. Mason, F.A. Stephenson, I.W. Levin, Polymorphic phase behavior of platelet-activating factor, Biophys. J. 49 (1986) 587–595.
[49] M. Harel, A. Aharoni, L. Gaidukov, B. Brumshtein, O. Khersonsky, R. Meged, H. Dvir, R.B. Ravelli, A. McCarthy, L. Toker, I. Silman, J.L. Sussman, D.S. Tawfik, Structure and evolution of the serum paraoxonase family of detoxifying and anti-atherosclerotic enzymes, Nat. Struct. Mol. Biol. 11 (2004) 412–419.
[50] K.A. Riske, R.M. Fernandez, O.R. Nascimento, B.L. Bales, M.T. Lamy-Freund, DMPG gel–fluid thermal transition monitored by a phospholipid labeled at the acyl chain end, Chem. Phys. Lipids 124 (2003) 69–80.
[51] P.W. van Dijck, B. de Kruijff, A.J. Verkleij, L.L. van Deenen, J. de Gier, Comparative studies on the effects of pH and Ca2+ on bilayers of various negatively charged phospholipids and their mixtures with phosphatidylcholine, Biochim. Biophys. Acta 512 (1978) 84–96.
[52] J.R. Giorgione, R. Kraayenhof, R.M. Ep, Interfacial membrane properties modulate protein kinase C activation: role of the position of acyl chain unsaturation, Biochemistry 37 (1998) 10956–10960.
[53] S.M. Davies, R.M. Epand, R. Kraayenhof, R.B. Cornell, Regulation of CTP: phosphocholine cytidylyltransferase activity by the physical properties of lipid membranes: an important role for stored curvature strain energy, Biochemistry 40 (2001) 10522–10531.
[54] N. Fuller, C.R. Benatti, R.P. Rand, Curvature and bending constants for phosphatidylserine-containing membranes, Biophys. J. 85 (2003) 1667–1674.
[55] G. Senisterra, R.M. Epand, Role of membrane defects in the regulation of the activity of protein kinase C, Arch. Biochem. Biophys. 300 (1993) 378–383.
[56] B. Kachar, N. Fuller, R.P. Rand, Morphological responses to calcium-induced interaction of phosphatidylserine-containing vesicles, Biophys. J. 50 (1986) 779–788.
[57] B. Fuhrman, N. Volkova, M. Aviram, Paraoxonase 1 (PON1) is present in postprandial chylomicrons, Atherosclerosis 180 (2005) 55–61.
[58] L. Gaidukov, D.S. Tawfik, High affinity, stability, and lactonase activity of serum paraoxonase PON1 anchored on HDL with ApoA-I, Biochemistry 44 (2005) 11843–11854.
[59] M. Navab, G.M. Anantharamaiah, S.T. Reddy, S. Hama, G. Hough, V.R. Grijalva, N. Yu, B.J. Ansell, G. Datta, D.W. Garber, A.M. Fogelman, Apolipoprotein A-I mimetic peptides, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 1325–1331.
[60] Z. Ahmed, S. Babaei, G.F. Maguire, D. Draganov, A. Kuksis, B.N. La Du, P.W. Connelly, Paraoxonase-1 reduces monocyte chemotaxis and adhesion to endothelial cells due to oxidation of palmitoyl, linoleoyl glycerophosphorylcholine, Cardiovasc. Res. 57 (2003) 225–231.
[61] S. Benitez, J.L. Sanchez-Quesada, V. Ribas, O. Jorba, F. Blanco-Vaca, F. Gonzalez-Sastre, J. Ordonez-Llanos, Platelet-activating factor acetylhydrolase is mainly associated with electronegative low-density lipoprotein subfraction, Circulation 108 (2003) 92–96.
[62] Y. Ishimoto, K. Yamada, S. Yamamoto, T. Ono, M. Notoya, K. Hanasaki, Group V and X secretory phospholipase A(2)s-induced modification of high-density lipoprotein linked to the reduction of its antiatherogenic functions, Biochim. Biophys. Acta 1642 (2003) 129–138.