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Lecithin: Cholesterol Acyltransferase Is Insufficient to Prevent Oxidative Modification of Low-Density Lipoprotein

Youhei Murakami,*[,1] Shin Kamiyama,[†,1,2] Zakir H. Howlader,* Tokuhisa Yamato,* Michio Komai,* and Yuji Furukawa*

Laboratory of Nutrition, Graduate School of Agricultural Science, and †Gene Research Center, Tohoku University, 1-1 Tsutsumidori, Amamiya-machi, Aoba-ku, Sendai 981-8555

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We tried to confirm the antioxidative capability of lecithin:cholesterol acyltransferase (LCAT) reported by Vohl *et al.* [*Biochemistry* (1999) 38, 5976–5981]. The enzyme solution protected LDL against oxidation. However, this protection was not due to LCAT enzyme, but to some unknown low-molecular-weight substance(s) in the solution; LCAT itself exerted little protective effect against LDL oxidation.

Key words: antioxidation, high-density lipoprotein, lecithin:cholesterol acyltransferase, low-density lipoprotein, oxidation.

It is known that oxidized low-density lipoprotein (ox-LDL) plays a central role in initiation of the process of atherosclerosis. LDL in the intima is oxidized by various active oxygen species released by macrophages and other cells in the arterial wall (*1*). Ox-LDL may be recognized by the scavenger receptors present on macrophages; its uptake by these receptors may promote the formation of foam cells and thus increase cholesterol accumulation in the arterial wall (*2*).

Recently, Vohl *et al.* suggested that lecithin:cholesterol acyltransferase (LCAT) [EC 2.3.1.43] possessed antioxidant activity against the formation of oxidized lipids during lipoprotein oxidation (*3*). LCAT is known to play an important role in the cholesterol reverse-transport system by converting the surface cholesterol of high-density lipoprotein (HDL) to cholesterol ester inside HDL (*4*). In the present study, we examined the protective capability of LCAT against oxidative modification of LDL using the purified enzyme.

Human plasma LCAT was purified as previously described (*5*). After 6 h incubation of LDL with Cu^{2+}, oxidative modification of LDL was detected as increases in both the thiobarbituric acid reactive substance (TBARS) value (Fig. 1A), and the electrophoretic mobility (Fig. 1B) resulting from lipid peroxidation and acquisition of a net negative charge, which reflects apo B-100 modification. In our experiment, the presence of the LCAT enzyme solution almost completely suppressed these alterations in LDL during oxidation (Fig. 1, A and B).

However, as also shown in Fig. 1, A and B, this protective action of the LCAT enzyme solution against oxidative modification of LDL was not prevented by the addition to the assay system of either of two inhibitors of the LCAT reaction [*viz.* 2 mM 5,5′-dithiobis(2-nitro-benzoic acid) (DTNB) or 1 mM diisopropyl-fluorophosphate (DFP)]. Furthermore, neither heat inactivation of LCAT in the enzyme solution (at 58°C for 30 min or at 100°C for 10 min) nor pronase digestion of protein had any effect on the antioxidative ability of the enzyme solution, even though each of these treatments completely inhibited the LCAT reaction (Fig. 1C). These data indicate that the ability of the enzyme solution to protect LDL against oxidative modification did not result from a reaction that depended on LCAT enzyme itself.

We therefore investigated whether the antioxidative effect of the enzyme solution is in fact due to LCAT enzyme or to some other substance(s). The enzyme solution was separated by ultrafiltration into three fractions according to molecular-weight (less than 1 kDa, 1 to 10 kDa, and over 10 kDa fractions). The antioxidative effect of each fraction was then examined. As shown in Fig. 2, A and B, the over 10 kDa fraction [which contained LCAT enzyme (67 kDa molecular-weight)] showed no ability to protect LDL from oxidative modification. This indicates that the antioxidative effect of the LCAT enzyme solution is not dependent on the presence of LCAT enzyme itself, but instead on some low molecular-weight (<1 kDa) substance(s) present in the solution.

Two years ago, Vohl *et al.* reported that LCAT has the ability to prevent both spontaneous oxidation of lipid, and oxidation catalyzed by Cu^{2+} and soybean lipoxygenase (*3*). They noted that the antioxidant activity of LCAT appeared to be enzymatic and that catalytic serine may mediate this activity as a reusable proton donor. Their data showing that chemical modification of the active serine residue with DFP completely inhibited the ability of LCAT to prevent lipid oxidation is in conflict with our finding that DFP had no effect on the antioxidant activity of the enzyme solution. A difference in the LCAT enzyme used may not be a factor in this disagreement because the enzyme we used had the same specific activity as that used by Vohl *et al.* Indeed, we obtained the same result as that shown for DFP in Fig. 1 when we repeated our experiment using LCAT enzyme purified by the procedure described by Vohl *et al.* (data not shown).

[1] The first two authors made equal contributions to this work.

[2] To whom correspondence should be addressed. Tel: +81-22-717-8875, Fax: +81-22-717-8877, E-mail: kammy@biochem.tohoku.ac.jp

Abbreviations: apo A-I, apolipoprotein A-I; DTNB, 5,5′-dithiobis(2-nitro-benzoic acid); HDL, high-density lipoprotein; LCAT, lecithin: cholesterol acyltransferase; LDL, low-density lipoprotein; ox-LDL, oxidized low-density lipoprotein.

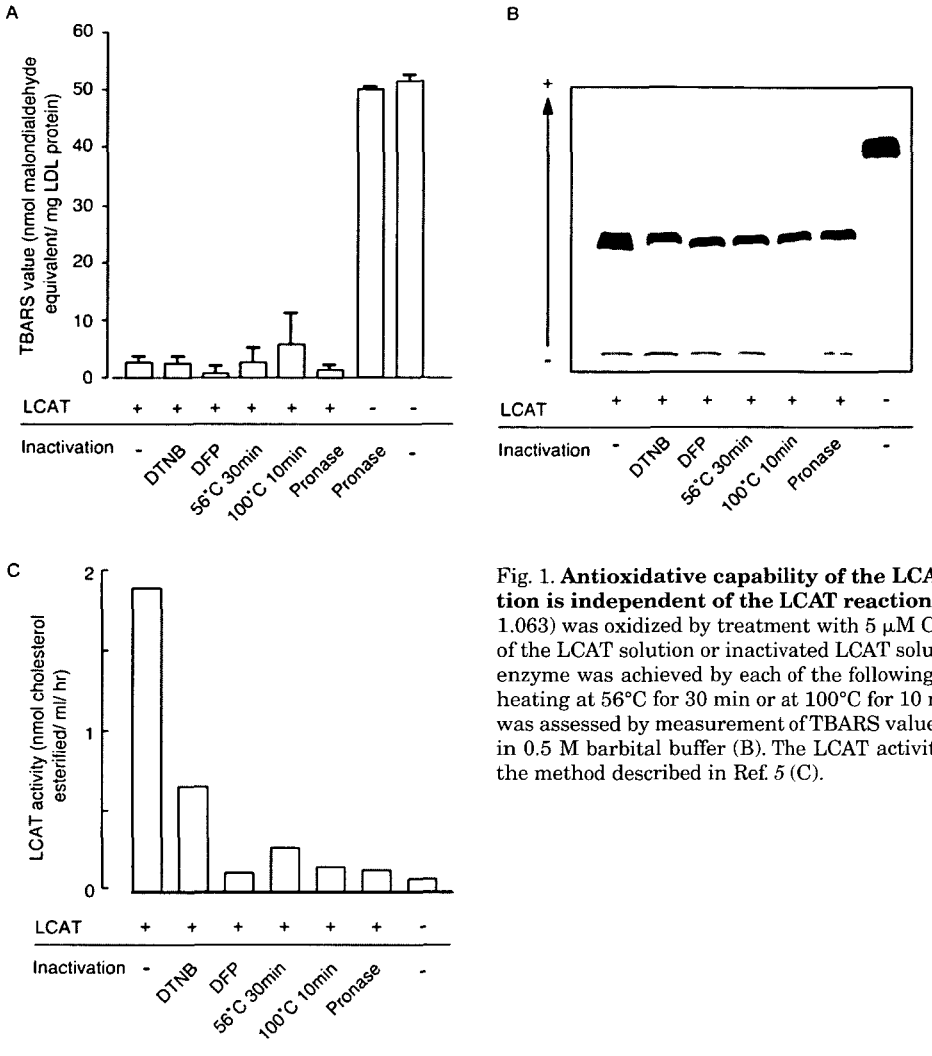

Fig. 1. **Antioxidative capability of the LCAT enzyme solution against LDL oxidation is independent of the LCAT reaction.** Freshly prepared human LDL (d = 1.006–1.063) was oxidized by treatment with 5 μM $CuSO_4$ at 37°C for 6 h in either the presence of the LCAT solution or inactivated LCAT solution, or their absence. Inactivation of LCAT enzyme was achieved by each of the following treatments: 10.4 mM DTNB, 10 mM DFP, heating at 56°C for 30 min or at 100°C for 10 min, or pronase digestion. Oxidation of LDL was assessed by measurement of TBARS values (A) or by 0.5% agarose gel electrophoresis in 0.5 M barbital buffer (B). The LCAT activity in each LCAT solution was examined by the method described in Ref. 5 (C).

The unknown substance preventing LDL oxidation interacted with the column contents on chelating affinity chromatography (Fig. 2C), but not on lectin chromatography or reverse-phase chromatography. This suggests that this substance prevents LDL oxidation by chelating the transition metal ions that initiate lipid peroxidation.

In conclusion, we failed to confirm the antioxidative effect of LCAT enzyme reported by Vohl *et al.* (*3*). Our result indicates that LCAT is insufficient to protect LDL from oxidative modification. However, we previously reported that LCAT reduced the adverse effects of ox-LDL (*6*), and that LCAT activity is inhibited by ox-LDL (*7*) and by oxidized phosphatidylcholine (*8*). Recent studies suggested a physiological role for LCAT in the metabolism of oxidized phosphatidylcholine in the plasma (*9, 10*). Furthermore, Itabe *et al.* reported metabolism of the oxidized phosphatidylcholines formed in ox-LDL by LCAT (*11*). These reports suggest that LCAT may reduce the cytotoxity of ox-LDL by metabolizing oxidized phosphatidylcholine. We anticipate that further investigations on the participation of LCAT in the metabolism of ox-LDL will yield very interesting results.

REFERENCES

1. Henriksen, T., Mahoney, E.M., and Steinberg, D. (1981) Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cells: recognition by receptors for acetylated low density lipoproteins. *Proc. Natl. Acad. Sci. USA* **78**, 6499–6503
2. Goldstein, J.L., Ho, Y.K., Basu, S.K., and Brown, M.S. (1979) Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc. Natl. Acad. Sci. USA* **76**, 333–337
3. Vohl, M.C., Neville, T.A., Kumarathasan, R., Braschi, S., and Sparks, D.L. (1999) A novel lecithin-cholesterol acyltransferase antioxidant activity prevents the formation of oxidized lipids during lipoprotein oxidation. *Biochemistry* **38**, 5976–5981
4. Glomset, J.A. (1968) The plasma lecithins:cholesterol acyltransferase reaction. *J. Lipid Res.* **9**, 155–167
5. Furukawa, Y., Urano, T., Hida, Y., Itoh, H., Takahashi, C., and Kimura, S. (1992) Interaction of rat lecithin-cholesterol acyltransferase with rat apolipoprotein A-I and with lecithin-cholesterol vesicles. *J. Biochem.* **111**, 413–418
6. Howlader, Z.H., Kamiyama, S., Murakami, Y., Ito, M., Komai, M., and Furukawa, Y. (2001) Lecithin:cholesterol acyltransferase reduces the adverse effects of oxidized low-density lipoprotein while incurring damage itself. *Biosci. Biotechnol. Biochem.* **65**, 2496–2503

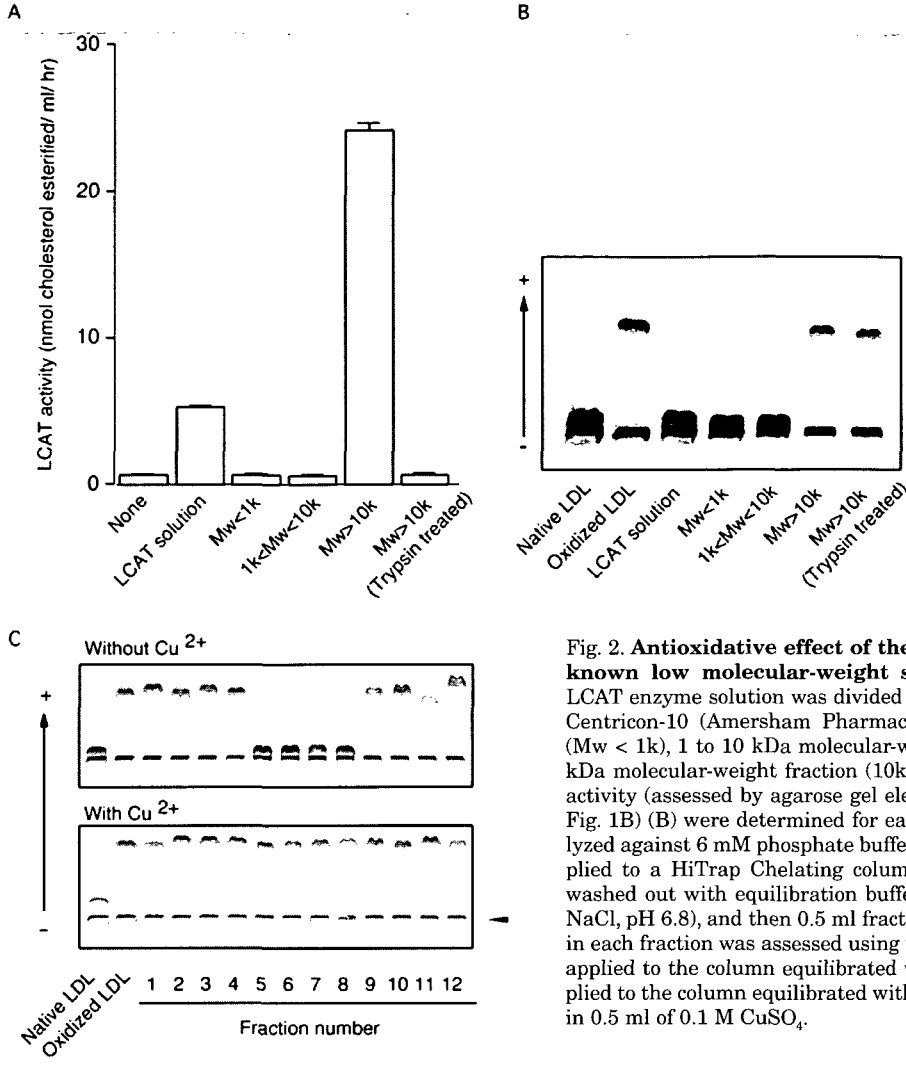

Fig. 2. **Antioxidative effect of the LCAT enzyme solution is due to an unknown low molecular-weight substance(s), not to LCAT enzyme.** The LCAT enzyme solution was divided into three fractions by ultrafiltration using a Centricon-10 (Amersham Pharmacia): under 1 kDa molecular-weight fraction (Mw < 1k), 1 to 10 kDa molecular-weight fraction (1k < Mw < 10k), and over 10 kDa molecular-weight fraction (10k < Mw). LCAT activity (A) and antioxidative activity (assessed by agarose gel electrophoresis using the same procedure as in Fig. 1B) (B) were determined for each fraction. C, the Mw < 1k fraction was dialyzed against 6 mM phosphate buffer containing 2.5 M NaCl, pH 6.8, and then applied to a HiTrap Chelating column (Amersham Pharmacia). The column was washed out with equilibration buffer (6 mM phosphate buffer containing 0.5 M NaCl, pH 6.8), and then 0.5 ml fractions were collected. The antioxidative activity in each fraction was assessed using the same procedure as in Fig. 1B. Top, sample applied to the column equilibrated with equilibration buffer. Bottom, sample applied to the column equilibrated with equilibration buffer after coupling with Cu^{2+} in 0.5 ml of 0.1 M $CuSO_4$.

7. Kamiyama, S., Kim, H.J., and Furukawa, Y. (1996) Inhibitory effects of oxidized low-density lipoprotein on the activity of plasma lecithin:cholesterol acyltransferase. *Biosci. Biotechnol. Biochem.* **60**, 580–583
8. Kamiyama, S., Yamato, T., and Furukawa, Y. (1998) Inhibitory effects of lipid oxidation on the activity of plasma lecithin: cholesterol acyltransferase. *Biosci. Biotechnol. Biochem.* **62**, 941–946
9. Nagata, Y., Yamamoto, Y., and Niki, E. (1996) Reaction of phosphatidylcholine hydroperoxide in human plasma: the role of peroxidase and lecithin:cholesterol acyltransferase. *Arch. Biochem. Biophys.* **329**, 24–30
10. Goyal, J., Wang, K., Liu, M., and Subbaiah, P.V. (1997) Novel function of lecithin-cholesterol acyltransferase. Hydrolysis of oxidized polar phospholipids generated during lipoprotein oxidation. *J. Biol. Chem.* **272**, 16231–16239
11. Itabe, H., Hosoya, R., Karasawa, K., Jimi, S., Saku, K., Takebayashi, S., Imanaka, T., and Takano, T. (1999) Metabolism of oxidized phosphatidylcholines formed in oxidized low density lipoprotein by lecithin-cholesterol acyltransferase. *J. Biochem.* **126**, 153–161