Low Density Lipoprotein Receptor-related Protein-mediated Membrane Translocation of 12/15-Lipoxygenase Is Required for Oxidation of Low Density Lipoprotein by Macrophages

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Oxidation of low density lipoprotein (LDL) is the key step for the development of atherosclerosis. The 12/15-lipoxygenase expressed in macrophages is capable of oxygenating linoleic acid esterified to cholesterol in the LDL particle, and thus this enzyme is presumed to initiate LDL oxidation. We recently reported that LDL receptor-related protein (LRP) was required for the enzyme-mediated LDL oxidation by macrophages and suggested the selective uptake of cholesterol ester from LDL to the plasma membrane (Xu, W., Takahashi, Y., Sakashita, T., Iwasaki, T., Hattori, H., and Yoshimoto. T. (2001) J. Biol. Chem. 276, 36454–36459). To elucidate precise mechanisms of lipoxygenase-mediated LDL oxidation, we investigated the intracellular localization of 12/15-lipoxygenase. The 12/15-lipoxygenase was predominantly detected in cytosol of resting peritoneal macrophages and of macrophage-like J774A.1 cells permanently transfected with the cDNA for the enzyme. When the cells were treated with LDL and subjected to subcellular fractionation, the 12/15-lipoxygenase was detected in the membranes with a concomitant decrease in cytosol as shown by Western blot analysis. The levels of the enzyme associated with the membrane reached maximum in 15 min after LDL addition and then decreased. However, the enzymatic activity of 12/15-lipoxygenase in the membrane fraction was very weak even after LDL treatment. This fact supports the suicide inactivation of the enzyme by the oxygenation of cholesterol ester transferred from the LDL particle to the plasma membrane. Immunohistochemical analysis using an antibody against 12/15-lipoxygenase revealed that the plasma membrane was the major site of the enzyme translocation by the LDL treatment. LDL-dependent 12/15-lipoxygenase translocation was inhibited by a blocking antibody against LRP. Furthermore, an enzyme translocation inhibitor, L655238, inhibited the LDL oxidation caused by the 12/15-lipoxygenase. We propose that cholesterol ester selectively transferred from the LDL particle to the plasma membrane via LRP is oxygenated by 12/15-lipoxygenase translocated to this membrane.

12/15-Lipoxygenase is a member of the lipoxygenase family, which incorporates one molecule of oxygen in regiospecific and stereospecific manners to unsaturated fatty acids such as arachidonic and linoleic acids (1–4). The enzyme consists of leukocyte-type 12-lipoxygenase found in rats, mice, cows, and pigs, and reticulocyte-type 15-lipoxygenase (15-lipoxygenase-1) expressed in humans and rabbits, oxygenating the position 12 and 15 of arachidonic acid, respectively (5). The notable feature of the 12/15-lipoxygenase is that the enzyme directly oxygenates not only free fatty acids but also complex substrates such as phospholipids, cholesterol ester, and the cholesterol ester present in the low density lipoprotein (LDL) particle (1–4). Oxidation of LDL is the first key step for the development of atherosclerosis (6, 7), and the roles of the 12/15-lipoxygenase in the process of LDL oxidation and the progress of atherogenesis have been extensively investigated. Recent study using 12/15-lipoxygenase-knockout mice (8) and the study using 12/15-lipoxygenase-transgenic mice (9) established that the enzyme was involved in the development of atherosclerosis, although contrary results were obtained using 12/15-lipoxygenase-transgenic rabbits (10, 11).

Using a macrophage-like cell line J774A.1, which did not have endogenous 12/15-lipoxygenase activity, we permanently transfected the cells with the 12/15-lipoxygenase cDNA and demonstrated that 12/15-lipoxygenase expressed in normal macrophages at a high level was required for LDL oxidation (12). However, the mechanism of extracellular LDL oxidation by intracellular 12/15-lipoxygenase has not been established. Recently, we revealed that the lipoxygenase-mediated LDL oxidation by macrophages required the binding of LDL to LDL receptor-related protein (LRP) but not to the LDL receptor, both of which are expressed on the surface of J774A.1 cells and are capable of binding native LDL (13).

The LDL is processed by the LDL receptor via receptor-mediated endocytosis in which cholesterol ester in the LDL particle is delivered to lysosomes where it is degraded (6). In contrast, the binding of LDL to LRP has been demonstrated to selectively take up the cholesterol ester from LDL in the plasma membrane without endocytosis and degradation of the LDL particle (14). For the efficient enzymatic oxygenation of the cholesterol ester transferred to the plasma membrane via LRP, the 12/15-lipoxygenase itself should also be localized in the plasma membrane or its neighborhood. However, the 12/15-lipoxygenase is predominantly present in cytoplasm and not...
in the membranes in various cells (15). Recent study has shown that the translocation of 12/15-lipoxygenase from the cytosol to the plasma membrane was observed in macrophages when incubated with apoptotic cells (16). Here we demonstrate that the binding of LDL to LRP expressed in normal macrophages and 12/15-lipoxygenase-expressing macrophage-like J774.A.1 cells translocates the enzyme from cytoplasm to the plasma membrane. The translocation is necessary for the cell-mediated oxidation of LDL. This study reveals a novel function of LRP in the development of atherosclerosis.

EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s modified Eagle’s medium (DMEM) was obtained from Nissui (Tokyo, Japan), fetal bovine serum from JRH Bioscience (St. Louis, MO), and 2-thiobarbituric acid and 1,1,3,3-tetramethoxypropane (bismalondialdehyde) from Wako (Osaka, Japan). Lipopolysaccharide-deficient serum from Sigma (St. Louis, MO), and 2-thiobarbituric acid and 1,1,3,3-tetramethoxypropane (bismalondialdehyde) from Wako (Osaka, Japan). (1-[14C] Arachidonic acid (2.1 MBq/mmol) and ECL Western blotting detection reagents from Amersham Biosciences (Bucks, UK), biotinylated and peroxidase-labeled anti-rabbit IgG from Vector (Burlingame, CA), L655238 from BIOMOL (Plymouth Meeting, PA), silica gel thin-layer plates from Merck (Darmstadt, Germany), polyvinylidene difluoride membranes from Millipore (Bedford, MA), Lab-Tek chamber slide from Nalgene (Naperville, IL), swine serum and horseradish peroxidase-conjugated streptavidin from Dakopatts (Carpenteria, CA), and Glicidether 100 from Selva Finebiochemica (Heidelberg, Germany). An antisem on 12/15-lipoxygenase was raised using purified recombinant rabbit piaule 12-lipoxygenase as an antigen as described previously (17). An anti-LDL receptor antibody was raised as described and purified to IgG using protein A-Sepharose (13). Human LDL was prepared from healthy volunteers and dialyzed against phosphate-buffered saline at 4 °C for 24 h before each experiment as described previously (12, 13). A murine macrophage-like cell line J774A.1 was kindly provided by Dr. Y. Sasaki of Shiga University of Medical Science. An expression vector, pEF-BOS having a elongation factor-1 α promoter, was kindly provided by Dr. G. Nagata of Osaka University. An anti-LRP antibody (19) was a generous gift from Dr. Joachim Herz of University of Texas Southwestern Medical Center.

Cell Culture—J774A.1 cells permanently transfected with the pEF-BOS vector carrying porcine leukocyte 12/15-lipoxygenase cDNA and mock-transfected cells were establish as describe previously (12). The cells were cultured at 37 °C with 5% CO2 in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin G and 100 μg/ml streptomycin sulfate, and subcultured every 2–3 days using a standard trypsin protocol. Mouse peritoneal macrophages were collected from C57BL/6 mice as described previously (20) except that thioglycollate was injected into mice before harvesting the cells.

Enzyme Preparation and Assay—The 12/15-lipoxygenase-expressing cells were cultured in 100-mm dishes in DMEM with 10% lipoprotein-deficient serum for 48 h, then LDL at 400 μg/ml was added to the medium. After incubation at 37 °C for various periods, cells were washed with ice-cold phosphate-buffered saline at pH 7.4 and suspended in 50 mM Tris-HCl buffer at pH 7.4 containing 1 mM EDTA. The cells were sonicated twice on ice, each for 5 s, at 20 kHz by a Branson sonifier model 250 (Danbury, CT), followed by ultracentrifugation at 265,000 × g at 4 °C for 2 h. The supernatant was referred to as cytosol, and the pellet resuspended in 50 mM Tris-HCl at pH 7.4 containing 1 mM EDTA was referred to as “membranes.”

12/15-Lipoxygenase activity was determined as described previously (12). Briefly, the assay and the membranes were incubated in a 200-μl reaction mixture containing 50 mM Tris-HCl buffer at pH 7.4 and 25 μM 1-[14C] Arachidonic acid (1.85 kBq). The reaction was carried out at 30 °C for 10 min with constant mixing and quenched by the addition of 1 ml of an ice-cold mixture of diethyl ether/methanol/1% citric acid (30:4:1, v/v). The ether layer was spotted onto a silica gel thin-layer plate and the plate was developed at 4 °C for 60 min with a solvent system of diethyl ether/petroleum ether/acetic acid (85:30:4, v/v). The radioactive products on the plate were detected and quantified by a Fujix BAS 1000 imaging analyzer (Tokyo, Japan). Protein concentration was determined by the method of Lowry et al. (21) with bovine serum albumin as a standard.

Western Blotting—The proteins in the cytosol and membranes were separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane, followed by blocking with 10% (w/v) nonfat dry milk in 20 mM Tris-HCl at pH 7.4 containing 136 mM NaCl and 0.1% Tween 20 for 1 h at room temperature. The washed membranes were incubated for 1 h at room temperature with an anti-12/15-lipoxygenase antibody at 1:1,000 dilution. 12/15-Lipoxygenase band was detected using a horseradish peroxidase-conjugated secondary antibody and ECL chemiluminesence reagents according to the manufacturer’s instruction. The density of 12/15-lipoxygenase band was quantified by National Institutes of Health Image 1.60 analysis software (Bethesda, MA). The intensity of the 12/15-lipoxygenase band increased linearly with the amount of the enzyme loaded onto the gel.

Thiobarbituric Acid Reactive Substance Assay—The 12/15-lipoxygenase-expressing cells (2 × 106) were preincubated for 48 h in DMEM containing 10% of lipoprotein-deficient serum followed by the addition of a translocation inhibitor, L655238, at various concentrations. After 1 h the cells were incubated with 400 μg/ml LDL in 100 μl of DMEM containing 10% lipoprotein-deficient serum in the presence of L655238 for 12 h, and the culture medium was subjected to TBARS assay as described previously (12).

Immunohistochemistry—The light- and electron-microscopic immunohistochemical procedures were performed as described (22). 12/15-Lipoxygenase-expressing cells and mouse resident macrophages collected from the peritoneal cavity were cultured for 48 h in the medium containing 10% lipoprotein-deficient serum in Lab-Tek chamber slide. The cells were then treated with LDL for 15 min followed by fixation in 0.1% phosphate buffer at pH 7.4 containing 4% paraformaldehyde on ice for 30 min and washed twice in phosphate-buffered saline at pH 7.4. For light microscope observation, the slides were first permeabilized by incubating (1 h) with 0.5% Triton X-100 in PBS, then treated with 3% hydrogen peroxide in methanol for 10 min, and then incubated with 5% normal swine serum for 30 min. Subsequently, the slides were incubated at room temperature overnight with an anti-12/15-lipoxygenase antisemur. For the negative control, the antibody was replaced with preimmune rabbit serum. The sites of immunoreaction were then visualized by incubating the slides successively with biotinylated anti-rabbit IgG diluted at 1:200 for 1 h, horseradish peroxidase-conjugated streptavidin diluted at 1:300 for 1 h, and with 0.01% 3,3′-diaminobenzidine tetrahydrochloride in the presence of 0.02% hydrogen peroxide in 50 mM Tris-HCl at pH 7.5 for 10–30 min.

For electron-microscopic immunocytochemistry, the immunostained slides were postfixed with 0.5% OsO4 for 20 min. After block-staining with 1% uranyl acetate for 30 min, the slides were dehydrated in graded ethanol series and embedded in an epoxy resin based on Glicidether 100. Ultrathin sections were prepared and subjected to observation with a Hitachi H-700 electron microscope (Tokyo, Japan).

RESULTS

Membrane Translocation of 12/15-Lipoxygenase by LDL—12/15-Lipoxygenase is predominantly localized in cytosol but not in the membranes (15). This was confirmed in macrophage-like J774.A.1 cells overexpressing 12/15-lipoxygenase and resident peritoneal macrophages by Western blot as shown in Fig. 1A. To investigate whether the subcellular localization of the 12/15-lipoxygenase is changed by LDL treatment, the enzyme-expressing cells or peritoneal macrophages were treated with LDL for various periods, and the cytosol and membranes were subjected to Western blot analysis. Fig. 1A shows that the band of 12/15-lipoxygenase at 75 kDa was detected not only in the cytosol but also in the membranes with a concomitant decrease of the enzyme level in the cytosol. Association of the enzyme to the membranes reached maximum at 15 min after LDL addition and then decreased. After 30 min the enzyme was no longer present in the membranes. Densitometric analysis revealed the increase in 12/15-lipoxygenase protein of the membranes after the LDL treatment for 5 and 15 min by 23- and 33-fold, respectively (Fig. 1B). The enzyme protein was increased by 14-fold in the membranes of macrophages by the treatment with LDL for 15 min. After 15-min treatment by LDL, the enzyme protein in the cytosol was decreased by 38 and 47% in 12/15-lipoxygenase-expressing cells and in macrophages, respectively. The results indicated that 12/15-lipoxygenase was transferred from cytosol to membranes by the LDL treatment in the resident macrophages as well as 12/15-lipoxygenase-expressing J774.A.1 cells.

We measured the enzyme activity in the cytosol and membranes in 12/15-lipoxygenase-expressing cells. As shown in Fig.
FIG. 1. Membrane translocation of 12/15-lipoxygenase in the enzyme-expressing cells and macrophages treated with LDL. A, a representative Western blot of 12/15-lipoxygenase protein in the cytosol and membranes in the 12/15-lipoxygenase-expressing J774A.1 cells and resident peritoneal macrophages in the presence or absence of 400 μg/ml LDL for indicated periods. The amounts, 10 μg of protein for the enzyme-expressing cells and 2 μg for peritoneal macrophages, were separated by SDS-PAGE and subjected to Western blot analysis as described under "Experimental Procedures." Arrows indicate the positive 12/15-lipoxygenase band at 75 kDa. B, densitometric analysis of 12/15-lipoxygenase protein in the cytosol and the membranes as compared with the density of the control cells. Ratios over the density for the cytosol of the cells incubated without LDL for 5 min are shown. Data represent means of three separate experiments, and the protein in the cytosol and the membranes as compared with the density of the control cells. Ratios over the density for the cytosol of the cells under "show significant difference from the cells treated without LDL at each time by Welch’s t test (p < 0.01). LOX, lipoxygenase.

2, the specific activity of the enzyme in the cytosol was decreased by 41% by the treatment with LDL for 15 min as compared with non-treated cells. The result was in good agreement with that from Western blot analysis (Fig. 1). The level of the increase of the enzyme activity in the membranes was significantly lower after LDL treatment. It is shown that cholesterol ester is selectively transferred from the LDL particle to the plasma membrane via LRP in Y1 murine adrenocortical cells (14) and in our 12/15-lipoxygenase-expressing cells and that linoleic acid esterified to cholesterol in the LDL particle is regio- and stereospecifically oxygenated by the 12/15-lipoxygenase-expressing cells (12). Thus, the above observations strongly support our contention that the 12/15-lipoxygenase associated with the membranes oxygenates cholesterol ester transferred to the membrane, because self-catalyzed inactivation of the 12/15-lipoxygenase, which should be observed in the enzyme reaction with cholesterol ester in the membrane, is known to occur (23). This would explain the much lower enzyme activity in membranes. The results indicate that LDL brings about translocation of 12/15-lipoxygenase from cytosol to membranes where the oxidation of cholesterol ester from LDL takes place.

LRP Mediates the Translocation of 12/15-Lipoxygenase—We previously reported the essential requirement of LRP for the cell-mediated oxidation of LDL in macrophages (13). To examine whether the LRP is also involved in the translocation of the enzyme, we employed an anti-LRP antibody that blocked the binding of LDL to LRP (19). The 12/15-lipoxygenase expressing cells and mouse resident peritoneal macrophages were preincubated in the presence of an anti-LDL receptor antibody or an anti-LRP antibody for 2 h. After 15-min incubation with LDL, the cytosol and membranes were subjected to Western blot analysis. As shown in Fig. 3 (A and C), the 12/15-lipoxygenase band in the membranes of the cells preincubated with an anti-LRP antibody was faint after LDL treatment as compared with that from the control. Consistent with this observation, the density of the 12/15-lipoxygenase band in the cytosol of the cells preincubated with an anti-LRP antibody was not significantly different from that of the cells that were not treated with LDL. Preincubation with an anti-LDL receptor antibody did not significantly affect the enzyme translocation by the LDL treat-

ment (Fig. 3). The results indicate that the translocation of 12/15-lipoxygenase is mediated by binding of LDL to the LRP but not to the LRP receptor.

Immunohistochemical Staining of 12/15-Lipoxygenase—To determine the intracellular localization of the 12/15-lipoxygenase after LDL treatment, the enzyme-expressing cells were subjected to immunohistochemical analysis using an antibody against the enzyme (Fig. 4). A different staining pattern of the 12/15-lipoxygenase was observed between the cells treated with and without LDL under light microscopy. In the non-treated cells, the enzyme was predominantly stained in cytoplasm of 12/15-lipoxygenase-expressing cells (Fig. 4, C and D). When the cells were treated with LDL for 15 min, the positive staining of 12/15-lipoxygenase was observed not only in cytoplasm but also in the plasma membrane of the enzyme-expressing cells (Fig. 4, A and B). Essentially the same results were obtained with LDL-treated resident peritoneal macrophages.

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To examine whether enzyme association with the membranes is required for the LDL oxidation, we employed a translocation inhibitor, L655238. This compound was first found to inhibit translocation of 5-lipoxygenase (24) but later shown to inhibit translocation of other lipoxygenases without affecting the enzyme activity per se (25). As shown in Fig. 5 (A and B), L655238 inhibited translocation of 12/15-lipoxygenase in the LDL-treated cells in a dose-dependent manner without affecting the enzyme activity. Fig. 5C shows that LDL oxidation determined as TBARS generation in the medium was blocked in a dose-dependent manner by the translocation inhibitor. The inhibitor at 10 μM, which completely suppressed the enzyme translocation (Fig. 5, A and B), inhibited the LDL oxidation to the level of mock-transfected cells (Fig. 5C). The results clearly indicate that the association of the enzyme with the plasma membrane is required for the LDL oxidation.

DISCUSSION

We demonstrate here that 12/15-lipoxygenase is translocated from cytosol to the membranes by LDL treatment in 12/15-lipoxygenase-expressing macrophage-like cells and resident peritoneal macrophages (Figs. 1 and 2). The translocated enzyme is preferentially localized in the plasma membrane (Fig. 4), strongly suggesting that the enzyme directly oxygenates cholesterol ester selectively transferred from the LDL particle to the plasma membrane. In fact, the LDL oxidation was inhibited by a translocation inhibitor, L655238 (Fig. 5). Regio- and stereospecific oxygenation of linoleic acid esterified to cholesterol in LDL by 12/15-lipoxygenase-expressing cells indicates that cholesterol ester is enzymatically oxygenated by the cells (12). The enzymatic oxygenation is presumed to be the first key step for the generation of the completely oxidized LDL, which is made in the subsequent steps, including non-enzymatic radical chain reaction (12, 23). The fact that 12/15-lipoxygenase translocation takes place in a very short period such as 5–15 min supports this notion (Fig. 1). Furthermore, such a short time course minimizes the oxygenation of phospholipids in plasma membrane, which may cause the cell injury. The weak activity of the membrane-associated enzyme after LDL treatment strongly suggests that the enzyme in the plasma membrane reacts with colocalized substrates, including cholesterol ester and then suicides. However, the reduction of the enzyme activity may be due to other mechanisms unrelated to suicide inactivation such as poor substrate availability or conformational changes of the enzyme. It is reported that 12/15-lipoxygenase preferentially oxygenates cholesterol ester in the plasma membrane, whereas phospholipids or even free fatty acids are not oxygenated, although they are present on the surface of the LDL particle (23). These results suggest that the specific oxygenation of cholesterol ester transferred to the plasma membrane by 12/15-lipoxygenase could take place. LRP is an LDL-binding receptor that selectively transfers cholesterol ester in the LDL particle (14). We show here that binding of LDL to LRP is also required for 12/15-lipoxygenase translocation (Fig. 3). 12/15-Lipoxygenase-expressing J774A.1 cells have both LRP and the LDL receptor, although the expression level of the LDL receptor is low (13). In contrast, normal macrophages express high level of LRP but do not express the LDL receptor (26, 27). The contribution of LRP but not of the LDL receptor to 12/15-lipoxygenase translocation supports the notion that the LDL-dependent translocation is also mediated by LRP in normal macrophages. In fact, the enzyme translocation is inhibited in mouse peritoneal macrophages lacking the LDL receptor by the anti-LRP antibody (Fig. 3, C and D). However, we cannot completely exclude a role for the LDL receptor in the translocation, because either type of cells used in our experiments express a little or no LDL receptor where an anti-LDL

The control experiments with preimmune rabbit serum in place of the antiserum against 12/15-lipoxygenase exhibited negative immunostaining (data not shown). The results indicate that the plasma membrane is at least one of the major sites where 12/15-lipoxygenase translocates after LDL treatment. To investigate the precise localization of the enzyme in the LDL-treated cells, we observed the immunostained cells with an electron microscopy. As shown in Fig. 4F, non-treated cells showed diffuse staining pattern in cytoplasm. In contrast, the plasma membrane was the major site where the 12/15-lipoxygenase was localized in LDL-treated cells, although membranes of some other intracellular organelles were also stained in addition to cytoplasm (Fig. 4E). It should be noted that the nuclear envelope was essentially not stained in LDL-treated cells.

Enzyme Translocation Is Required for LDL Oxidation—To
Translocation of 12/15-Lipoxygenase by LDL

Fig. 5. Translocation inhibitor L655238 inhibits LDL oxidation. A, 12/15-lipoxygenase-expressing cells were preincubated with L655238 at indicated concentrations for 1 h and treated with LDL for 15 min. Cytosol and membranes were subjected to Western blot analysis. B, the densitometric analysis was carried out, and the ratios over the density for the cytosol of the cells incubated without LDL are shown. Data represent the means of three separate experiments, and bars denote standard error. Asterisks show significant difference from the cells treated without LDL in each preparation by Welch’s t test (p < 0.01). Open circles show the 12/15-lipoxygenase activity in the cytosol prepared from the enzyme-expressing cells measured with [1-14C]arachidonic acid as a substrate in the presence of indicated concentrations of L655238. Data show the means of triplicate experiments, and bars denote standard errors. C, LDL at 400 μg/ml was added to the culture medium of 12/15-lipoxygenase-expressing cells (closed circles) and mock-transfected cells (an open circle) preincubated with indicated concentrations of L655238 for 1 h. The oxidized LDL in the medium was measured as TBARS after 12 h. Data are shown as means of quadruplicate experiments after subtraction of no-cell control, and bars denote standard errors.

receptor antibody would not be expected to have an effect. Coupling of selective uptake of cholesterol ester with 12/15-lipoxygenase translocation would cause efficient oxygenation of linoleic acid esterified to cholesterol. The mechanisms of the efflux of oxygenated cholesterol ester to the LDL particle are now under extensive investigation in our laboratory. The cholesterol ester in the high density lipoprotein is selectively transferred to the plasma membrane by scavenger receptor class B type I (28). The same receptor is shown to mediate cholesterol efflux to high density lipoprotein (29, 30). It may be possible that LRP also mediates the efflux of oxygenated cholesterol ester from the plasma membrane to the LDL particle. The mechanism of translocation of 12/15-lipoxygenase in macrophages is not known (16), although the N-terminal C2-like domain in the enzyme is proposed to be responsible for the enzyme binding to the membrane phospholipids in a calcium dependent way (31). In fact, our finding that the translocation of 12/15-lipoxygenase is inhibited by L655238 suggests the similar translocation mechanism to that of 5-lipoxygenase. L655238 was first developed as an inhibitor of 5-lipoxygenase-activating protein, which was later shown to function as a substrate transfer protein promoting the use of arachidonic acid and other unsaturated fatty acids (32). In 5-lipoxygenase, the N-terminal C2-like domain is demonstrated to be a calcium-dependent membrane-targeting domain without requirement of any special docking protein (33). We have reported that 12-lipoxygenase in human platelets is activated by membrane translocation when stimulated by thrombin or calcium ionophore A23187 caused translocation of the enzyme (data not shown), although different results have been reported (35).

LRP is known to couple with a G protein-coupled receptor (36, 37) and phospholipase A2 binds preferentially to the nuclear envelope (33, 35), whereas that in protein kinase Ca and phospholipase Cδ prefers targeting to the plasma membrane (36, 37) in a calcium-dependent way. In fact, our preliminary results using 12/15-lipoxygenase-expressing cells suggested that the calcium ionophore A23187 caused translocation of the enzyme (data not shown), although different results have been reported (35).
REFERENCES
1. Takahashi, Y., and Yoshimoto, T. (2002) Res. Adv. Cancer 2, 221–229
2. Funk, C. D. (2001) Science 294, 1871–1875
3. Brash, A. R. (1999) J. Biol. Chem. 274, 23679–23682
4. Kuhn, H., and Thiele, B. J. (1999) FEBS Lett. 449, 7–11
5. Funk, C. D. (1996) Biochim. Biophys. Acta 1304, 65–84
6. Brown, M. S., and Goldstein, J. L. (1986) Science 232, 34–47
7. Wittum, J. L., and Steinberg, D. (1991) J. Clin. Invest. 88, 1785–1792
8. Cyrus, T., Wittum, J. L., Rader, D. J., Tangirala, R., Fazio, S., Linton, M. F., and Funk, C. D. (1999) J. Clin. Invest. 103, 1597–1604
9. Harats, D., Shaish, A., George, J., Mulkins, M., Kurinaka, H., Levkovitz, H., and Sigal, E. (2000) Arterioscler. Thromb. Vasc. Biol. 20, 2100–2105
10. Shen, J., Kuhn, H., Petho-Schramm, A., and Chan, L. (1995) FASEB J.
11. Funk, C. D., and Cyrus, T. (2001) Trends Cardiovasc. Med. 11, 163–169
12. Swarnakar, S., Beers, J., Strickland, D. K., Azhar, S., and Williams, D. L. (2000) J. Biol. Chem. 275, 21121–21128
13. Xu, W., Takahashi, Y., Sakashita, T., Hattori, H., and Yoshimoto, T. (2000) J. Biol. Chem. 276, 36454–36459
14. Miller, Y. I., Chang, M. K., Funk, C. D., Feramisco, J. R., and Witztum, J. L. (1999) J. Biol. Chem. 274, 19341–19349
15. Kowal, R. C., Herz, J., Goldstein, J. L., Esser, V., and Brown, M. S. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5810–5814
16. Mizushima, S., and Cho, W. (1999) J. Biol. Chem. 274, 3575–3579
17. Ozeki, Y., Nagamura, Y., Ito, H., Unemi, F., Kimura, Y., Iwagawa, T., Kambayashi, J., Takahashi, Y., and Yoshimoto, T. (1999) Br. J. Pharmacol. 128, 1699–1704
18. Yamamoto, M., Fan, L., Wakayama, T., Amass, O., and Iseki, S. (2001) Anat. Rec. 262, 213–220
19. Swarnakar, S., Beers, J., Strickland, D. K., Azhar, S., and Williams, D. L. (2000) J. Biol. Chem. 275, 21121–21128
20. Cho, W. (2001) J. Biol. Chem. 276, 8312–8316
21. Belkner, J., Stender, H., and Kuhn, H. (1998) J. Biol. Chem. 273, 23225–23232
22. Evans, J. F., Leville, C., Maneini, J. A., Prasit, P., Therien, M., Zamoni, R., Gauthier, J. Y., Fortin, R., Charleson, P., MacIntyre, D. E., Rasmussen, B., Bach, T. J., Meurer, R., Guay, J., Vickers, P. J., Rouzer, C. A., Gillard, J. W., and Miller, D. K. (1991) Mol. Pharmacol. 40, 22–27
23. Moestrup, S. K., Gliemann, J., and Pallesen, G. (1992) Cell Tissue Res. 269, 375–382
24. Brash, A. R. (1999) J. Biol. Chem. 274, 1875–1879
25. Kambayashi, J., Takahashi, Y., and Yoshimoto, T. (2002) Translocation of 12/15-Lipoxygenase by LDL
