Biochemical and Biophysical Research Communications 434 (2013) 594–599

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Lectin-like oxidized LDL receptor-1 is palmitoylated and internalizes ligands via caveolae/raft-dependent endocytosis

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ARTICLE INFO

Article history:
Received 19 March 2013
Available online 10 April 2013

Keywords:
Lectin-like oxidized LDL receptor-1 (LOX-1)
Oxidized low-density lipoprotein
Palmitoylation
Caveolae

ABSTRACT

Lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) is an endothelial scavenger receptor that is important for oxidized low-density lipoprotein uptake. LOX-1 functions as an oligomer; however, little is known about the oligomeric complex and ligand processing after recognition by LOX-1. Here, we found that LOX-1 recognized and internalized ligands through the caveolae/raft-dependent endocytosis pathway in human coronary artery endothelial cells. Furthermore, we demonstrated that LOX-1 was palmitoylated and that both cysteine 36 and cysteine 46 were necessary for the recruitment of LOX-1 into raft microdomains and for its ligand uptake ability.

1. Introduction

Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is a member of the scavenger receptor family [1] and functions as an oxidized low-density lipoprotein (OxLDL) receptor on vascular endothelial cells [2]. LOX-1 recognizes OxLDL and initiates endothelial dysfunction, which is believed to be a crucial early step in atherosclerosis [3]. The extracellular domain of LOX-1 consists of a C-type lectin-like domain (CTLD) and a neck domain, which contains Cys140, an amino acid that forms interchain disulfide bonds and enables LOX-1 to exist as a covalent homodimer [4]. This homodimer can form higher noncovalent oligomeric complexes, and consistent with this, LOX-1 has been proposed to function as an oligomer [5,6]. The molecular density of LOX-1 on the cell surface is thought to be closely related to its ability to form oligomers [6], and thus, it is possible that LOX-1 is recruited to microdomains in the cell membrane, where it forms a functional complex. However, despite the importance of LOX-1, the mechanism through which it forms an oligomeric complex and the processing of LOX-1 ligands after recognition by LOX-1 remain unknown.

Lipid rafts are membrane microdomains rich in sphingolipids and cholesterol and are considered tightly packed signaling platforms [7]. Although recent studies have indicated that lipid rafts are not tightly packed stable microdomains, as described previously, they are known to be important in cell signaling, often through the organization of cell surface receptors, signaling molecules, and adaptor molecules into membrane complexes [8].

Here, we investigated the localization of LOX-1 in lipid rafts and the contribution of palmitoylation to LOX-1 activity.

2. Materials and methods

2.1. Materials

1,1′-Dioctadecyl-3, 3,3′, 3′-tetramethylindodicarbocyanine perchlorate (DiD), Alexa 555-conjugated cholera toxin subunit B (CT-B), Alexa 555-conjugated anti-mouse IgG, and Alexa 633-conjugated anti-rabbit IgG were purchased from Molecular Probes (Eugene, OR), and acetylated low-density lipoprotein (AcLDL) was from Biomedical Technologies (Rockville, MD). The anti-LOX-1 polyclonal antibody (ab60178) and LOX-1 neutralizing antibody (23C11) were from Abcam (Cambridge, MA); anti-caveolin 1 (Cav1) monoclonal antibody was from Novus Biologicals (Littleton, CO); Biotin-HPDP (N-{6-(biotinamido)hexyl}-3′-(2′-pyridyldithio)propionamide) was from Pierce Biotechnology (Rockford, IL); nystatin, genistein, and 2-bromopalmitate (2BP) were from Sigma (St. Louis, MO); HEK293 cell lines and CHO cell lines were from

Abbreviations: LDL, low-density lipoprotein; LOX-1, lectin-like oxidized low-density lipoprotein receptor-1; OxLDL, oxidized low-density lipoprotein; HCAEC, human coronary artery endothelial cells; Cav1, caveolin-1; AcLDL, acetylated low-density lipoprotein; CT-B, cholera toxin subunit B; CM, chloroform-methanol; NEM, N-ethylmaleimide; Biotin-HPDP, N-{6-(biotinamido)hexyl}-3′-(2′-pyridyldithio) propionamide; HA, hydroxylamine; DRMs, detergent-resistant membranes; 2BP, 2-bromopalmitate.

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http://dx.doi.org/10.1016/j.bbrc.2013.03.120

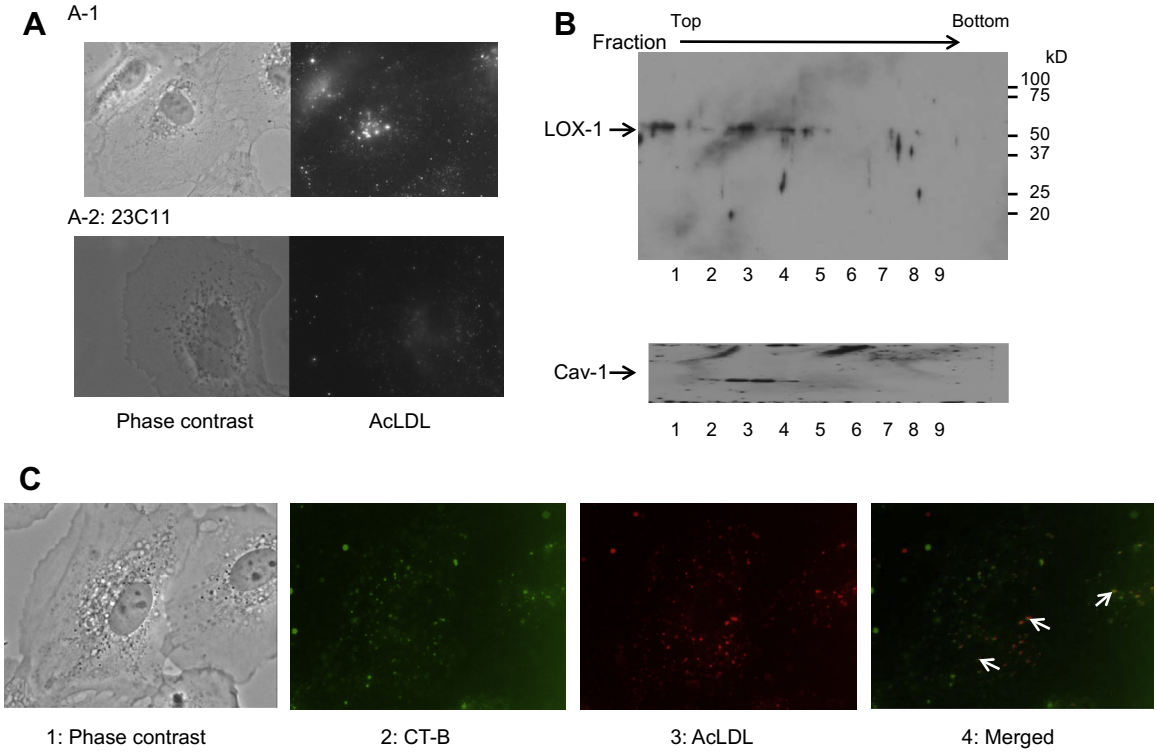

Fig. 1. Isolation of LOX-1 in the detergent-resistant membrane fraction, and co localization of AcLDL with CT-B. (A-1) DiD–AcLDL uptake by HCAECs. Cells were observed as described in Section 2. (A-2) DiD–AcLDL uptake by HCAECs pretreated with the LOX-1 neutralizing antibody 23C11. (B) Brij 98-solubilized membranes from HCAECs were fractionated by a sucrose gradient. Solubilized membranes (lane 1) and each fraction (lanes 2–9) were resolved by SDS–PAGE and analyzed by immunoblotting using the specific antibodies indicated. (C) HCAECs were incubated with Alexa 555-conjugated CT-B and DiD–AcLDL for 10 min and fixed with 2% formaldehyde. (1) Phase contrast; (2) image of Alexa 555-conjugated CT-B; (3) DiD–AcLDL; and (4) merged image of CT-B and DiD–AcLDL. Arrows indicate the colocalization.

RIKEN Cell Bank (Tsukuba, Japan); and human coronary artery endothelial cells (HCAECs) were from LONZA (Walkersville, MD).

2.2. Isolation of detergent-resistant membranes

Detergent-resistant membranes (DRMs) were prepared by floatation on sucrose density gradients by ultracentrifugation of Brij 98 lysates, as previously described [9]. Briefly, the membrane fraction was prepared from the post-nuclear fraction by ultracentrifugation (100,000*g* for 1 h), solubilized in buffer containing 1% Brij 98 for 5 min at 37 °C, and fractionated by a sucrose gradient. Fractions were collected from the top of the gradient, and equal protein amounts (10 μg protein/lane for LOX-1, 1 μg/lane for Cav1) from each fraction were analyzed by immunoblotting using anti-LOX-1 or anti-Cav1 antibodies, followed by HRP-conjugated goat anti-rabbit or mouse IgG. The signals were detected using an ECL detection system (GE Healthcare, Buckinghamshire, UK).

2.3. Immunofluorescence staining

DiD-labeled AcLDL was prepared from human LDL as previously described [5]. Cells were grown on cover slips for 2 days prior to the experiments, incubated with DiD–AcLDL (0.4 μg/mL) and/or CT-B (0.4 μg/mL) at 37 °C in a humidified atmosphere containing 5% CO_2 for the indicated time, fixed with formaldehyde (2% in PBS), mounted, and imaged using a Leica DM IRE2 microscope. For indirect immunofluorescence staining, the fixed cells were stained for 2 h at room temperature with the primary antibody (mouse monoclonal anti-Cav1 at 1:1000, or rabbit polyclonal anti-LOX-1 at 1:200) in blocking buffer (PBS with 5% BSA). The samples were rinsed with blocking buffer, then exposed to the Alexa-555-conjugated anti-mouse IgG (5 μg/mL in blocking buffer) or Alexa-633 conjugated anti-rabbit IgG for 1 h at room temperature, and mounted.

2.4. Ligand uptake assay

Cells were grown on cover slips for 2 days prior to the experiments, and the ligand uptake assay was performed as previously described [10]. Images of more than 50 cells from 3 independent experiments were acquired. The fluorescence intensity of each cell was determined from the mean pixel values of the whole cell using MetaMorph software (Universal Imaging). The effects of inhibitors were evaluated by pre-incubation with each inhibitor: nystatin (final concentration, 100 μM) for 1 h or genistein (final concentration, 200 μM) for 2 h.

2.5. Plasmid construction

Plasmids carrying CFP-LOX-1 or CFP-mutant LOX-1 were prepared as previously described [5]. HEK293 and CHO cells were transfected using Lipofectamine Plus (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol, and stable cell lines were selected using G418 (2 mg/mL).

2.6. Identification of palmitoylation

Palmitoylation of LOX-1 was determined according to acyl-biotinylated exchange methods [11]. Briefly, cells were grown on 10-cm culture dishes, solubilized by 0.4 mL cell lysis buffer (150 mM NaCl, 5 mM EDTA, and 50 mM Tris; pH 7.4), and incubated at 4 °C for 1 h. After centrifugation (250*g* for 5 min), the proteins were precipitated by chloroform–methanol (CM) precipitation. Precipitated proteins were resolved using 50 μL of SDS buffer (4% SDS,

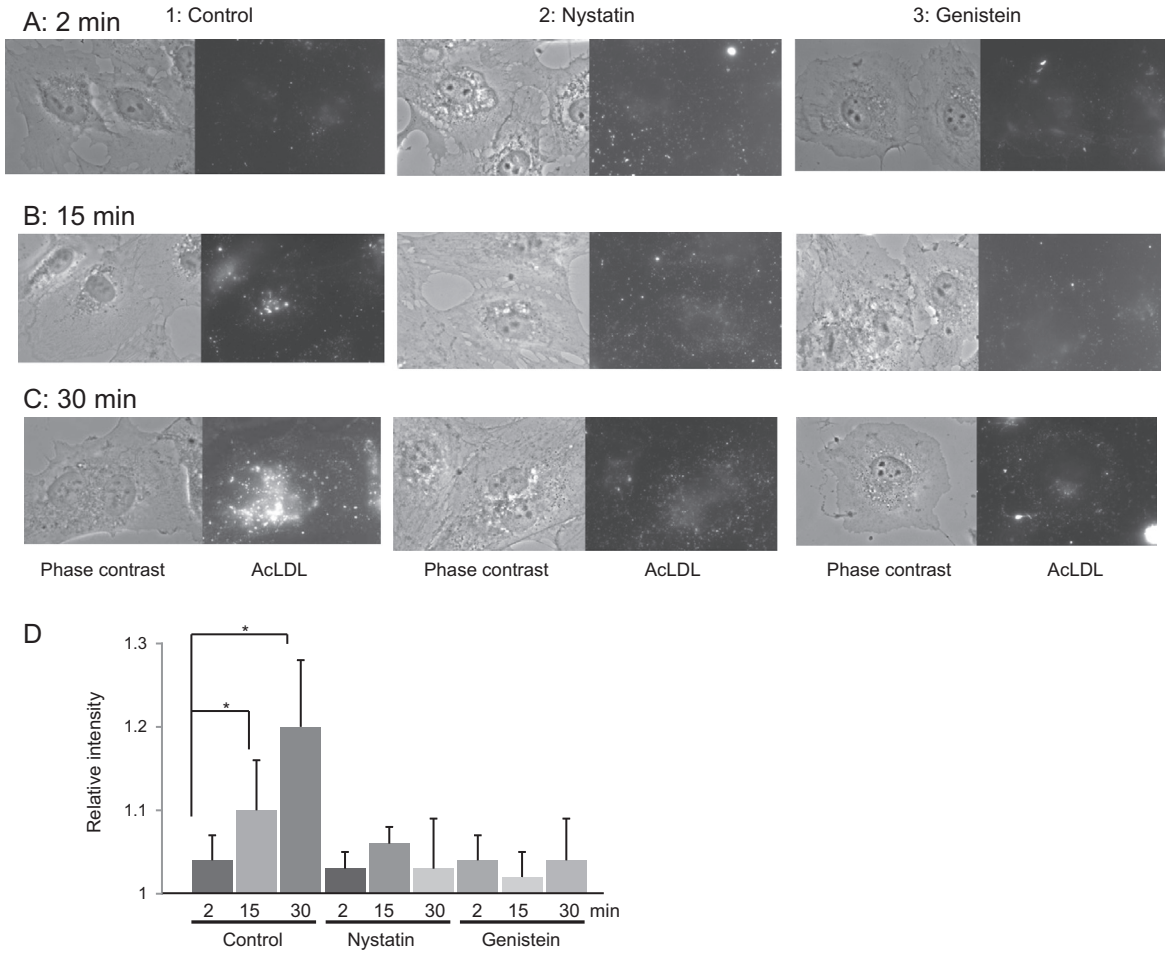

Fig. 2. Effects of nystatin and genistein on internalization of DiD–AcLDL. HCAECs were pretreated with nystatin or genistein. Cells were imaged following incubation with DiD–AcLDL for (A) 2 min, (B) 15 min, or (C) 30 min. (D) Images of more than 50 cells were acquired from 3 independent experiments. The fluorescence intensity was quantitatively evaluated using region-measurement analysis (MetaMorph). Results are expressed as the mean ± SD. Ratios of the amounts of DiD–AcLDL around the perinuclear area to those near the plasma membrane are shown.*$P < 0.05$ compared with the 2-min incubation.

50 mM EDTA, and 50 mM Tris) containing 10 mM N-ethylmaleimide (NEM) and incubated at 37 °C for 10 min. Next, 0.15 mL lysis buffer (0.2% Triton X-100, 1 mM NEM, and 1 mM PMSF) was added and incubated at 4 °C for 16 h. During this step, free thiols were blocked with NEM. The resulting mixture was CM-precipitated thrice to completely remove the NEM. The precipitated protein was resolved in 0.25 mL of 4% SDS buffer and incubated at 37 °C for 10 min. The solution was then divided into 2 tubes. One tube was incubated with (+) hydroxylamine (HA) buffer (0.7 M HA, 1 mM HPDP-biotin, 0.2% Triton X-100, 1 mM PMSF, and a protease inhibitor cocktail), and the other was treated with (−) HA buffer (50 mM Tris, 1 mM HPDP-biotin, 0.2% Triton X-100, 1 mM PMSF, and a protease inhibitor cocktail). During this step, HA cleaved the Cys-palmitoyl thioester linkages, and the newly exposed thiols were labeled with biotin. Samples were then subjected to 3 sequential CM precipitations, and the biotinylated protein was purified using streptavidin agarose. The bound protein was subjected to immunoblotting using anti-LOX-1 antibodies.

For inhibition of palmitoylation, 100 μM 2BP in DMSO was added to HCAEC cultures. Eight hours later, cell lysates were prepared as described above.

2.7. Statistical analysis

Student's *t*-tests were used for comparison of 2 data sets, and ANOVA was used for multiple data sets. *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. LOX-1 is isolated in DRMs with Cav1

To avoid ambiguity due to variation in the extent of LDL oxidization, AcLDL, whose affinity for LOX-1 is comparable with that of OxLDL [12], was used as an alternative ligand. As shown in Fig. 1A, HCAECs took up DiD–AcLDL, whereas HCAECs pretreated with anti-LOX-1 neutralizing antibodies exhibited a marked decrease in the uptake of DiD–AcLDL (17.4 ± 3.7%). This result indicated that LOX-1 was the main receptor for the uptake of OxLDL in HCAECs.

Next, we analyzed whether LOX-1 was isolated in DRMs. DRMs can be isolated by their insolubility in Brij 98 and separated from the disordered membrane environments by sucrose density gradient centrifugation [9]. A substantial proportion of LOX-1 was found in the light fraction (DRMs), and these fractions were enriched in the raft marker Cav1 (Fig. 1B).

3.2. Internalized AcLDL colocalizes with CT-B

CT-B binds ganglioside GM 1 located in the lipid raft and can be internalized by caveolae/raft-dependent endocytosis to endosomes or caveosomes [13]. Therefore, we next investigated the localization of DiD–AcLDL and Alexa 555-conjugated CT-B to examine whether the ligand recognized by LOX-1 was internalized via a caveolae/raft-dependent pathway. HCAECs were incubated with DiD–AcLDL and CT-B on ice for 10 min, followed by an additional

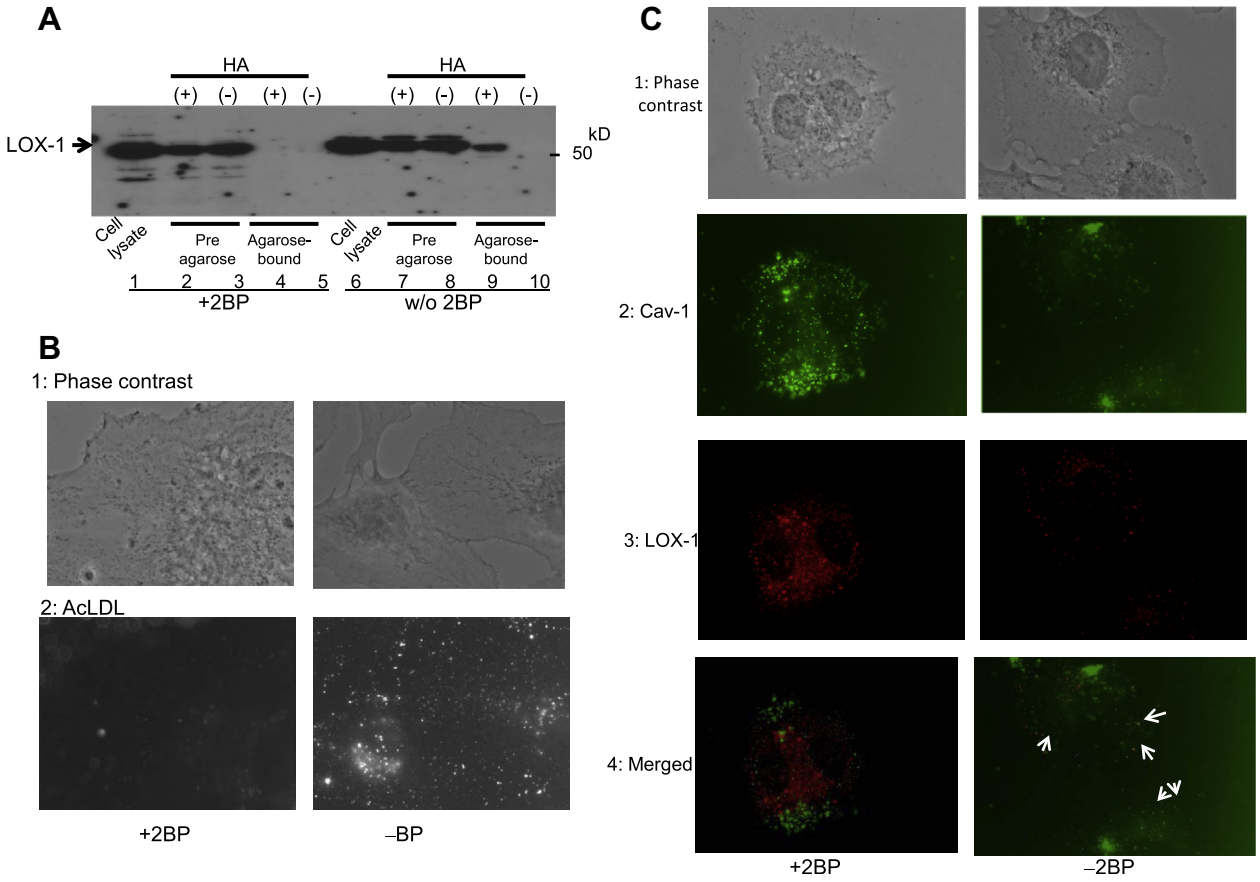

Fig. 3. Effects of 2BP on DiD–AcLDL uptake and localization of LOX-1. (A) HCAECs grown on six 10-cm culture dishes were subjected to acyl-biotinylated exchange methods, as described in Section 2. Lanes 1–5: HCAECs treated with 2BP in DMSO for 8 h, lanes 6–10: HCAECs treated with 1% DMSO as a control. Lanes 1 and 6: cell lysates; lanes 2, 3, 7, and 8: samples before adding streptavidin-agarose; lanes 4, 5, 9, and 10: streptavidin-agarose-bound samples; lanes 2, 4, 7, and 9: after NEM treatment followed by biotinylation under HA; lanes 3, 5, 8, and 10: biotinylation without HA. (B) DiD–AcLDL uptake by HCAECs treated with 2BP. (1) Phase contrast, (2) fluorescence of internalized DiD–AcLDL. Left panel: HCAECs treated with 2BP, right panel: HCAECs treated with 1% DMSO. (C) Indirect immunofluorescence staining of HCAECs. Left panel: HCAECs treated with 2BP, right panel: HCAECs treated with DMSO. (1) Phase contrast, (2) caveolin-1, (3) LOX-1, (4) merged.

incubation for 10 min at 37 °C. After the 10-min incubation, 68 ± 9.6% of DiD–AcLDL was colocalized with CT-B (Fig. 1C). This result indicated that DiD–AcLDL was internalized via the caveolae/raft microdomain.

3.3. Nystatin and genistein inhibit DiD–AcLDL internalization

To further explore the internalization process, we investigated the effects of nystatin and genistein on DiD–AcLDL uptake. Nystatin mediates cholesterol sequestration and inhibits the formation of caveolae [14], whereas genistein is known to inhibit caveolae-dependent endocytosis [15]. HCAECs were pre-incubated with nystatin or genistein, followed by incubation with DiD–AcLDL. Clear uptake of DiD–AcLDL was detected after a 15-min incubation in control cells (Fig. 2B-1), and after the 30-min incubation, DiD–AcLDL accumulated around the nuclei (Fig. 2C-1). Comparatively, cells pretreated with nystatin bound the ligand, but little ligand was detected in the perinuclear area after the 15-min incubation, and almost all the ligand remained near the plasma membrane (Fig. 2C-2). Similar results were obtained using cells treated with genistein (Fig. 2C-3). Since these inhibitors induced some cell damage, causing a decrease in ligand binding, quantitative analysis of the internalization of ligands was performed to calculated the ratio of ligands around the perinuclear region to that near plasma membrane at the indicated times. The ratio of ligands around the perinuclear region did not increase in nystatin- or genistein-treated cells, whereas the ratio significantly increased in control cells (Fig. 2D). These results were consistent with the CT-B studies and showed that DiD–AcLDL was recognized by LOX-1 and was internalized via a caveolae/raft-dependent endocytosis pathway.

3.4. LOX-1 is palmitoylated, affecting LOX-1 localization

Increasing evidence has shown that post-translational palmitoylation influences protein recruitment to lipid raft microdomains [16]. Therefore, we next investigated whether protein modifications regulated the localization and ligand-uptake ability of LOX-1. We treated HCAECs with 2BP, a palmitate analog that blocks palmitoylation of proteins [17], to probe the palmitoylation of LOX-1 and evaluated the effects of palmitoylation on LOX-1 localization. The expression of LOX-1 in 2BP-treated HCAECs was confirmed by immunoblotting (Fig. 3A, lanes 1 and 6). The palmitoylation of LOX-1 was detected by the acyl-biotinyl exchange method. LOX-1 signals were detected in samples treated with or without HA (Fig. 3A, lanes 2, 3, 7, and 8), indicating that there was no loss of LOX-1 during acyl-biotinyl exchange. After palmitoylation of LOX-1, samples treated with NEM followed by HA were biotinylated, bound to streptavidin-agarose, and detected by immunoblotting. LOX-1 signals were detected in HA(+) samples without 2BP treatment (Fig. 3A, lane 9). In contrast, no signal was detected in samples treated without HA (Fig. 3A, lane 10) or samples prepared from 2BP-treated cells (Fig. 3A, lanes 4 and 5). These results indicated that LOX-1 was palmitoylated.

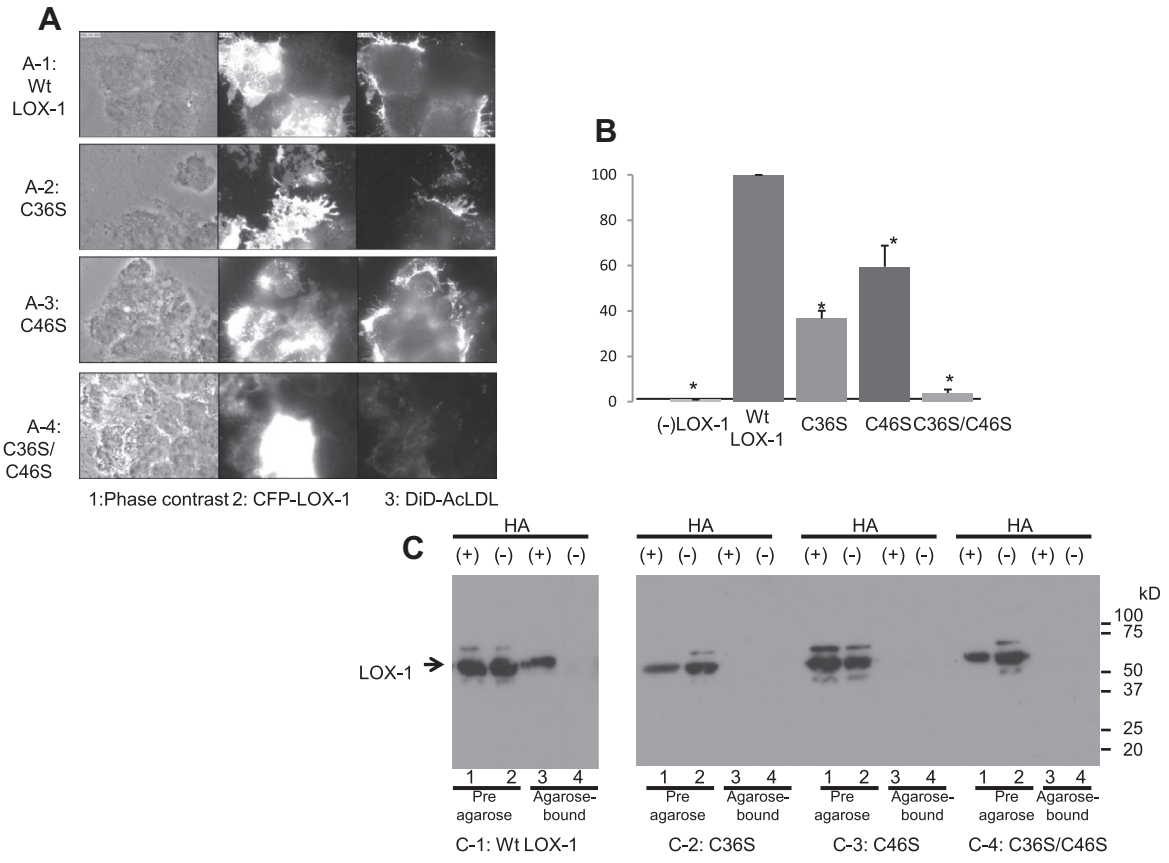

Fig. 4. Analysis of ligand uptake ability and palmitoylation of mutant LOX-1. (A) HEK293 transiently expressing Wt or mutants LOX-1 were incubated with DiD–AcLDL for 15 min at 37 °C and then fixed with 2% formaldehyde. (A-1) Wt, (A-2) C36S mutant, (A-3) C46S mutant, and (A-4) double-mutant C36S/C46S LOX-1. Left panel: phase contrast, central panel: CFP-LOX-1, right panel: DiD–AcLDL. (B) Images of more than 50 cells were acquired from 3 independent experiments. The fluorescence intensity was quantitatively evaluated using region-measurement analysis (MetaMorph). The expression level of LOX-1 was calculated from the CFP intensity, and the level of AcLDL uptake was calculated from the DiD intensiy. The level of AcLDL uptake was averaged for the expression level of LOX-1. Results are expressed as the mean ± SD. Percentages of internalized DiD–AcLDL compared with that observed using Wt LOX-1. $^{*}P < 0.05$ compared with Wt LOX-1. (C) Cell lysates prepared from CHO cell lines stably expressing Wt or mutants LOX-1 were treated according to the acyl-biotinylated exchange method. (C-1) Cell lysate from Wt LOX-1, (C-2) C36S mutant, (C-3) C46S mutant, (C-4) C36S/C46S double mutant. (−) HA: treated without HA, (+): treated with HA. Lanes 1 and 2: cell lysate before treatment with streptavidin-agarose; lanes 3 and 4: streptavidin-agarose-bound samples.

Next, we evaluated the effects of LOX-1 on DiD–AcLDL uptake. As shown in Fig. 3B, 2BP treatment significantly decreased DiD–AcLDL uptake in HCAECs (by 17.8 ± 5.4%). In addition, treatment with 2BP altered the localization of LOX-1 (Fig. 3C). Indeed, 74 ± 9% of LOX-1 was colocalized with Cav1 (Fig. 3C, right panel), whereas after 2BP treatment, only 8.9% of LOX-1 was colocalized with Cav1, a significant decrease (Fig. 3C, left panel).

3.5. Palmitoylation of C36 and C46 is necessary for proper plasma membrane/raft localization

Palmitoylation occurs at cysteine residues, and there are 2 cysteine residues in the potential cytoplasmic region and transmembrane domain (TMD) of LOX-1. One is C36, located close to the TMD, and the other is C46, located within the TMD (Supplementary Fig. S1). To investigate the contribution of these cysteine residues, the HEK293 cells were transiently transfected with CFP-tagged wild type (Wt) or LOX-1 mutants carrying cysteine residues substituted with serine (C36S, C46S, and double-mutant C36S/C46S). We first evaluated the ligand uptake ability of each mutant LOX-1. Since no CFP signals or DiD–AcLDL uptake were detected in HEK293 cells, we concluded that the CFP signal and the DiD–AcLDL uptake from stable cell lines were dependent on LOX-1. The substitution of serine for C36 decreased the uptake of DiD–AcLDL (Fig. 4A, right panel). Moreover, the substitution of both cysteine residues caused a dramatic decrease in DiD–AcLDL uptake. When ligand uptake ability was calculated from fluorescence intensity from the internalized DiD–AcLDL (Fig. 4B), a significant decrease was detected for C36S (decreased to 30% that of Wt). C46S also caused an over 40% decrease, while the double mutant resulted in only 5% uptake compared to Wt. These results indicated that both C36 and C46 contribute the ligand uptake ability of LOX-1.

We established CHO cell lines which stably expressed C36S, C46S, and C36S/C46S, but the expression levels of the mutants were low (Supplementary Fig.S2, B, central panel), and therefore, little uptake of DiD–AcLDL was detected (Supplementary Fig.S2, B, right panel). Next, we examined whether C36S/C46S were localized in DRMs. Low expression of mutant LOX-1 was observed by immunoblotting, as compared to Wt LOX-1 (Supplementary Fig.S2, C). Wt LOX-1 was isolated in DRMs, similar to the result in HCAECs. In contrast, the localization of double-mutant C36S/C46S LOX-1 to DRMs was not detected.

3.6. C36 and C46 contribute to the palmitoylation status of LOX-1

Finally, we examined the palmitoylation state of each mutant. We prepared lysates from six 10-cm dishes for each mutant because of the low expression of LOX-1 protein. Signals from Wt and mutant LOX-1 were detected in samples before adding streptavidin-agarose (Fig. 4C, lanes 1 and 2). After streptavidin-agarose treatment, signals were detected only in sample prepared from Wt LOX-1 treated with HA (Fig. 4C-1, lane 3). This result suggested

that overexpressed LOX-1 was palmitoylated. In contrast, no signal was detected from streptavidin-agarose-bound samples of C36S (Fig. 4C-2, lane 3), C46S (Fig. 4C-3, lane 3), or C36S/C46S mutants (Fig. 4C-4, lane 3). Therefore, we concluded that both C36 and C46 contributed to the palmitoylation status of LOX-1.

4. Discussion

In this study, we demonstrated that LOX-1 was localized to DRMs with Cav1 and internalized AcLDL via the same pathway as CT-B in HCAECs. Although CT-B is known to be internalized by other microdomains as well as caveolae, internalization of AcLDL was inhibited by both nystatin and genistein, which inhibit caveolae-dependent endocytosis [14,15]. Thus, our data demonstrated that AcLDL was internalized via caveolae/raft microdomains. A previous study demonstrated that OxLDL, recognized by LOX-1, is internalized by dynamin-dependent, clathrin-independent pathways [18]. The caveolae endocytosis pathway is dynamin dependent [19], and nystatin selectively disrupts caveolae/lipid raft endocytosis, but has no effect on clathrin-dependent endocytosis [14]. Moreover, a very recent study reported that LOX-1 is predominantly localized in caveolae/lipid rafts in HEK293 and COS7 cells, and statin, a potent cholesterol lowering drug, promotes the spational disorganization of LOX-1 in the plasma membrane and a loss of OxLDL internalization [20]. Therefore, the results presented here are consistent with those of previous studies.

N-linked glycosylation of LOX-1 is known to play a key role in the cell surface expression of LOX-1 [21]. Moreover, palmitoylation, another type of post-translational modification, plays a significant role in the trafficking and function of many receptors. Among scavenger receptors, palmitoylation of CD36 has been reported [22]. Although palmitoylation of CD36 is not required for maturation and surface expression, the palmitoylation mutant could not locate in lipid rafts and reduced the efficiency of ligand uptake. Here, we demonstrated that palmitoylation was also necessary for the correct localization of LOX-1 into lipid rafts, in turn mediating its ligand uptake ability.

Finally, we found that both C36 and C46 contributed to the correct localization of LOX-1. There are 9 cysteine residues in human LOX-1; 6 are within the CTLD, a highly conserved ligand recognition domain in the extracellular region. Although C140 at the neck domain is specific to human LOX-1, it has an important role in the formation of interchain disulfide bonds. Multiple sequence comparison of the cytoplasmic domain and the TMD showed that C46 within the TMD is conserved in LOX-1 from 6 different species, and C36 close to the TMD is conserved in humans, rabbits, and rats. When we evaluate the ligand uptake ability of mutants using transiently expressing mutants LOX-1, C36S had decreased ligand uptake activity and C36S/C46S double mutation led to complete inhibition of ligand uptake. Thus, we expect that the enzyme that catalyzes the palmitic acid attachment cannot access C46; therefore, we hypothesize that C36 is the palmitoylated site and that the conserved C46 is important for proper folding and palmitoylation of C36. Moreover, the expression of the Cys mutants is much lower than that of the wild LOX-1. This result indicates that the lack of palmitoylation causes a degradation of LOX-1.

In summary, we propose that LOX-1 is located in raft microdomains, internalized OxLDL via caveolae/raft-dependent endocytosis, and delivers OxLDL to the endosome. Palmitoylation plays an important role in the correct localization of LOX-1.

Acknowledgments

We thank Setsuko Niimi for technical assistance. This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science to M.S.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.03.120.

References

[1] U.P. Steinbrecher, Receptors for oxidized low density lipoprotein, Biochim. Biophys. Acta 1436 (1999) 279–298.
[2] T. Sawamura, N. Kume, T. Aoyama, H. Moriwaki, H. Hoshikawa, Y. Aiba, T. Tanaka, S. Miwa, Y. Katsura, T. Kita, T. Masaki, An endothelial receptor for oxidized low-density lipoprotein, Nature 386 (1997) 73–77.
[3] M. Chen, T. Masaki, T. Sawamura, LOX-1, the receptor for oxidized low-density lipoprotein identified from endothelial cells: implications in endothelial dysfunction and atherosclerosis, Pharmacol. Ther. 95 (2002) 89–100.
[4] Q. Xie, S. Matsunaga, S. Niimi, S. Ogawa, K. Tokuyasu, Y. Sakakibara, S. Machida, Human lectin-like oxidized low-density lipoprotein receptor-1 functions as a dimer in living cells, DNA Cell Biol. 23 (2004) 111–117.
[5] I. Ohki, T. Ishigaki, T. Oyama, S. Matsunaga, Q. Xie, M. Ohnishi-Kameyama, T. Murata, D. Tsuchiya, S. Machida, K. Morikawa, S. Tate, Crystal structure of human lectin-like, oxidized low-density lipoprotein receptor 1 ligand binding domain and its ligand recognition mode to OxLDL, Structure 13 (2005) 905–917.
[6] S. Matsunaga, Q. Xie, M. Kumano, S. Niimi, K. Sekizawa, Y. Sakakibara, S. Komba, S. Machida, Lectin-like oxidized low-density lipoprotein receptor (LOX-1) functions as an oligomer and oligomerization is dependent on receptor density, Exp. Cell Res. 313 (2007) 1203–1214.
[7] K. Simons, E. Ikonen, Functional rafts in cell membranes, Nature 387 (1997) 569–572.
[8] K. Simons, M.J. Gerl, Revitalizing membrane rafts: new tools and insights, Nat. Rev. Mol. Cell Biol. 11 (2010) 688–699.
[9] A.O. Hueber, A.M. Bernard, Z. Herincs, A. Couzinet, H.T. He, An essential role for membrane rafts in the initiation of Fas/CD95-triggered cell death in mouse thymocytes, EMBO Rep. 3 (2002) 190–196.
[10] M. Kumano-Kuramochi, Y. Shimozu, C. Wakita, M. Ohnishi-Kameyama, T. Shibata, S. Matsunaga, Y. Takano-Ishikawa, J. Watanabe, M. Goto, Q. Xie, S. Komba, K. Uchida, S. Machida, Identification of 4-hydroxy-2-nonenal-histidine adducts that serve as ligands for human lectin-like oxidized LDL receptor-1, Biochem. J. 442 (2012) 171–180.
[11] J. Wan, A.F. Roth, A.O. Bailey, N.G. Davis, Palmitoylated proteins: purification and identification, Nat. Protoc. 2 (2007) 1573–1584.
[12] M. Chen, K. Inoue, S. Narumiya, T. Masaki, T. Sawamura, Requirements of basic amino acid residues within the lectin-like domain of LOX-1 for the binding of oxidized low-density lipoprotein, FEBS Lett. 499 (2001) 215–219.
[13] M. Kirkham, R.G. Parton, Clathrin-independent endocytosis: new insights into caveolae and non-caveolar lipid raft carriers, Biochim. Biophys. Acta 1745 (2005) 273–286.
[14] J.T. Korhonen, M. Puolakkainen, A. Haveri, A. Tammiruusu, M. Sarvas, R. Lahesmaa, Chlamydia pneumoniae entry into epithelial cells by clathrin-independent endocytosis, Microb. Pathog. 52 (2012) 157–164.
[15] B.S. Davies, C.N. Goulbourne, R.H. Barnes 2nd, K.A. Turlo, P. Gin, S. Vaughan, D.J. Vaux, A. Bensadoun, A.P. Beigneux, L.G. Fong, S.G. Young, Assessing mechanisms of GPIHBP1 and lipoprotein lipase movement across endothelial cells, J. Lipid. Res. 53 (2012) 2690–2697.
[16] I. Levental, M. Grzybek, K. Simons, Greasing their way: lipid modifications determine protein association with membrane rafts, Biochem. 49 (2010) 6305–6316.
[17] I. Delint-Ramirez, D. Willoughby, G.V. Hammond, L.J. Ayling, D.M. Cooper, Palmitoylation targets AKAP79 protein to lipid rafts and promotes its regulation of calcium-sensitive adenylyl cyclase type 8, J. Biol. Chem. 286 (2011) 32962–32975.
[18] J.E. Murphy, R.S. Vohra, S. Dunn, Z.G. Holloway, A.P. Monaco, S. Homer-Vanniasinkam, J.H. Walker, S. Ponnambalam, Oxidised LDL internalisation by the LOX-1 scavenger receptor is dependent on a novel cytoplasmic motif and is regulated by dynamin-2, J. Cell Sci. 121 (2008) 2136–2147.
[19] Q. Yao, J. Chen, H. Cao, J.D. Orth, J.M. McCaffery, R.V. Stan, M.A. McNiven, Caveolin-1 interacts directly with dynamin-2, J. Mol. Biol. 348 (2005) 491–501.
[20] S. Matarazzo, M.C. Quitadamo, R. Mango, S. Ciccone, G. Novelli, S. Biocca, Cholesterol-lowering drugs inhibit lectin-like oxidized low-density lipoprotein-1 receptor function by membrane raft disruption, Mol. Pharmacol. 82 (2012) 246–254.
[21] X. Shi, S. Niimi, T. Ohtani, S. Machida, Characterization of residues and sequences of the carbohydrate recognition domain required for cell surface localization and ligand binding of human lectin-like oxidized LDL receptor, J. Cell Sci. 114 (2001) 1273–1282.
[22] R.F. Thorne, K.J. Ralston, C.E. de Bock, N.M. Mhaidat, X.D. Zhang, A.W. Boyd, G.F. Burns, Palmitoylation of CD36/FAT regulates the rate of its post-transcriptional processing in the endoplasmic reticulum, Biochim. Biophys. Acta 2010 (1803) 1298–1307.