LDL-mediated interaction of Lp[a] with HepG2 cells: a novel fluorescence microscopy approach

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Abstract We studied the topography of Lp[a]–LDL–cell interactions by means of fluorescence microscopy, using fluorescence-labeled lipoproteins. In contrast to known methods which are based on noncovalent labeling of lipoproteins by positively charged amphiphiles, the protein moiety of LDL and Lp[a] was covalently labeled with either BODIPY-succinimide-ester (green) or rhodamine X iodoacetamide (red). The interaction of the fluorescent lipoproteins with cultured HepG2 cells was studied using a confocal laser scanning fluorescence microscope. LDL and Lp[a], each labeled with a different dye, could be examined separately within a mixture of both lipoproteins during their interaction with HepG2 cells. At 4°C, the majority of both fluorescent particles co-localized and only a few separate LDL- or Lp[a]-binding domains could be observed. Quantification of the amount of fluorescent lipoprotein associated with the cell surface at 4°C showed that binding of Lp[a] was increased in the presence of LDL under these conditions, probably via formation of an Lp[a]–LDL complex. At 37°C, LDL and Lp[a] were taken up by the cells within 10 min. Again the majority of LDL and Lp[a] particles co-localized intracellularly. Only minor amounts of LDL and Lp[a] could be observed separately. As the entire fluorescence of labeled Lp[a] co-localized with excess of LDL in cells, and taking into account the high tendency of LDL-Lp[a] association in solution and on cell surfaces, it is concluded that a significant portion of the internalized Lp[a] is taken up into the cells by the LDL receptor via LDL by a hitchhiking-like process.—**Hofer, G., E. Steyrer, G.M. Kostner, and A. Hermetter.** LDL-mediated interaction of Lp[a] with HepG2 cells: a novel fluorescence microscopy approach. *J. Lipid Res.* 1997. **38:** 2411–2421.

Supplementary key words atherosclerosis • endocytosis • lipoprotein metabolism • hepatic lipoprotein uptake

Lipoprotein[a] (Lp[a]) is an independent risk factor for arteriosclerosis (1–5). Lp[a] consists of a low density lipoprotein (LDL)-like particle and a highly glycosylated protein apolipoprotein [a], which is linked to apoB-100 via one disulfide bridge (6, 7) and varies in size, depending on the donor (8). Its interaction with cultured cells is not completely understood. The investigations reported so far show partially divergent results (9–19) with respect to receptors that are involved in the removal of Lp[a] from blood and for the interactions with cells in vitro. Both Lp[a] binding to the cell surface and uptake by the cell may include LDL receptor-dependent and LDL receptor-independent mechanisms (20). In addition, these processes are probably interrelated with Lp[a]-LDL interaction (21), because it is known that Lp[a] binds to LDL (22).

We chose fluorescence microscopy to study in a more direct way whether Lp[a]–cell interaction is mediated by LDL. Each type of lipoprotein was covalently labeled with its own respective dye, so that interactions between fluorescent Lp[a] and LDL could be observed when a mixture of both was incubated with cultured cells. We found that binding of Lp[a] by HepG2 cells correlates to a major extent with the amount of LDL present in the same incubation mixture. More LDL was bound and taken up by HepG2 cells than Lp[a], and Lp[a] and LDL binding to the cells were synergistic.

MATERIALS AND METHODS

Materials

Rhodamine-X-iodoacetamide dipotassium salt (RHO) and 4,4-difluoro-5,7-dimethyl-4-bora-3α,4α-diaza-3-indacenepropionic acid succinimidyl ester (BDP) were purchased from Molecular Probes, Eugene, OR. All

Abbreviations: BDP, 4,4-difluoro-5,7-dimethyl-4-bora-3α,4α-diaza-3-indacene-propionic acid succinimidyl ester; LDL, low density lipoprotein; Lp[a], lipoprotein[a]; LPDS, lipoprotein-deficient serum; RHO, rhodamine-X-iodoacetamide dipotassium salt.

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other chemicals were obtained from Merck, Darmstadt, Germany, except where otherwise indicated.

Isolation of lipoproteins

Low density lipoprotein (LDL) and lipoprotein [a] (Lp[a]), isoform S1, were isolated from plasma of fasting normolipemic volunteers selected according to their plasma Lp[a] concentrations. Lp[a] was purified essentially as described earlier (19). Immediately after blood drawing and centrifugation, the plasma was stabilized with 3 mmol/L EDTA and sodium azide (1 mg/ml) and subjected to density gradient ultracentrifugation in an SW-40 rotor (Beckman) for 24 h at 40,000 rpm (23). The fraction at d 1.070–1.125 g/ml was passed through an immunoaffinity column containing polyclonal rabbit anti[a] IgG. After washing the fraction, adsorbed Lp[a] was eluted with glycine-hydrochloride buffer, pH 2.5, yielding preparations of more than 98% purity. In parallel experiments, Lp[a] was prepared by lysine-Sepharose chromatography (24). LDL was purified from a fraction corresponding to d 1.025–1.055 g/ml of the density gradient and centrifuged under identical conditions.

All buffers and solutions used for lipoprotein preparation contained EDTA (3 mmol/L) and sodium azide (1 mg/ml) and were kept under nitrogen atmosphere. All purification steps were performed at 4°C, and preparations were used within 1 week. The purity of Lp[a] and LDL fractions was assayed by double-decker rocket immunoelectrophoresis and SDS polyacrylamide gel electrophoresis as described elsewhere (9, 25, 26).

Protein concentration was measured according to Lowry et al. (27) in the presence of 0.5% (w/w) sodium dodecyl sulfate.

Preparation of labeled lipoproteins

Lipoprotein (700 μg protein) was dissolved in 500 μl phosphate-buffered saline (PBS). A chloroform solution containing the reactive dye (Rhodamine-X-iodoacetamide or BODIPY-succinimidyl ester) was dried under argon. The dye was dissolved in 10 μl DMSO and added to the lipoprotein suspension (the molar ratio of dye to lipoprotein was 5/1). Incubations were carried out at room temperature for 3 h under argon, followed by removal of excess label by gel chromatography on a Sephadex G-25 column. Protein recovery was higher than 90%.

The fluorophore-to-protein ratio was determined from the emission intensity of RHO at 590 nm (excitation 564 nm), the absorbance of BDP at 510 nm, and by protein analysis according to Lowry et al. (27). For the analysis of RHO emission, a standard curve with known RHO concentrations in the same buffer was recorded.

Agarose gel electrophoresis

LE-Agarose (FMC BioProducts, Rockland, ME) was used to prepare 0.5% (w/v) agarose gels. Electrophoresis was carried out at 80 V for 45 min at 4°C. The fluorescence-labeled lipoproteins (BDP, green and RHO, red) were visualized on the gels under a strong UV-light screen and photographed. Thereafter, the particles were stained for proteins with Coomassie brilliant blue (Sigma, St. Louis, MO).

Polyacrylamide gel electrophoresis

Electrophoresis in 4% polyacrylamide gels was performed according to Laemmli (28). Samples were incubated in electrophoresis running buffer without mercaptoethanol for 5 min at 95°C. Electrophoresis was carried out at 180 V. After separation, proteins were visualized with Coomassie brilliant blue.

Ligand blotting

Solubilization of membrane proteins with Triton X-100 from bovine adrenal cortex, SDS-electrophoresis, and transfer of proteins onto nitrocellulose by electroblotting have previously been described (19). Nitrocellulose strips (5-mm wide) containing identical amounts of membrane proteins were incubated with lipoproteins, specified in caption of Fig. 3, in a pH 7.4 buffer (referred to as ligand blotting buffer, LBB) containing 5% skim milk, 20 mmol/L Tris-HC1, 90 mmol/L NaCl, 2 mmol/L $CaCl_2$ for 2 h at 20°C. Thereafter, the nitrocellulose strips were washed twice in LBB for 10 min, followed by incubation with either polyclonal rabbit anti B- or anti A-I-IgG (10 μg/ml, in LBB). LDL-receptor was detected by sequential incubation with a commercially available mouse monoclonal anti-LDL-receptor (Ab-1) IgG2a-fraction (Oncogene Science Inc., Cambridge, MA) (dilution 1:1.000; in LBB), and rabbit anti-mouse IgG for 2 h each. Visualization of bound lipoproteins and identification of LDL-receptor were performed by incubation with horseradish peroxidase (HRP)-labeled protein-A (Amersham, Buckinghamshire, England) for 2 h at 20°C followed by ECL-light detection and autoradiography.

Fluorescence spectroscopy

Lipoprotein binding to or uptake by HepG2 cells was determined from label fluorescence intensity. HepG2 cells were grown in 6-well plates. After 3 days, the medium containing 10% fetal calf serum (FCS) was replaced by a medium containing lipoprotein-deficient serum (LPDS). On day 5, the cells were incubated with the labeled lipoproteins (30 μg protein/ml) in LPDS-medium for 2 h at 4°C or 10 min at 37°C. Then the cells were washed 3 times with PBS and solubilized in 1 ml of

a 1% (w/v) Triton X-100 solution in PBS buffer. An aliquot was used for protein determination; the rest was used for the spectroscopic analysis (29). Rhodamine fluorescence was measured at 590 nm and BODIPY fluorescence was determined at 515 nm, using excitation wavelengths of 568 nm and 500 nm, respectively. For the quantification of labeled lipoproteins associated with cells, fluorescence intensities were compared to standard curves of RHO or BDP in 1% Triton X-100. All fluorescence intensity measurements were carried out on a Shimadzu RF-540 spectrofluorometer.

Fluorescence microscopy

Lipoprotein–cell interactions were studied with a confocal laser scanning microscope from Bio-Rad equipped with a Krypton/Argon laser (emission lines at 488 nm, 568 nm and 647 nm) equipped with single and dual channel filter sets. Ektachrome 100 color slide films were used for documentation of the resulting images. Cells, grown on round cover slips, were incubated with the labeled lipoproteins (30 μg protein/ml) at 4°C for 2 h or at 37°C for 10 min, followed by extensive washing with PBS buffer. The cells were examined under the microscope either in a perfusion cell chamber or were fixed with 3.5% formaldehyde before microscopic examination. Living cells were kept in a cell chamber under externally thermostated Hanks buffer. Formaldehyde-fixed cells were mounted on a slide with Mowiol 4-88 from Hoechst (Frankfurt am Main, Germany) as described by Harlow and Lane (30). As output from the microscope we obtained two images, one from each emission color channel. These images represent the interaction of one lipoprotein, labeled with the respective dye, with the cells (see Figs. 4A and 5A). For experiments with two differently labeled lipoproteins, we had to superimpose these images to visualize the real behavior of the lipoproteins (see Figs. 4B and 5B).

Cell culture

HepG2 cells were grown in Dulbecco's minimal essential medium (DMEM) (Sigma, St. Louis, MO) supplemented with 10% FCS (Sebak, Suben, Austria) as previously reported. For quantification studies, the cells were seeded in 6-well plates and grown to approximately 80% confluency. For microscopy, the cells were grown on ethanol-washed, heat-sterilized round cover slips (thickness 0.155–0.185 mm, Chance Propper, Smethwick, England) to approximately 30% confluency. The cells were routinely checked for viability using the Trypan blue (0.5 g in 100 ml water; Merck Diagnostica, Germany) exclusion test. The absence of mycoplasma infections was assessed using DAPI (4′,6-diamidine-2-phenylindole-dihydrochloride, Boehringer Mannheim, Germany).

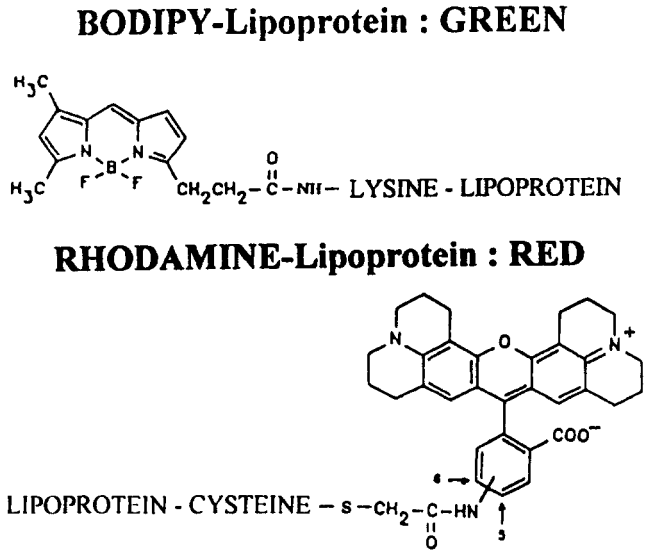


Fig. 1. Schematic diagram of lipoprotein fluorescence labeled with BODIPY-hydroxysuccinimide ester and Rhodamine X-iodoacetamide.

RESULTS

Fluorescence-labeled lipoproteins

We used Rhodamine-X-iodoacetamide (RHO)- and BODIPY-succinimidyl ester (BDP)-labeled lipoproteins (**Fig. 1**) to study their interaction with cultured cells. RHO, a red dye (emission maximum: 590 nm), preferably reacts with free sulfhydryl groups on the protein (31). Under appropriate labeling conditions we found molar RHO-to-protein ratios of 1 for Lp[a] and 2 for LDL. BDP, a green dye (emission maximum: 513 nm), preferably reacts with lysine amino groups of proteins (32). As free amino groups of apoB-100 are involved in the binding to the LDL receptor (33), protein labeling had to be kept very low. Typical molar label-to-protein ratios of 3 to 5 were obtained for both lipoproteins under reaction conditions where undesired side reactions (hydrolysis of reactive labels) were slow enough. The respective labeling rate corresponds to approximately 2% of the amino groups available on apoB-100 (33, 34).

In order to demonstrate that the labeling procedure had not altered lipoprotein integrity, we analyzed the fluorescent particles by agarose gel electrophoresis. The fluorescence-labeled lipoproteins showed only slightly higher electrophoretic mobilities as compared to natural lipoproteins (**Fig. 2**). Ligand blotting experiments confirmed that the labeled particles were still recognized by the LDL receptor from bovine adrenal cortex (**Fig. 3**), indicating that the labeling procedure did not interfere with their functional integrity.

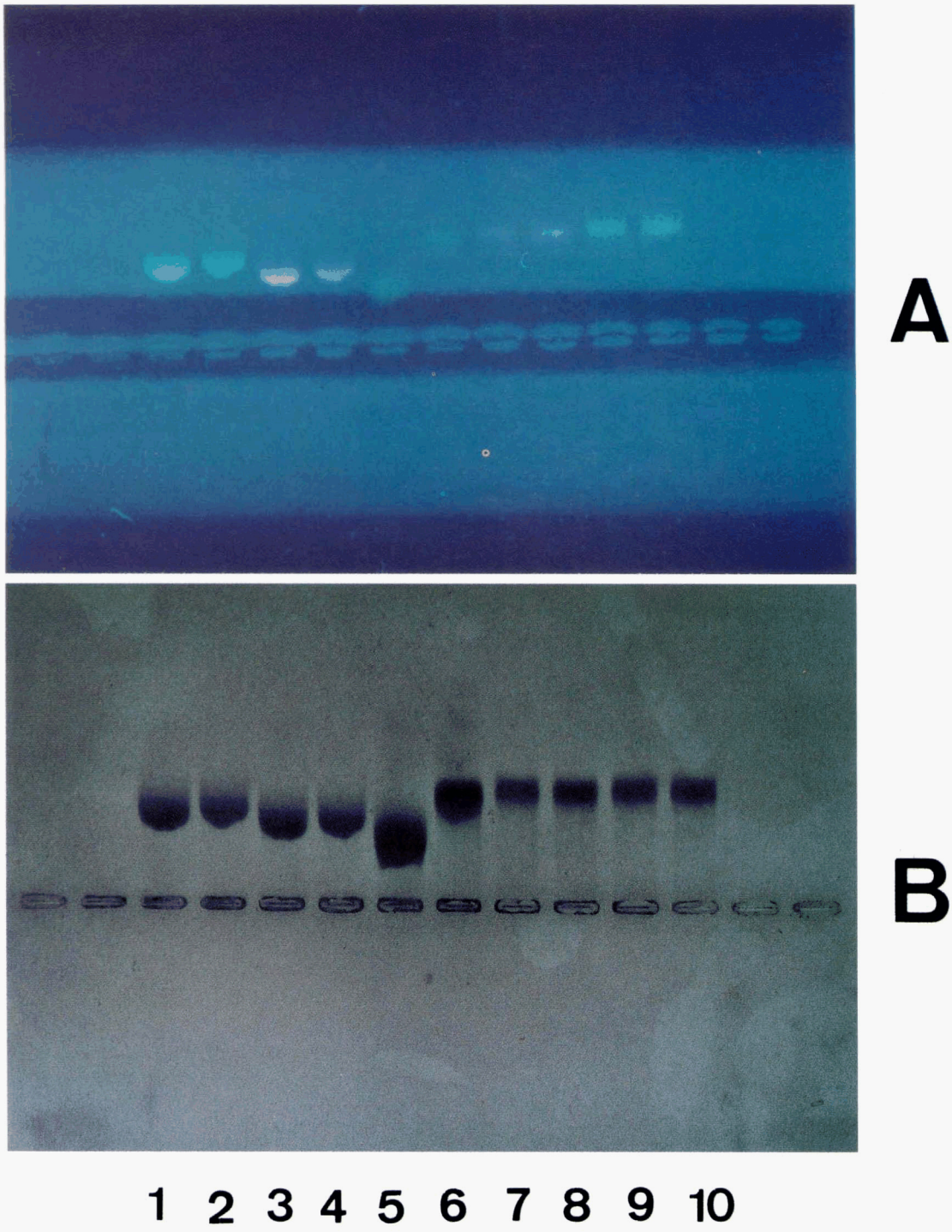


Fig. 2. Agarose gel electrophoresis of labeled and unlabeled lipoproteins. Panel A shows a fluorescence image and panel B shows the gel after protein staining with Coomassie blue. From the left: lanes 1 and 2, BDP-labeled LDL; lanes 3 and 4, RHO-labeled LDL; lane 5, reference-LDL; lane 6, reference-Lp[a]; lanes 7 and 8, RHO-labeled Lp[a]; lanes 9 and 10, BDP-labeled Lp[a].

Interaction of fluorescent lipoprotein with HepG2 cells

Incubation of labeled lipoproteins with HepG2 cells at 4°C for 2 h revealed that both LDL and Lp[a] bound to the cell surface irrespective of the dye used. LDL fluorescence appeared to be more clustered than Lp[a] fluorescence (data not shown) when the lipoproteins were used in separate experiments. In a mixture of

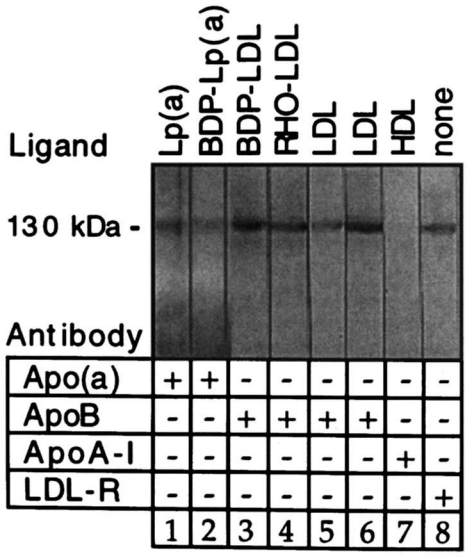


Fig. 3. Ligand blotting of LDL-receptor from bovine adrenal cortex. Nitrocellulose strips containing identical amounts of solubilized membrane proteins from bovine adrenal cortex were incubated with fluorescence-labeled or unlabeled ligands as indicated in Methods. Lane 1, unlabeled Lp[a] (25 μg/ml); lane 2, BDP-Lp[a] (25 μg/ml); lane 3, BDP-LDL (25 μg/ml); lane 4, RHO-LDL (25 μg/ml); lane 5, unlabeled LDL (18 μg/ml); lane 6, unlabeled LDL (25 μg/ml); lane 7, HDL (25 μg/ml); lane 8, monoclonal anti LDL-receptor (Ab-1). Conditions for incubation with lipoproteins and polyclonal antibodies are described in Methods. LDL-receptor was detected by incubation with a monoclonal antibody (Ab-1) and goat anti-rabbit IgG as a second antibody. Bound ligands and LDL-receptor, respectively, were visualized by incubation with HRP-labeled protein A and ECL-light detection. Autoradiography was for 2 min.

identical amounts (30 μg protein/ml) of both lipoproteins Lp[a] and LDL bound simultaneously to the cell surface (**Fig. 4A**). When both images were superimposed, an image was obtained showing that the major fractions of LDL and Lp[a] were co-localized (Fig. 4B) as demonstrated by the appearance of yellow spots. A false color image (Fig. 4C) revealed that more LDL bound to the cells as compared to Lp[a].

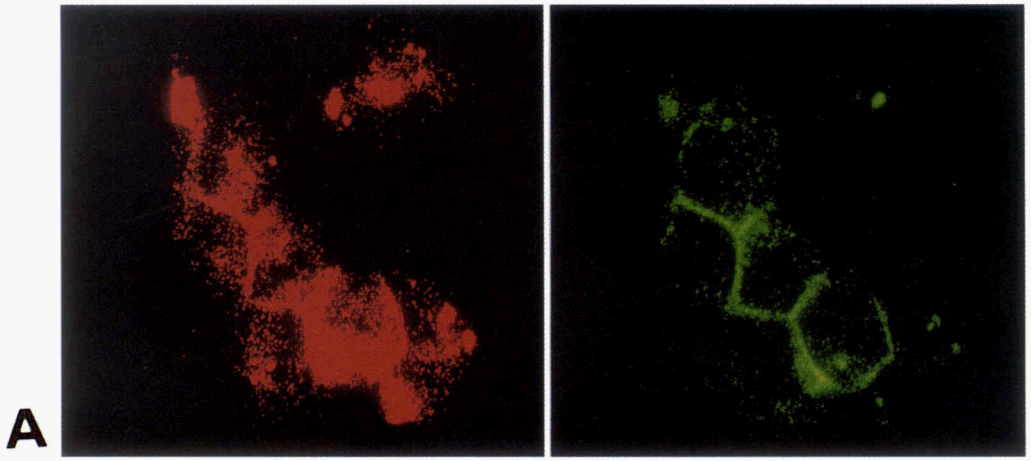
As expected, after incubation of a mixture of the labeled lipoproteins with HepG2 cells at 37°C for 10 min, LDL and Lp[a] were taken up by the cells (**Fig. 5**). Again the major fraction of LDL and Lp[a] co-localized. In addition, the same image shows Lp[a] (green) and LDL (red) in separate pools that are, however, much more finely dispersed throughout the cells as compared to the much larger pools (yellow) containing both lipoproteins (Figs. 5A and 5B). According to a false color image (Fig. 5C), much more LDL than Lp[a] was internalized by HepG2 cells. The same results were obtained with mixtures of RHO-LDL and BDP-Lp[a] and vice versa with BDP-LDL and RHO-Lp[a].

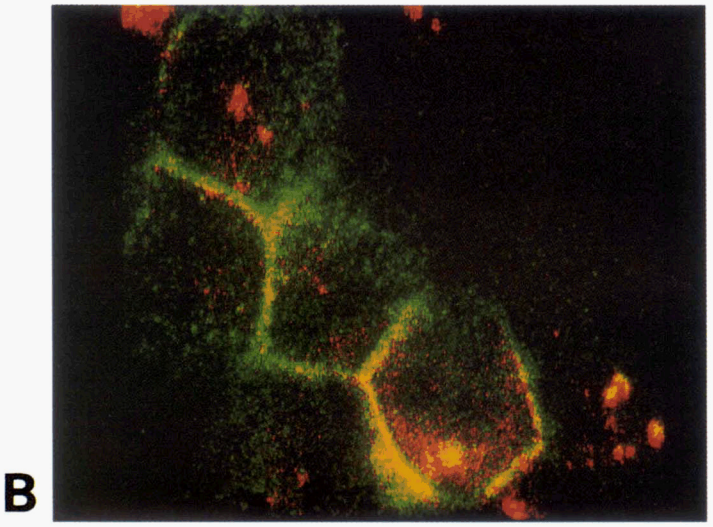
LDL and Lp[a] binding to and internalization by cells were expressed in terms of label fluorescence intensity. At 4°C, slightly higher amounts of Lp[a] than LDL, expressed in ng/mg cell protein, bound to the cells at concentrations of 30 μg total apolipoprotein/ml (**Fig. 6**), in agreement with data obtained from radioactive experiments (35). In this context it is important to note that under our experimental conditions (30 μg apoprotein/ml), the cell surface is already saturated with LDL, whereas Lp[a] binding is still non-saturable. In this respect titration of the cell surface with increasing amounts of fluorescent lipoproteins at 4°C gave the same results as compared to radiolabeled lipoprotein (data not shown). In direct competition experiments, an excess of labeled lipoprotein prevented the fluorescent lipoprotein from binding to the cells at 4°C (Fig. 6A) (35).

In mixtures containing equal amounts of labeled and unlabeled particles (30 μg total apolipoprotein/ml, respectively), binding of fluorescent Lp[a] at 4°C is considerably enhanced in the presence of LDL (Fig. 6A), probably as a consequence of Lp[a]–LDL–complex formation on the cell surface. In contrast, Lp[a] did not significantly affect LDL binding to a noticeable degree (Fig. 6A). Enhancement of Lp[a] binding by LDL is substantially reduced in the presence of proline or tranexamic acid. These compounds are known to dissociate Lp[a]-LDL complexes (in solution). LDL binding is not affected by the presence of Lp[a] and is only slightly influenced in the presence of both amino acids. (Fig. 6A). Internalization of fluorescent lipoproteins at 37°C was affected differently in the presence of unlabeled particles (Fig. 6B). Unlabeled LDL interfered more efficiently than unlabeled Lp[a] with the uptake of fluorescent lipoproteins. Uptake of labeled LDL was reduced by equal amounts of unlabeled Lp[a] in the incubation mixture by 15%. Unexpectedly, unlabeled LDL decreased the uptake of labeled Lp[a] into the cells by 30%.

DISCUSSION

The process of Lp[a] removal from plasma is poorly understood. Previous reports indicate that Lp[a] can bind to the LDL receptor (19), to glycosaminoglycans (36), to the LDL receptor-related protein (LRP) (37), and to a few other proteins (38, 39). Furthermore, binding of LDL to Lp[a] was observed (22) and Lp[a]-mediated binding of LDL to receptor-negative fibroblasts, probably via cell surface glycosaminoglycans (21), has been reported. Taken together, these observations indicate that LDL can influence the uptake of





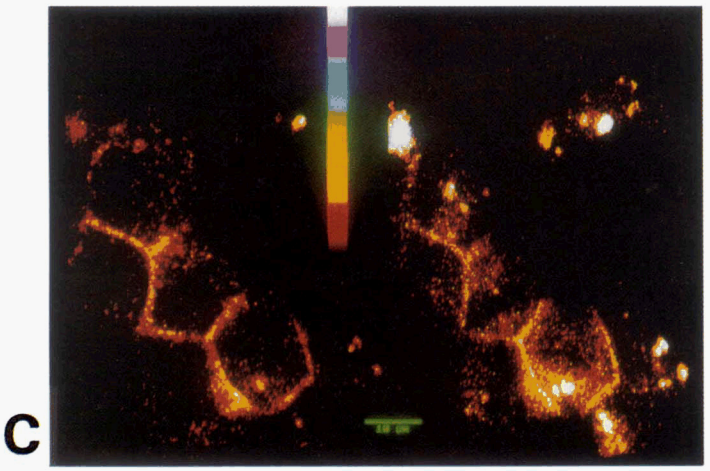


Fig. 4. Confocal micrographs of fluorescent lipoproteins. thirty μg protein/ml of each lipoprotein (Lp[a]; green, LDL; red) were incubated with HepG2 cells at 4°C for 2 h. A: Shows separated images of LDL and Lp[a]; B: shows the merged image that reveals the co-localization of both lipoproteins; C: is a false color image reflecting the relative quantities of each lipoprotein (LDL: right, and Lp[a]: left).

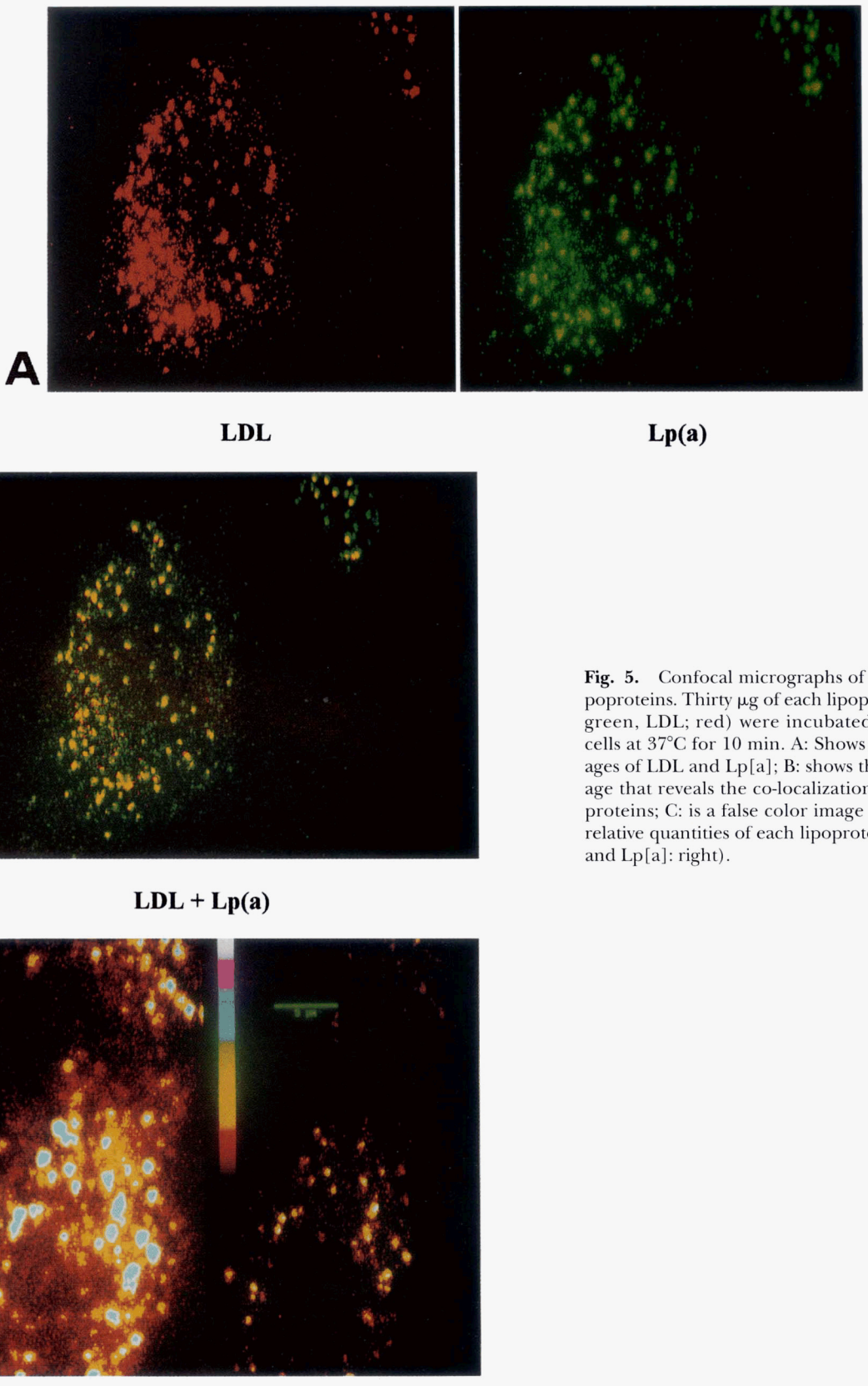


Fig. 5. Confocal micrographs of fluorescent lipoproteins. Thirty μg of each lipoprotein (Lp[a]; green, LDL; red) were incubated with HepG2 cells at 37°C for 10 min. A: Shows separated images of LDL and Lp[a]; B: shows the merged image that reveals the co-localization of both lipoproteins; C: is a false color image reflecting the relative quantities of each lipoprotein (LDL: left, and Lp[a]: right).

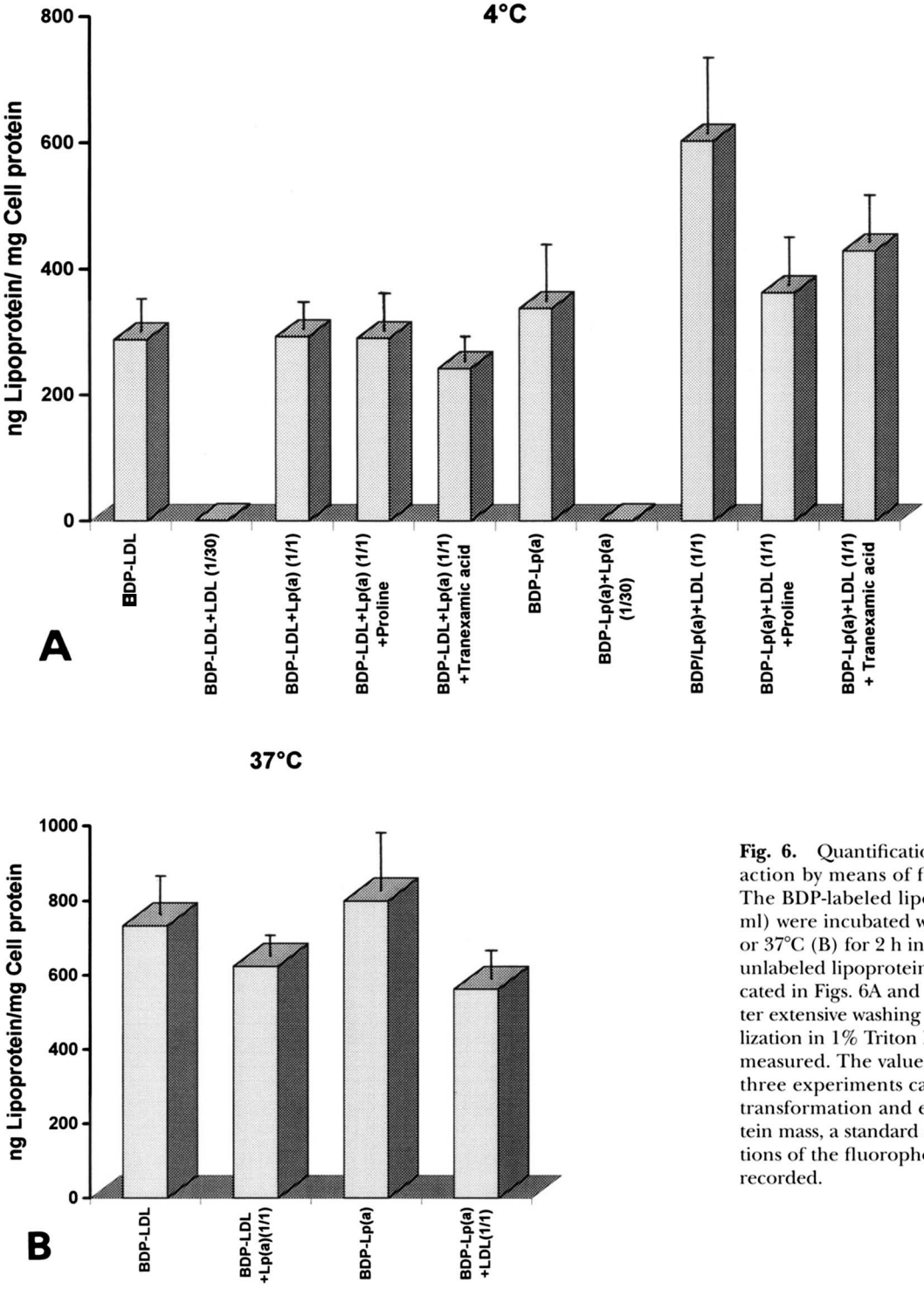


Fig. 6. Quantification of lipoprotein–cell interaction by means of fluorescence spectroscopy. The BDP-labeled lipoproteins (30 μg protein/ml) were incubated with HepG2 cells at 4°C (A) or 37°C (B) for 2 h in the presence or absence of unlabeled lipoproteins at the protein ratios indicated in Figs. 6A and 6B (30 μg protein/ml). After extensive washing with PBS buffer and solubilization in 1% Triton X-100, the fluorescence was measured. The values represent mean values of three experiments carried out in triplicate. For transformation and expression in terms of protein mass, a standard curve with given concentrations of the fluorophore in 1% Triton X-100 was recorded.

Lp[a] by cells and vice versa via specific (receptor-mediated) and nonspecific mechanisms.

This study aimed at obtaining more information on the morphological distribution of the interactions of Lp[a] with cultured cells in the presence of LDL, which reflects a physiological situation to a certain extent. For this purpose, we studied the topography of Lp[a]–LDL–cell interactions by means of fluorescence microscopy, using fluorescence-labeled lipoproteins. So far, lipophilic dyes had been used for non-covalent labeling of lipoproteins (40–45). However, this method suffers from at least two shortcomings when applied to studies

of lipoprotein metabolism by cells. First, the respective label(s) impart a positive charge on the lipoproteins. Under standard labeling conditions, up to 30 positive charges (30 labels) are incorporated in a particle (45), which might interfere with lysine-mediated interaction of the labeled lipoproteins with cell receptors. Second, non-covalently associated dyes are subject to exchanges between lipid (membrane or lipoprotein) surfaces. As a consequence, the fluorescence of the label does not necessarily reflect the localization of the lipoprotein under these circumstances. For these reasons, we decided to prepare fluorescent lipoproteins covalently labeled at their protein moiety. In this case, label fluorescence is representative for the particles until it becomes degraded (e.g., by cells). HepG2 cells seemed to represent an appropriate model system for our studies on lipoprotein–cell interactions as lipoprotein degradation is slow in these cells (46) and the binding and import of lipoproteins into the cells can be unambiguously observed within an appropriate time window.

Two different fluorochromes (RHO and BDP) were chosen for LDL and Lp[a] labeling. Therefore, both differently labeled components within mixtures containing LDL and Lp[a] could be examined separately during their interaction with HepG2 cells. Labeling of protein sulfhydryl groups by RHO-iodoacetamide does not change the net particle charge. In contrast, protein derivatization with BODIPY-succinimide ester results in a loss of 1 positive lysine charge per label, resulting in a total loss of 3 positive charges at labeling rates of 3/1 (label/protein). In addition, the labeling procedure itself may lead to some minor oxidative modification as compared to the native lipoproteins. According to our control experiments (ligand blotting, electrophoresis, competition with unlabeled lipoproteins), however, the biological properties of the labeled particles seem to be unaffected under these labeling conditions.

We examined the interaction of the fluorescence-labeled lipoproteins by laser scanning microscopy, a high resolution method that enabled us to scan through the cell and to determine whether a fluorescent (lipoprotein) spot was on the cell surface or inside the cell. Both fluorescent lipoproteins were bound to the cells at 4°C. The majority of both particles co-localized and only a few distinct LDL- or Lp[a]-binding domains could be observed. This suggests that both lipoproteins bind to the same sites on the cell surface, either in very close vicinity or as an LDL-Lp[a] complex (22). On the basis of our microscopic resolution, none of these possibilities can be excluded. However, taking into account the high tendency of LDL-Lp[a] association (22) in solution, we may assume that LDL and Lp[a] also bind to each other when associated with cell surfaces (35, 47). At 37°C, both lipoproteins were taken up. Substantially more LDL than Lp[a] was internalized when a mixture containing equal amounts of both were incubated with HepG2 cells. However, this is at variance with the observation that LDL enhances binding of Lp[a] to HepG2 cells at 4°C. Most likely a certain fraction of Lp[a] associated with LDL in cell-bound form is not available for (receptor-mediated) internalization. The major amount of Lp[a] internalized at 37°C co-localized with the excess of LDL within the cells (Fig. 5B). Thus, it is probable that the respective fraction of the internalized Lp[a] was taken up into the cells by the LDL receptor via LDL by a hitchhiking-like process (35). The hypothesis that Lp[a] first has to lose its apolipoprotein [a] moiety to allow the subsequent uptake of the remaining LDL-like particle (48) could not be examined by our method. For this purpose, further investigations using other techniques will be necessary.

While both lipoprotein receptors and cell surface proteoglycans are responsible for lipoprotein binding, only receptors are ultimately involved in their final uptake by the cell (49). Thus the possibility exists that different binding sites are available for Lp[a] and LDL, but the number of receptors for their uptake is rather limited. This might explain the observation that, although Lp[a] binding is enhanced in the presence of LDL at 4°C, internalization of Lp[a] is not improved by LDL at 37°C. In line with these data, Snyder et al. (50) found that Lp[a] bound to the surface of hepatocytes, macrophages, and human fibroblasts with an affinity similar to that of LDL, but with a much lower receptor occupancy. From the latter study it was concluded that the hepatic LDL receptor pathway is not significantly involved in the clearance of Lp[a].

LDL is exclusively taken up by the LDL receptor, but the mechanism for Lp[a] uptake is not so well understood. It is a poor competitor of LDL for the LDL receptor and, therefore, should be less effectively captured by this system if LDL is present in the incubation mixture. This assumption is in agreement with our findings and with results of other laboratories that Lp[a] may reduce uptake of LDL by liver cells less effectively than vice versa. As LDL affinity to the LDL receptor is higher, Lp[a] uptake is inhibited more efficiently by LDL than is LDL uptake by Lp[a]. This results in a more efficient uptake of LDL, probably serving as a vehicle for Lp[a] transport to a significant extent. The latter assumption would also be in line with our microscopic observations, demonstrating the morphology of the simultaneous uptake of LDL and Lp[a] into cells for the first time.

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