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Fluorescence Labeling and Interaction of Atherogenic Lipoproteins with Cultured Cells

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We prepared lipoprotein (a) and LDL covalently labeled with either BODIPY or rhodamine. A dual wavelength method was used for the microscopic observation of both lipoproteins during their interaction with HepG2 cells. Since a large proportion of Lp(a) colocalized with LDL on the cell surface and inside the cells, it was concluded that Lp(a) uptake into cells is mediated by LDL via internalization of LDL.

KEY WORDS: BODIPY; Rhodamine; lipoprotein (a); low density lipoprotein; confocal fluorescence microscopy.

Lipoprotein (a) [Lp(a)] consists of a low-density lipoprotein (LDL)-like particle and a highly glycosylated protein, apoprotein (a) [1]. Lp(a) is an independent risk factor for atherosclerosis [2]. The interaction of this lipoprotein with cultured human cells is a largely unknown process. Both Lp(a) binding to the cell surface and Lp(a) uptake into the cell may have at least two components, a LDL receptor-dependent and a LDL receptor-independent component [3]. In addition, these processes are probably interrelated with LDL–Lp(a) interactions [4]. It is known that LDL binds to Lp(a) [5] and thus the respective lipoprotein–lipoprotein interaction might play a role in the interaction of both lipoproteins with cells.

To address these questions we prepared and characterized fluorescently labeled Lp(a) and LDL. In the literature, LDL labeling was described as a rather time-consuming process, using 3,3'-dioctadecylindocarbocyanine iodide as a noncovalent lipid marker. One fluorophore molecule imposes one additional positive charge on the particle surface, and it cannot be excluded that label molecules are transferred from labeled lipoproteins to cells via diffusion through the aqueous phase [6]. In our procedure the protein moiety of the lipoproteins was covalently labeled either with BODIPY [7]

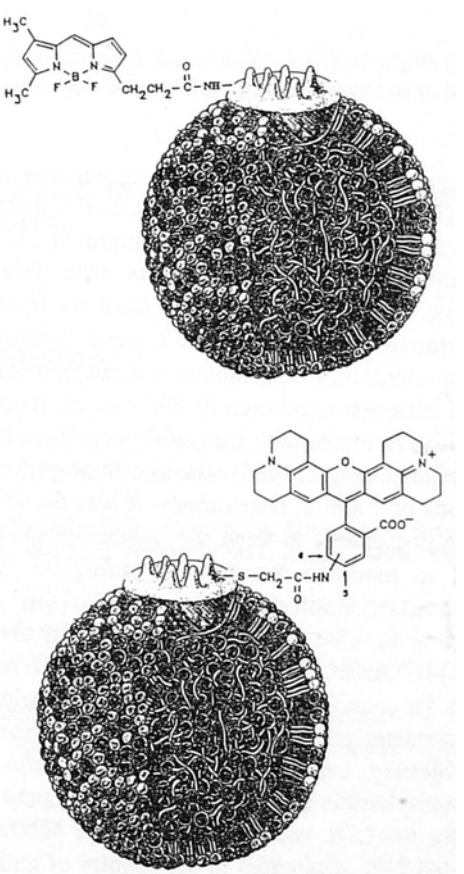

Fig. 1. Schematic representation of labeled lipoproteins. Top: BODIPY–lipoprotein (green fluorescence). Bottom: Rhodamine–lipoprotein (red fluorescence).

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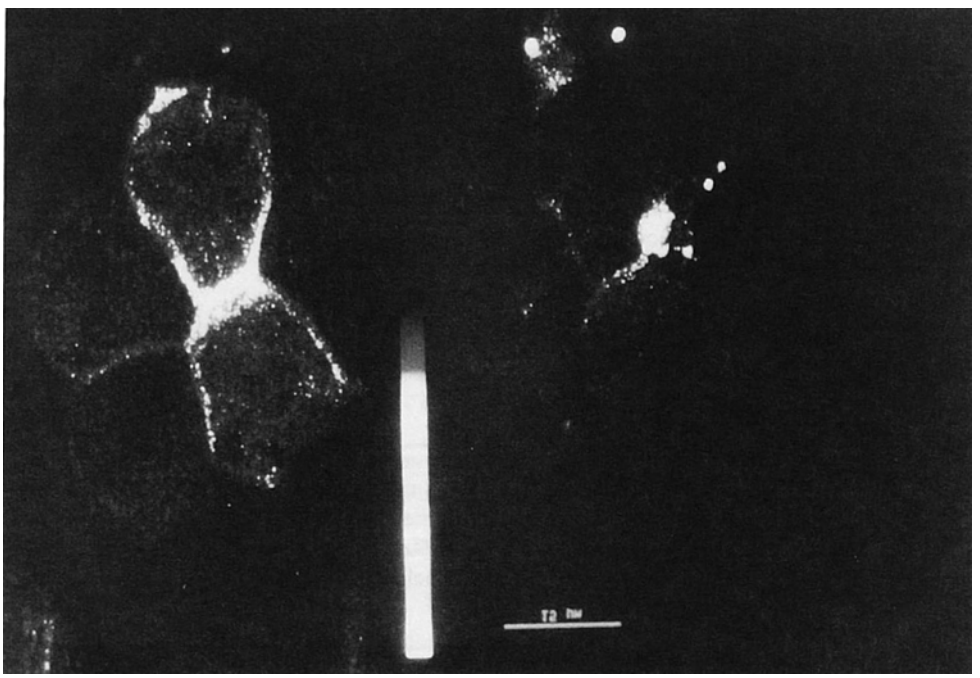

A B

Fig. 2. Binding of BODIPY–LDL (A) and rhodamine–Lp(a) (B) to HepG2 cells. A confocal fluorescence micrograph. Cells were incubated with a mixture of the labeled lipoproteins at 4°C for 2 h. The image shows that only the cell surface was stained under these conditions.

hydroxysuccinimide ester or with rhodamine iodoacetamide [8] within 3 h (see Fig. 1). BODIPY shows green fluorescence, with an emission maximum at 513 nm. Its succinimide ester derivative reacts with free amino groups on the proteins. Using this label we found label-to-lipoprotein ratios of between 3 and 5 irrespective of the lipoprotein used. Rhodamine is a red fluorescent dye with an emission maximum at 590 nm. Its iodoacetamide derivative reacts with free sulfhydryl groups. In this case labeled Lp(a) and LDL showed fluorophore-to-protein ratios of 1 and 2, respectively. It was the aim of our labeling procedures to keep the degree of labeling low enough to maintain the specific biological properties (e.g., receptor binding) of the particle. The question whether or to what extent both apoproteins are labeled in Lp(a) is currently the subject of further investigations.

So far, our fluorescently labeled lipoproteins have been characterized by agarose gel electrophoresis and ligand blotting. Labeling of the lipoproteins did not alter the electrophoretic mobility of LDL and Lp(a). The affinity for the LDL receptor from bovine adrenal cortex of labeled LDL was equal to the affinity of native LDL. Taken together, these observations support that fluorescence labeling did not alter the physicochemical or biological properties of the lipoproteins under investigation.

Interaction of the labeled lipoproteins with cultured HepG2 cells was studied using a laser scanning microscope. If LDL and Lp(a) were incubated with cells, both lipoproteins bound to the cell surface at 4°C (see Fig. 2) and were internalized at 37°C (see Fig. 3). In all cases, larger amounts of LDL were found to be associated to the cells compared with Lp(a).

When mixtures of LDL and Lp(a), each labeled with a different dye (e.g., "red" Lp(a) and "green" LDL), were incubated with cells at 4°C, both lipoproteins bound to the cell surface. However, only minor amounts of LDL and Lp(a) could be detected separately. The major amount of both lipoproteins colocalized under these conditions. At 37°C, mixtures of both lipoproteins were internalized by the cells. Now most of the labeled LDL and Lp(a) colocalized inside the cell (endosomal-like structure). False color images of the same samples showed semiquantitatively that much more LDL than Lp(a) was bound to and taken up by the cells (see Figs. 2 and 3).

Binding and internalization by HepG2 cells of labeled LDL and Lp(a) and mixtures of both were quantified spectrophotometrically after cell disintegration by Triton X-100. Protein masses of LDL and Lp(a) at 4 and 37°C, respectively, expressed as nanograms of lipopro-

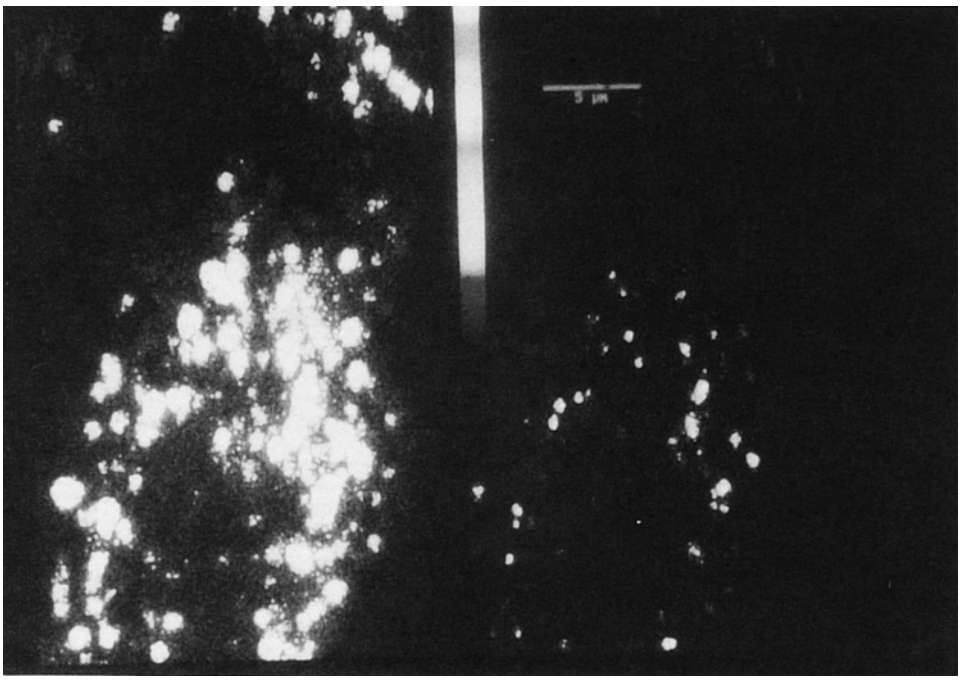

Fig. 3. Internalization of BODIPY–LDL (A) and rhodamine–Lp(a) (B) by HepG2 cells. A confocal fluorescence micrograph. Cells were incubated with a mixture of the labeled lipoproteins at 37°C for 10 min. The image shows that at this temperature, the lipoproteins are taken up by the cells into endosomal-like structures of a high fluorescence intensity.

tein per milligram of cell protein were very similar. However, if we take into account that Lp(a) contains approximately twice as much protein per particle compared with LDL, we may conclude that only half the molar amount of Lp(a) compared with LDL is associated with the cells. When we used mixtures of labeled and unlabeled lipoproteins (e.g., LDL labeled and Lp(a) unlabeled, and vice versa), competition was observed at 4 and at 37°C according to the reduction in cellular uptake and binding of the labeled lipoproteins in the presence of the unlabeled particles.

From these observations we conclude that LDL–Lp(a) interactions may contribute significantly to Lp(a)–cell interaction in that Lp(a) binds to LDL, followed by internalization via the LDL receptor into the HepG2 cells.

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