Properties of Incorporation, Redistribution, and Integrity of Porphyrin–Low-Density Lipoprotein Complexes

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ABSTRACT: In the photodynamic therapy of cancer, research has focused on the influence of lipoproteins (particularly the low-density lipoprotein, LDL) on the fate and transport of the porphyrin mixture. We have studied the interaction between LDL and a series of well-defined tetraphenylporphinesulfonates, TPPS-1, TPPS-2A, and TPPS-4. Compounds with at least two unsulfonated phenyl groups were found to associate with LDL (TPPS-1 and TPPS-2A), whereas sulfonation of all four rings abolished lipoprotein binding. As shown with agarose gel electrophoresis, association of doubly charged TPPS-2A molecules with LDL strongly influences LDL's charge. Because a change in charge may alter LDL's biological behavior, the effect of increasing amounts of TPPS-2A molecules per LDL on its biological reactivity was examined. In vitro studies with Hep G2 cells indicated that up to 250 molecules of TPPS-2A per LDL left LDL receptor recognition unchanged. In vivo studies on the fate of ^{125}I-LDL–TPPS-2A particles in rats showed that complexes with molar ratios up to 1:100 were processed like native LDL. It is concluded that tetraphenylporphines of a partial hydrophilic–hydrophobic nature are most optimal for spontaneous association with lipoproteins. These porphyrin structures will utilize lipoproteins as biological transport vehicles whereby up to 100 molecules per lipoprotein particle will not change the biological behavior of the particles so that LDL receptor-dependent uptake by tumor cells under these conditions is warranted.

The association of drugs with blood components including lipoproteins has gained much interest during the last decade. Many lipophilic drugs appear to show a tendency to bind to serum lipoproteins: cyclosporin A (Lemaire & Tillement, 1982), polychlorobiphenyls (Lesca et al., 1987), steroids (Hobbelen et al., 1975) and benzo[*a*]pyrene (Shu & Nichols, 1979) are among the many compounds reported to bind to these blood components.

Because of the capacity of serum low-density lipoprotein (LDL)[1] and high-density lipoprotein (HDL) to bind drugs, these particles may be regarded as depots for storage of these compounds. In addition to the delayed release of a drug from lipoproteins, the association may lead to the utilization of the specific lipoprotein uptake pathways which exist in the body. So the accumulation of some porphyrin preparations in tumor tissue is thought to be the consequence of a delivery mediated by the LDL receptors (Barel et al., 1986; Candide et al., 1986). High amounts of specific receptors for LDL per tumor cell are reported for various cancer cell types (Vitols et al., 1984, 1985; Norata et al., 1984).

Most studies of the interaction of porphyrins with LDL and LDL-mediated delivery to tumor tissues have focused on preparations like hematoporphyrin derivative (HpD) (Kessel, 1986) and photofrin II (P2) (Candide et al., 1986). However, these preparations are mixtures of mono-, di-, tri-, and polymeric derivatives of hematoporphyrin, and it is not yet known which components of HpD or P2 associate with LDL. To gain more insight in the relationship between the molecular structure of porphyrins in relation to their interaction with LDL, we have investigated a series of synthetic monomeric sulfonated tetraphenylporphines that differ only in the number of sulfonate moieties per molecule. These molecules are called TPPS-1, TPPS-2A, and TPPS-4, possessing respectively one, two adjacent, or four sulfonate groups on the four phenyl side groups (Figure 1).

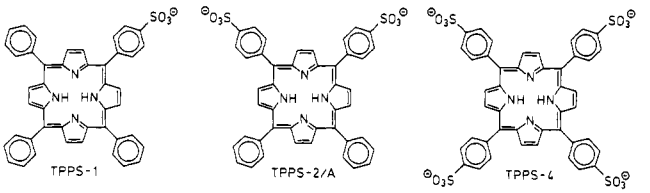

FIGURE 1: Structures of TPPS-1, TPPS-2A, and TPPS-4.

Association of porphyrins with LDL may well affect the charge, tertiary structure, and LDL receptor-binding properties of the lipoprotein. Therefore we have investigated, in addition to the incorporation efficiency, the change in charge and the in vivo behavior of the porphyrin–LDL complexes. Furthermore, the possible modification of the LDL receptor recognition was studied by performing competition studies for LDL receptor binding. It is anticipated that the presently obtained results will lead to the development of a more optimal design for the photodynamic therapy of cancer.

MATERIALS AND METHODS

Materials. ^{125}I, sodium salt (98.5% pure), was obtained from Amersham International, Amersham, U.K. Human serum albumin and agarose were purchased from Sigma Chemical Co., St. Louis, MO. Rabbit anti-apo B serum was from Behring, Marburg, Germany. Fetal calf serum was obtained from Boehringer Mannheim, Mannheim, Germany, and Dulbecco's modified Eagle's medium was from Flow Laboratories, Irvine, Scotland. Potassium bromide was purchased from J. T. Baker, Deventer, The Netherlands.

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[1] *Abbreviations*: LDL, low-density lipoprotein; HDL, high-density lipoprotein; LPDS, lipoprotein-deficient serum; TPPS, tetraphenylporphinesulfonate (the number following denotes the number of sulfonate groups: in the case of TPPS-2A, two adjacent); DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; PBS, phosphate-buffered saline.

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Tetraphenylporphyrin (TPP) and its sulfonated analogues (TPPS-1, TPPS-2A, and TPPS-4, purity >90% as determined by thin-layer chromatography) were obtained from Porphyrin Products, Inc., Logan, UT.

Lipoproteins. LDL was isolated from human plasma at density $1.024 < d < 1.055$ g/mL by two repetitive centrifugations according to Redgrave et al. (1975) as previously described (Van Berkel et al., 1985). The LDL preparation contained solely apolipoprotein B (99.97%), and no degradation products were noticeable when the preparation was checked by electrophoresis in sodium dodecyl sulfate (SDS) gels. Radioiodination of LDL was done according to the ^{125}I-iodine monochloride method described by Bilheimer et al. (1972). Lipoprotein-deficient serum (LPDS) was isolated as the bottom fraction (density > 1.21 g/mL) from human plasma after centrifugation as previously described (Van Berkel et al., 1985).

Loading of LDL with Porphyrins. Porphyrins became associated with LDL when the components were mixed and subsequently incubated at 37 °C. Incubation times of 30 min resulted in complete association of TPPS-1 and TPPA-2A with LDL. Incubations were carried out under N_2 in the dark (aluminum foil). Small aliquots (10–50 μL) of concentrated stock solution of TPPS-1 in DMSO (1.5 mg/mL) were added to 360 μg of LDL (with a trace of ^{125}I-LDL) kept in phosphate-buffered saline (PBS)/1 mM EDTA and diluted with the same buffer, resulting in a final DMSO content of 5%. This solution was further used for in vitro and in vivo experiments. In control experiments, 5% DMSO did not modify native LDL as assessed by agarose gel electrophoresis, density ultracentrifugation, and in vivo behavior.

Stock solutions of TPPS-2A and TPPS-4 (1.5 mg/mL) were in PBS 1 mM EDTA, pH 7.4, and were directly added to LDL (2 mg/mL) in order to yield samples of the desired TPPS:LDL ratios.

Density Gradient Ultracentrifugation. LDL–TPPS complexes (molar ratio 1:100) were prepared as described above and analyzed by ultracentrifugation. To the sample (1.8 mL), containing 0.7 nmol of the LDL–porphyrin complex (1:100), 1106 mg of solid KBr and PBS 1 mM EDTA to a final volume of 4.0 mL were added. Consecutive layers of 3.0, 3.0, and 3.0 mL of KBr solution (1.063, 1.019 and 1.0063 g/mL, respectively) were then added, and the tubes were centrifuged in a Beckman ultracentrifuge at 40 000 rpm (6 × 15 swing-out rotor) for 22 h at 4 °C. By taking 500-μL samples, starting at the bottom of the tube, the gradient was subdivided according to density. Samples (100 μL) of the fractions were counted for ^{125}I-radioactivity and diluted with 2.5 mL of PBS/1 mM EDTA, pH 7.4, for fluorescence measurements. All porphyrins showed excitation maxima at 415 nm and emission peaks at 650 nm (Kessel et al., 1987). Samples were measured for fluorescence on a Perkin-Elmer LS-5B luminescence spectrometer with 5-nm slit width and PBS/1 mM EDTA, pH 7.4, or DMSO as solvent. Following the described protocol, KBr did not quench fluorescence of the porphyrins as was pointed out by control experiments.

For the redistribution experiments, essentially the same procedure was followed except that 1.8 mL of the LDL–porphyrin complex (1:100) was incubated with 0.8 mL of freshly prepared human serum for 2.0 h at 37 °C (under N_2, aluminum foil) before the ultracentrifugation step.

Agarose Gel Electrophoresis. Aliquots of porphyrin–^{125}I-LDL complexes were subjected to electrophoresis in agarose gels at pH 8.8 (Tris–trippuric acid buffer). After electrophoresis, the gel was cut into segments which were counted for ^{125}I activity and subsequently extracted with 2 × 1.5 mL of PBS 1 mM EDTA, pH 7.4, for fluorescence measurements. In another experiment (Figure 7), the gel was dried, exposed to Kodak X-OMAT films, and photographed for porphyrin fluorescence.

Immunoaffinity Chromatography. Rabbit antiserum to apolipoprotein B was utilized for the preparation of a Sepharose 4B immunoaffinity column. The procedure as described by McConathy et al. (1985) was followed to construct the immunosorber. LDL–TPPS–2A complex (100 μg) was loaded on a 1.0- × 2.5-cm immunosorber column and a 50 mM Tris buffer containing 0.15 M NaCl and 0.01% EDTA, pH 7.4, was utilized to elute unretained fractions at a flow rate of 14.6 mL/h. After 23 mL, the retained fractions were eluted with 3 M sodium thiocyanate. All eluted fractions were then assayed for radioactivity and fluorescence as described above.

Culturing of Hep G2 Cells. The Hep G2 cell line, derived from a human hepatocyte tumor, was obtained from Dr. B. B. Knowles (Wistar Institute to Anatomy and Physiology, Philadelphia, PA). The cells were cultured at 37 °C in 25-cm^2 flasks (Costar) containing 0.2 mL of culture medium/cm^2 supplemented with 10% heat-inactivated fetal calf serum, penicillin, and streptomycin under CO_2 air (1:19). The medium was renewed twice a week.

Competition between LDL–TPPS-2A Complexes and Iodinated LDL for the LDL Receptor. Four to five days before the experiment, Hep G2 cells were trypsinized and transferred to 2-cm^2 Costar dishes. Twenty hours before the assay, the medium was replaced with Dulbecco's modified Eagle's medium containing 1% human serum albumin (preincubation medium) to enhance cellular LDL receptor expression (Havekes et al., 1986). Just prior to the experiment, the cells were washed and incubated with the preincubation medium three times (15, 15, and 30 min). Cells were then incubated for 4 h at 37 °C with 10 μg/mL ^{125}I-LDL in the presence of increasing concentrations of unlabeled native LDL or LDL–TPPS-2A particles. After 4 h, the wells were cooled to 4 °C and washed with PBS/2.5 mM Ca^{2+}/0.2% bovine serum albumin (3×), followed by three final washes with PBS/2.5 mM Ca^{2+}. For determination of cellular association of ^{125}I-LDL, the cells were dissolved in 500 μL of 0.1 N NaOH and sonicated. Of this homogenate, 350 μL was assayed for radioactivity and subsequently for protein according to the method of Lowry et al. (1951) with bovine serum albumin as standard.

In Vivo Studies. ^{125}I-LDL (90 μg) was preincubated with 7.5–150 μg of TPPS-2A (according to molar ratios 1:50–1:1000) in 600 μL of PBS 1 mM EDTA, pH 7.4, for 30 min at 37 °C.

Male Wistar rats (250–300 g) under Nembutal anesthesia received injections of 0.5 mL of sample in the vena cava. At regular intervals, blood samples were taken from the vena cava at least 1 cm below the site of injection and liver lobules were excised. Blood samples were centrifuged and 200 μL of serum was counted for ^{125}I radioactivity. Liver lobules were weighed and also counted for ^{125}I radioactivity. For determination of liver uptake, measured values were corrected for the amount of serum present in the tissue samples (Caster et al., 1955).

RESULTS

Incorporation of Porphyrins into LDL. Porphyrins were incorporated into ^{125}I-LDL by the direct addition of the porphyrin solution to LDL, followed by a 30-min incubation at 37 °C whereby the initially formed precipitate was dissolved completely. When analyzed by density ultracentrifugation,

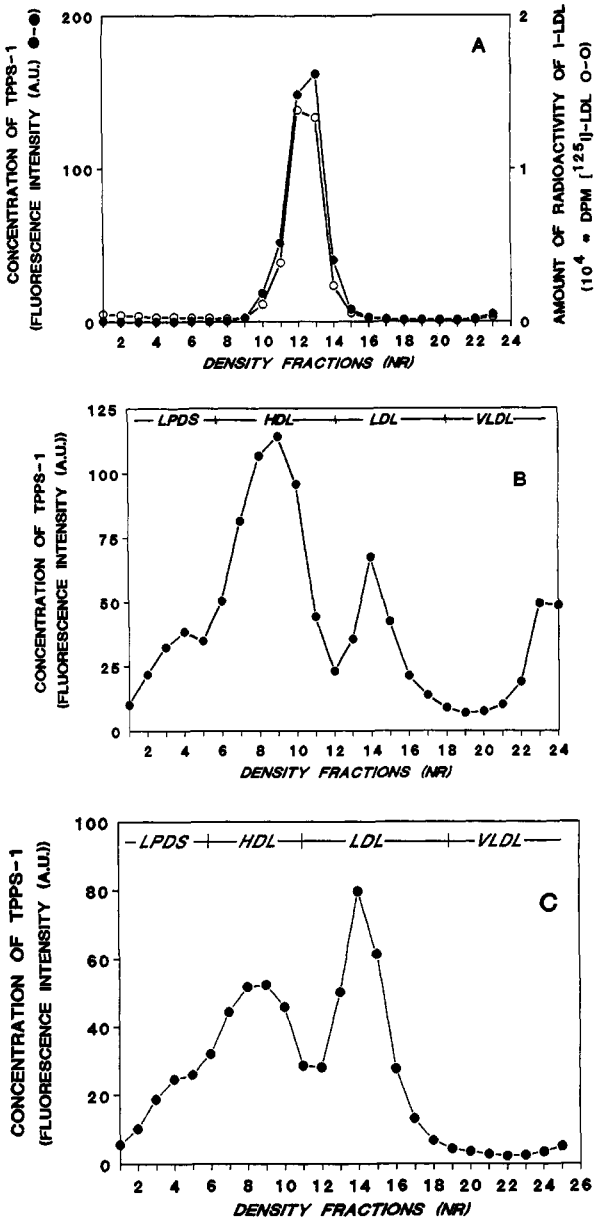

FIGURE 2: Density ultracentrifugation of TPPS-1 after incubation with LDL (A) or human serum (B) or redistribution of TPPS-1 from preformed TPPS-1–LDL complexes to serum (lipo)proteins after incubation at 37 °C (C). In panel C, preformed TPPS-1–LDL complexes were incubated with human serum for 2 h at 37 °C. The gradient was then analyzed for fluorescence as described in the Materials and Methods section.

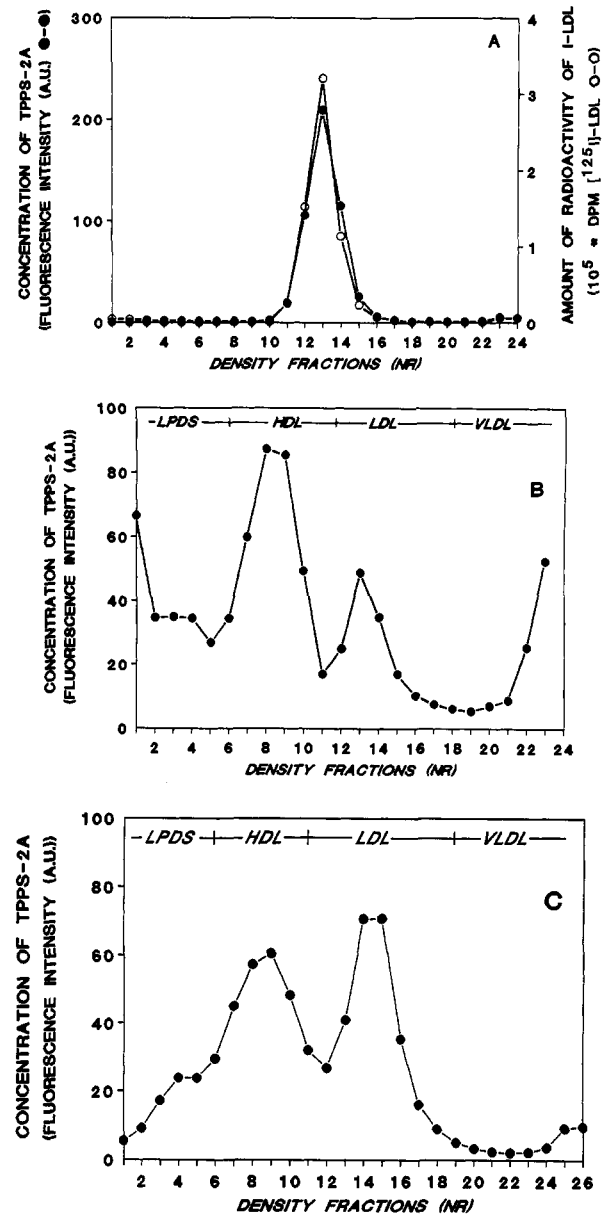

FIGURE 3: Density ultracentrifugation of TPPS-2A after incubation with LDL (A) or human serum (B) or redistribution of TPPS-2A from preformed TPPS-2A–LDL complexes to serum (lipo)proteins after incubation at 37 °C (C). In panel C, preformed TPPS-2A–LDL complexes were incubated with human serum for 2 h at 37 °C. The gradient was then analyzed for fluorescence as described in the Materials and Methods section.

TPPS-1 and TPPS-2A were recovered as single porphyrin–LDL peaks (Figures 2A and 3A). When stock solutions of TPPS-1 or TPPS-2A were subjected to density ultracentrifugation (without the presence of serum or any lipoproteins), both TPPS-1 and TPPS-2A were found to form a pellet on the bottom of the centrifuge tube, while virtually no fluorescence could be measured in the obtained density fractions (results not shown). TPPS-4 after incubation with LDL distributed over a less specified range of density fractions than TPPS-1 or TPPS-2A (Figure 4A). This distribution pattern, however, was identical to the distribution pattern for TPPS-4 in the absence of lipoproteins (results not shown). Addition of LDL to a solution of TPPS-4 did not change the physical–chemical behavior of either the LDL or the TPPS-4. All preparations presented are of molar ratios 1:100 (LDL:porphyrin).

Distribution of Porphyrins over Human Serum Fractions. TPPS-1 and TPPS-2A, directly incubated with human serum, showed primarily association with the HDL and LDL density fractions (Figures 2B and 3B). TPPS-1 associated approximately 61%, 20%, and 8% with the HDL, LDL, and chylomicron–remnant–VLDL fractions, respectively. For TPPS-2A, the percentages were 51%, 20%, and 7%, respectively. The association of TPPS-1 with the density fraction >1.21 g/mL was virtually absent, while TPPS-2A showed apparently a higher affinity for this fraction. In view of the complete precipitation of free TPPS-2A during ultracentrifugation in the absence of serum, the presence of TPPS-2A with serum indicates that TPPS-2A does interact with serum proteins, possibly albumin. Recovery of TPPS-4 after addition of serum was essentially limited to the fraction with density >1.21 g/mL (Figure 4B).

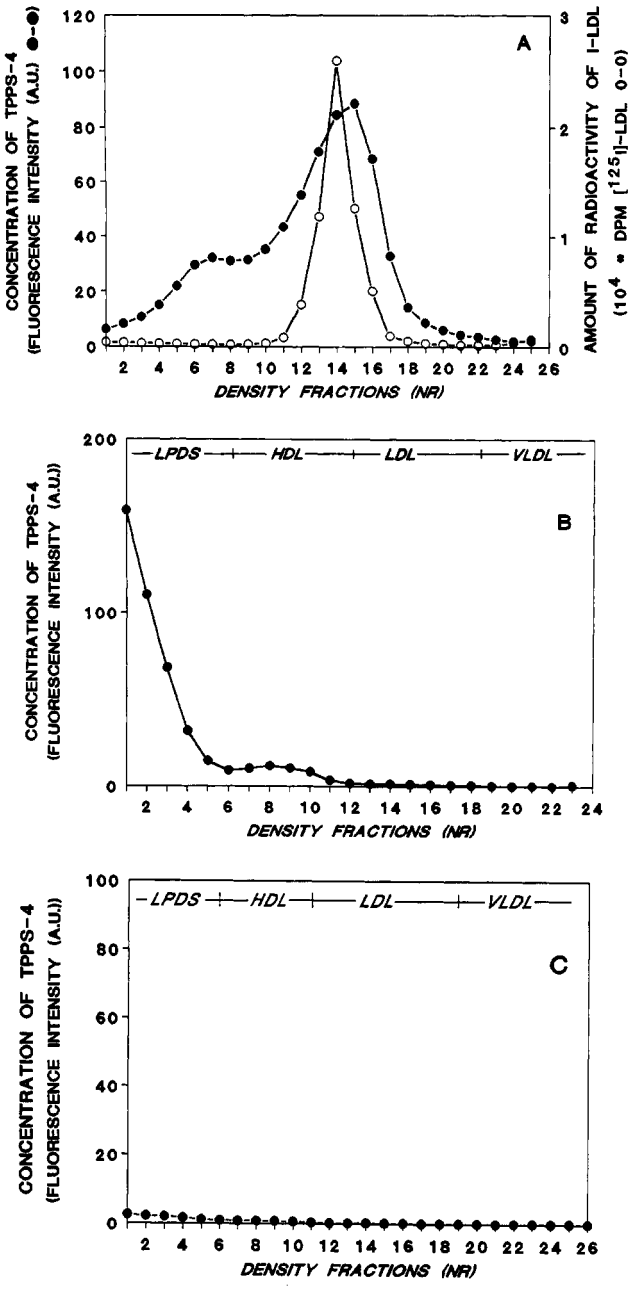

FIGURE 4: Density ultracentrifugation of TPPS-4 after incubation with LDL (A) or human serum (B) or redistribution of TPPS-4 from preformed TPPS-4–LDL complexes to serum (lipo)proteins after incubation at 37 °C (C). In panel C, preformed TPPS-4–LDL complexes were incubated with human serum for 2 h at 37 °C. The gradient was then analyzed for fluorescence as described in the Materials and Methods section.

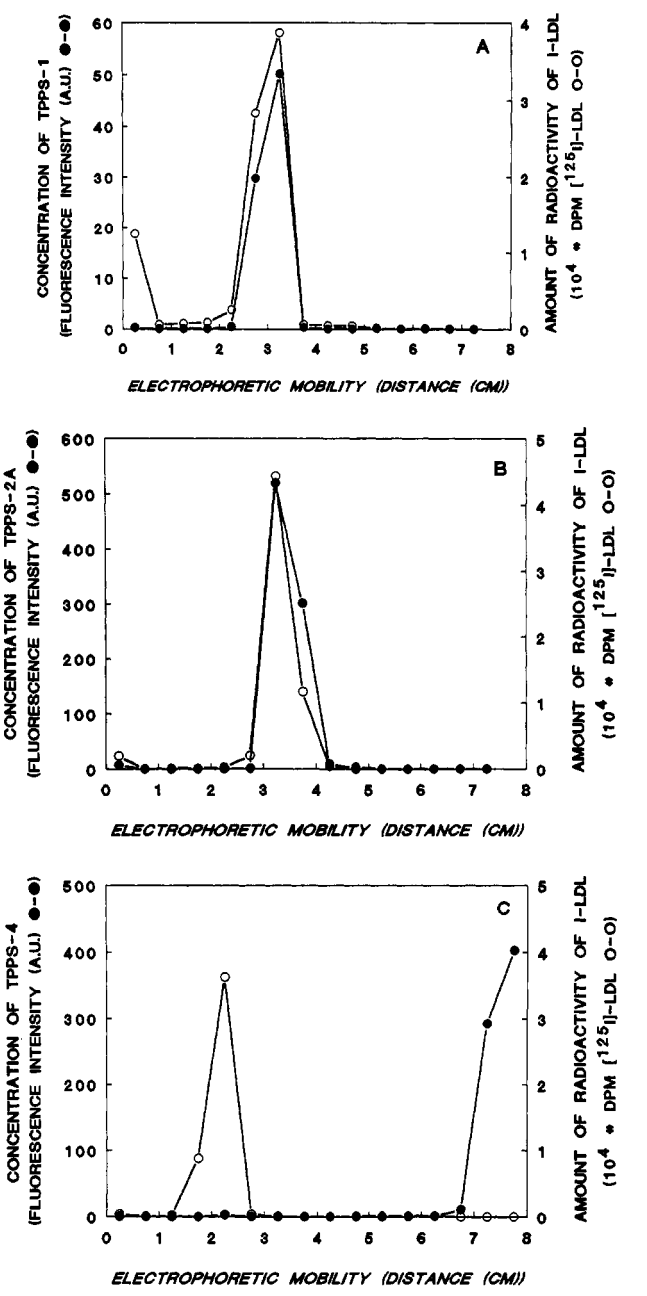

FIGURE 5: Agarose gel electrophoresis of TPPS-1 (A), TPPS-2A (B), or TPPS-4 (C) after incubation for 30 min with ^{125}I-LDL at 37 °C. After electrophoresis, the gels were cut in slices and analyzed for fluorescence (●) and ^{125}I-radioactivity (O). For all porphyrins, the incubations were carried out at molar ratios of 100:1 (porphyrin: LDL) before agarose gel electrophoresis was performed.

Redistribution of Porphyrins from LDL to Human Serum Fractions. In these experiments, LDL–porphyrin preparations as shown in Figures 2A and 3A were subsequently incubated with human serum for 2 h at 37 °C. Redistribution patterns of TPPS-1 and TPPS-2A were virtually identical (Figures 2C and 3C). Both compounds (re)distributed almost exclusively to the HDL and LDL serum fractions. In contrast with direct incubation of serum with TPPS-1 and TPPS-2A, no association with the density fractions >1.21 g/mL and <1.0063 g/mL was observed.

Agarose Gel Electrophoresis. The porphyrin–LDL mixtures were further analyzed by agarose gel electrophoresis. Migration patterns (Figure 5) indicate LDL–porphyrin complex formation for TPPS-1 and TPPS-2A, as the porphyrins move in unison with LDL in the electrophoretic field. As was observed with density ultracentrifugation, TPPS-4 did not comigrate with ^{125}I-LDL.

Immunoaffinity Chromatography. Because TPPS-2A is highly water soluble and easily incorporated into LDL, we have examined the LDL–TPPS-2A complex in greater detail. The LDL–TPPS-2A preparation (1:100) was loaded on an anti-apo B affinity column. Only a small amount of TPPS-2A was recovered unassociated with LDL while the great majority of TPPS-2A and LDL were recovered as a single peak after the elution buffer was changed to 3 M SCN^-, by which the LDL–antibody interaction is interrupted (Figure 6). Repeat chromatography did not lead to additional release of TPPS-2A.

Effects of Varying LDL–TPPS-2A Ratios on the Electrophoretic Mobility. Incubation of LDL with increasing

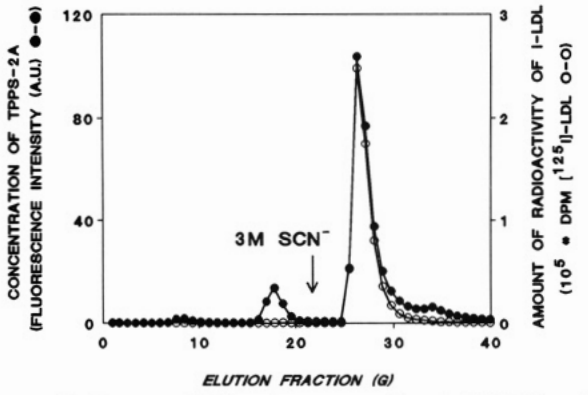

FIGURE 6: Immunoaffinity chromatography of TPPS-2A after incubation for 30 min at 37 °C with ^{125}I-LDL. The sample was loaded on an anti-apo-B–Sepharose column and the elution buffer was replaced by 3 M SCN^- after 23 mL. (●) TPPS-2A fluorescence; (○) ^{125}I-LDL radioactivity.

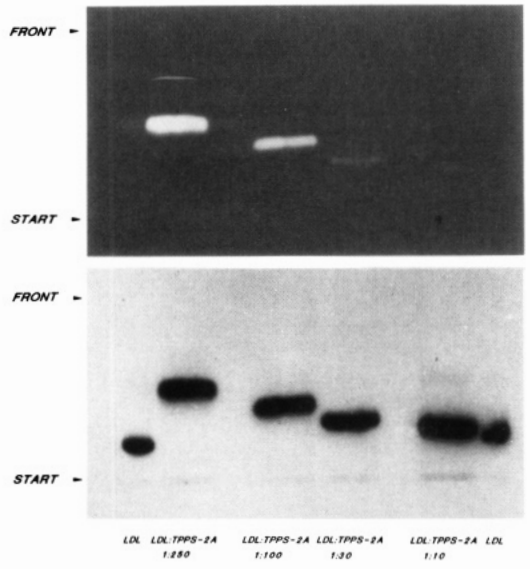

FIGURE 7: Visualization of agarose gel electrophoresis of TPPS-2A–LDL preparations with increasing TPPS-2A loading. LDL was incubated with the indicated molar ratios of TPPS-2A for 30 min at 37 °C and subjected to agarose gel electrophoresis. After electrophoresis, the gel was dried and examined for TPPS-2A fluorescence (upper panel) and by autoradiography to indicate ^{125}I-radioactivity (lower panel).

amounts of TPPS-2A is parallelled by an increasing migration of the complex on agarose gels (Figure 7). This phenomenon became already apparent with the agarose gel electrophoresis experiments in Figure 5, where both LDL–TPPS-1 and LDL–TPPS-2A (1:100 molar ratios, Figure 5A, B) showed increased migration as compared with native LDL (Figure 5C). A similar influence on migration was also observed for LDL–P2 complexes (Candide et al., 1986). For all LDL–TPPS-2A molar ratios applied, ^{125}I radioactivity and porphyrin fluorescence were located at similar positions in the gel. Furthermore, incorporation of higher amounts of TPPS-2A did not result in a smear of LDL on the gels but still relatively narrow bands were preserved.

Biological Reactivity of LDL–TPPS-2A Complexes. The effect of the association of TPPS-2A with LDL on LDL receptor recognition was examined in competition experiments with Hep G2 cells. Hep G2 cells form a hepatocellular carcinoma cell line with well-defined expression of LDL receptors (Havekes et al., 1986; Wu et al., 1984). Upon incubation of iodinated LDL with Hep G2 cells, LDL receptor recognition can be competed for by increasing concentrations

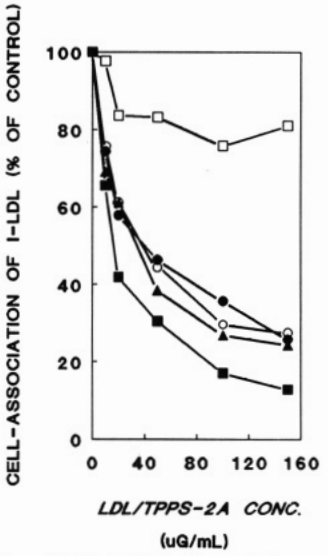

FIGURE 8: Ability of LDL–TPPS-2A particles to compete with the association of ^{125}I-LDL with Hep G2 cells. Hep G2 cells were incubated for 4 h at 37 °C with 10 μg/mL ^{125}I-LDL and indicated amounts of (●) native LDL, (○) LDL–TPPS-2A (1:50), (▲) LDL–TPPS-2A (1:100), (■) LDL–TPPS-2A (1:250), and (□) LDL–TPPS-2A (1:1000). Native LDL followed the same procedure as used to prepare the TPPS-2A–LDL complexes.

of unlabeled LDL (Figure 8). LDL–TPPS-2A preparations with molar ratios of 1:50, 1:100, and 1:250 were equally as effective in competition for iodinated LDL binding to the LDL receptor as native LDL. However, after the incorporation of 1000 molecules of TPPS-2A per LDL, no effective competition could be observed anymore (Figure 8).

In Vivo Behavior of LDL–TPPS-2A Complexes. In rats, LDL shows a slow decay [$t_{1/2}$ = 4–6 h (Craig et al., 1982)], whereby a low percentage of the injected dose is recovered in liver durig the initial 30 min after injection (Nagelkerke et al., 1984). Association of 50–100 molecules of TPPS-2A per LDL resulted in serum decay and liver uptake that is identical with that of native LDL (Figure 9). It has been well-established that rat liver LDL receptors can recognize human LDL (Harkes & Van Berkel, 1983; Rudling, 1987). However, with a molar ratio of 1:250 an enhanced liver uptake of 20–25% of the injected dose at 10 min after injection is noticed (Figure 9b) which is parallelled by an increased decay from serum (Figure 9a). Higher porphyrin loadings (1:1000) enhance disappearance from serum and liver uptake even further (Figure 9).

DISCUSSION

In the past few years, there has been an increasing interest in the influence of lipoproteins on the in vivo fate of therapeutic agents (Counsell & Pohland, 1982; Shaw et al., 1987). Especially in the photodynamic therapy, LDL is thought to play a major role in the accumulation of photosensitizing agents in human tumor tissue (Mazière et al., 1990).

In the present investigation, we have examined the interaction of LDL and a series of well-defined synthetic porphyrins that differed solely in the number of sulfonate groups per molecule. It can be argued not only that LDL can influence the biological properties of porphyrins but also that the binding of porphyrins may affect the lipoprotein itself.

Of the sulfonated porphyrins examined in this study, TPPS-1 and TPPS-2A, containing 1 or 2 sulfonate groups, possessed the highest affinity for lipoproteins. Both compounds associate quantitatively upon addition of the porphyrin solution to LDL

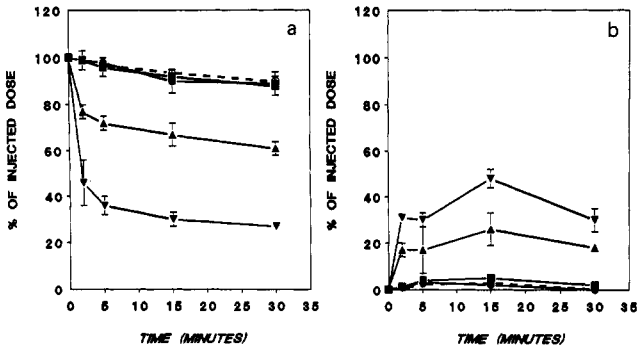

FIGURE 9: Serum decay and liver association of LDL–TPPS-2A particles with increasing molar ratios. ^{125}I-LDL–TPPS-2A particles with increasing loading of TPPS-2A were injected in male Wistar rats. During the initial 30 min, both serum ^{125}I-radioactivity (A) and liver association (B) were measured. (●) LDL–TPPS-2A (1:50); (■) LDL–TPPS-2A (1:100); (▲) LDL–TPPS-2A (1:250); (▼) LDL–TPPS-2A (1:1000), (○) native LDL. Native LDL followed the same procedure as used to prepare the TPPS-2A–LDL complexes.

and subsequent incubation at 37 °C. Density ultracentrifugation and agarose gel electrophoresis indicated that a single porphyrin–LDL complex was formed, in contrast with TPPS-4, which lacked affinity for the lipoprotein.

After incubation of the three porphyrins with human serum, TPPS-1 and TPPS-2A were found to bind to all lipoprotein classes, with TPPS-2A having a somewhat larger fraction recovered in the albumin fraction than TPPS-1. Substituting all phenyl side groups of the porphyrin with sulfonate groups apparently abolishes its capacity to bind to lipoproteins, as TPPS-4 was only recovered in the albumin fraction. Also, Kessel et al. (1987) and Kongshaug et al. (1989) indicated that upon in vitro incubation of porphyrins with serum a decrease in polarity leads to a lower association with albumin. It must, however, be stated that TPPS-4 is a selective tumor-localizing agent while lacking affinity for lipoproteins.

Redistribution of porphyrin from LDL to other serum (lipo)-proteins is identical for TPPS-1 and TPPS-2A. In contrast with the direct incubation with serum, TPPS-2A did not bind to the albumin fraction or HDL to the same extent after initial incorporation into LDL followed by a incubation with serum. These data indicate that some redistribution in vitro of TPPS-2A formerly complexed to LDL does occur, but that this process is not complete within the applied incubation time (2 h).

The first indication that association of porphyrins may influence the properties of LDL itself was found upon agarose gel electrophoresis of LDL–TPPS-2A preparations with increasing porphyrin loading. Association of 100 molecules of TPPS-2A per LDL increases the number of negative charges by 200, leading to a substantially increased migration rate. As the negatively charged sulfonate groups are likely to be positioned outside of LDL's lipid core, the two unsubstituted phenyl moieties will be associated with the phospholipid/cholesterol shell of the lipoprotein. A similar localization of amphiphilic compounds in LDL has been observed earlier in the case of a tris-galactosylated cholesterol derivative (Van Berkel et al., 1985).

As the charge of LDL is changed substantially by association with porphyrins, the integrity of its biological properties has to be verified. LDL–TPPS-2A particles with molar ratios up to 1:250 were equally as effective for competition in binding to the LDL receptor on Hep G2 cells as native LDL, indicating that LDL receptor recognition was still intact. Extremely high molar ratios (1:1000) resulted in loss of receptor recognition.

Earlier data have pointed out that in vitro data obtained for LDL–drug complexes are not necessarily representative for similar results at the physiological level (De Smidt & Van Berkel, 1990). Therefore, we have examined the in vivo fate of the same LDL–TPPS-2A preparations as used in the in vitro studies. The in vivo studies appeared to give a more sensitive estimate of the integrity of LDL, as LDL–TPPS-2A particles with molar ratios above 1:100 already expressed a substantially higher uptake by the liver. We might suggest that, although LDL receptor recognition is apparently still intact, additional uptake mechanisms in the liver, i.e., scavenger receptors, may already be triggered by the enhanced negative charge of the particle, due to incorporation of the negatively charged porphyrins. We have shown earlier (Van Berkel et al., 1991) that a change in relative electrophoretic mobility of LDL of 1.23 is already sufficient for inducing scavenger receptor recognition [which can be blocked by poly(inosinic acid)].

From the present studies it may be concluded that lipoproteins can form an important class of transport vehicles for therapeutic agents, especially in the delivery of lipophilic antineoplastic drugs to tumor tissue by the LDL receptor-mediated pathway since enhanced LDL receptor expression levels have been published for several different types of tumors. Determination of the optimal loading number per lipoprotein particle in order to acheive the desired uptake sites seems however obligatory, because association of compounds with lipoproteins, specifically under higher loading conditions, may affect the biological properties of the lipoprotein carrier. Furthermore, the association of drugs with lipoproteins is highly dependent on the amphiphilic behavior so that the utilization of the LDL receptor-mediated uptake pathway in order to achieve specific drug accumulation in tumor tissue will be more adequately utilized if these properties are taken into account, i.e., as by the photodynamic therapy of cancer.

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