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Synthesis and Properties of a Nonexchangeable Radioiodinated Phospholipid†

Alan J. Schroit

ABSTRACT: An efficient method for the synthesis and purification of *N*-[3-(3-[^{125}I]iodo-4-hydroxybenzyl)propionyl]-phosphatidylethanolamine (^{125}I-phenylpropionyl-PE), a nonexchangeable iodinated lipid of high specific radioactivity, is described. The technique involves acylation of phosphatidylethanolamine with *N*-succinimidyl 3-(3-[^{125}I]iodo-4-hydroxyphenyl)propionate (monoiodinated Bolton–Hunter reagent) and purification by thin-layer chromatography. Quantitative incorporation of the iodinated lipid into vesicles prepared by a variety of techniques was observed to occur. With these vesicles, no transfer of the labeled lipid to other vesicles or cells occurred, irrespective of vesicle composition or the presence of other transferable lipids in the same bilayer membranes. This, ^{125}I-phenylpropionyl-PE appears to be an accurate liposome tracer and can be used in a variety of in vitro and in vivo assays that require high levels of sensitivity unobtainable with most other lipid-labeling techniques.

It is generally accepted that lipid vesicles can interact with cells by various mechanisms, notably by adsorption to cell surfaces, by fusion, and/or by lipid transfer. Although the definition of these various events is straightforward, experimental evidence that unambiguously demonstrates these phenomena is difficult to obtain (Pagano & Weinstein, 1978; Poste, 1980; Pagano et al., 1981b).

Most of the available information on the mechanism involved in vesicle–cell interactions has come from experiments in which vesicles are "labeled" with aqueous space markers or with radiolabeled and/or fluorescent phospholipids. Encapsulated markers used for this purpose include compounds such as sugars (Batzri & Korn, 1975; Huang & Pagano, 1975; Poste & Papahadjopoulos, 1976), antibiotics (Gregoriadis & Neerunjun, 1975; Tyrrell et al., 1977), proteins (Magee et al., 1974; Weissmann et al., 1977), carboxyfluorescein (Weinstein et al., 1977), and technetium (Dunnick et al., 1976) or indium (Mauk & Gamble, 1979). Although these methods are straightforward, definitive interpretation of results is, for the most part, complicated since vesicles are invariably leaky, and the loss of entrapped material varies considerably with a multitude of diverse factors such as lipid and cell type, temperature, and medium employed. Similar problems are also encountered with certain radiolabeled and fluorescent phospholipid markers, especially when one considers that phospholipids can exchange with other vesicles and cell membranes (Pagano & Huang, 1975; Sandra & Pagano, 1979; Struck & Pagano, 1980; Pagano et al., 1981a,b).

A reliable radiolabeled nonexchangeable phospholipid marker could resolve some of these problems. In line with observations that the addition of a cyclical moiety to the primary amino group of phosphatidylethanolamine abrogates its exchange properties (Struck & Pagano, 1980), we describe here the synthesis, purification, and applications of an iodinated nonexchangeable phosphatidylethanolamine derivative, *N*-[3-(3-[^{125}I]iodo-4-hydroxybenzyl)propionyl]dipalmitoylphosphatidylethanolamine (^{125}I-phenylpropionyl-PE)[1] (Figure 1), of high specific activity.

Experimental Procedures

Materials and Routine Procedures. DOPC, DOPE, DPPC, DPPE, *N*-NBD-PE, and C_6-NBD-PC were purchased from Avanti Biochemicals (Birmingham, AL). *N*-Rh-PE was prepared by reacting lissamine rhodamine B sulfonyl chloride (Molecular Probes, Plano, TX) with DPPE as previously described (Struck et al., 1981). ^{125}I-BHR (sp act. ~2000 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Phospholipase A_2 (hog pancreas) was a product of Boehringer Mannheim and was used as described by Kates (1972). Radiation was measured with an Amersham/Searle Model 1185 automatic γ counter. Fluorescence was quantified with a Farrand MK II spectrophotofluorometer.

Cells. Monolayer cultures of the UV-radiation-induced UV-2237 fibrosarcoma syngeneic to C3H mice were grown to confluency in Eagle's minimum essential medium supplemented with L-glutamine and 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2 in air. Cell sus-

† From the Cancer Metastasis and Treatment Laboratory, NCI-Frederick Cancer Research Facility, Frederick, Maryland 21701. *Received April 28, 1982.* This work was sponsored by the National Cancer Institute, Department of Health and Human Services, under Contract NO1-CO-75380 with Litton Bionetics, Inc. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commerical products, or organizations imply endorsement by the U.S. Government.

[1] Abbreviations: ^{125}I-phenylpropionyl-PE, *N*-[3-(3-[^{125}I]iodo-4-hydroxybenzyl)propionyl]dipalmitoylphosphatidylethanolamine; ^{125}I-BHR, *N*-succinimidyl 3-(3-[^{125}I]iodo-4-hydroxyphenyl)propionate, monoiodinated Bolton–Hunter reagent; DOPC, dioleoylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; DPPE, dipalmitoylphosphatidylethanolamine; *N*-NBD-PE, *N*-(7-nitro-2,1,3-benzoxadiazol-4-yl)phosphatidylethanolamine; C_6-NBD-PC, 1-acyl-2-[6-[(7-nitro-2,1,3-benzoxadiazol-4-yl)-amino]caproyl]phosphatidylcholine; *N*-Rh-PE, *N*-(lissamine rhodamine B sulfonyl)dioleoylphosphatidylethanolamine; PBS, Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline, pH 7.2; LUV, large unilamellar vesicles; SUV, small unilamellar vesicles.

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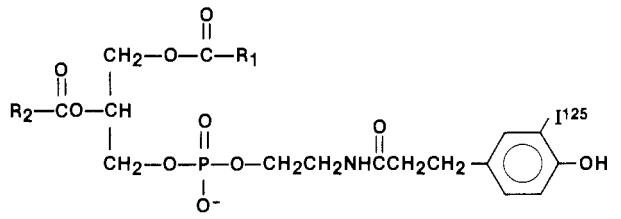

FIGURE 1: Chemical structure of *N*-[3-(3-[^{125}I]iodo-4-hydroxybenzyl)propionyl]phosphatidylethanolamine (^{125}I-phenylpropionyl-PE). R1 and R2, palmitic acid.

pensions were prepared by brief trypsinization at 22 °C. The single cell suspension was then extensively washed with PBS and kept on ice until used.

Preparation of ^{125}I-phenylpropionyl-PE. ^{125}I-phenylpropionyl-PE was synthesized by reacting ^{125}I-BHR (1 mCi $\cong$ 4.5 × 10^{-10} mol) with 100 μg of DPPE in 200 μL of $CHCl_3$/MeOH (1/2) containing 1 μL of redistilled triethylamine for 18 h at 0 °C. Five milliliters of $CHCl_3$/MeOH/H_2O (2/2/1.8) was then added, and the mixture was vigorously mixed. After centrifugation, the lower organic phase was removed to a clean tube and washed with water; the ^{125}I-phenylpropionyl-PE was purified by preparative thin-layer chromatography on activated silica gel 60 thin-layer plates (Merck & Co., Inc., Rahway, NJ) in $CHCl_3$/MeOH/acetone/acetic acid/H_2O (5/1/2/1/0.5) (see Results). The presence of ^{125}I-containing compounds was assessed by autoradiographic analysis using Kodak XR-5 film.

Lipid Vesicles. Unilamellar vesicles of various sizes were prepared by ethanol injection (Kremer et al., 1977) cholate dialysis (Littman et al., 1979), or ultrasonication (Huang, 1969) as previously described. (1) Ethanol injection: LUV were made by drying 10 mg of total lipid (DOPC with trace amounts of ^{125}I-phenylpropionyl-PE, *N*-NBD-PE, C_6-NBD-PC, and/or *N*-Rh-PE) under a stream of dry nitrogen gas and further dried under vacuum. The lipids were then dissolved in 250 μL of absolute ethanol and injected with stirring into 3.2 mL of PBS. The resulting vesicle preparation was subsequently dialyzed overnight against several liters of buffer. SUV were prepared by the same general procedure except that a total of 0.5 mg of lipid in 167 μL of ethanol was injected into 2.2 mL of PBS. (2) Cholate dialysis: LUV were prepared by adding 1 mL of 1.5% sodium cholate (Sigma Chemical Co., St. Louis, MO) in PBS to 10 mg of dry lipid. The suspension was then sonicated in a bath-type sonifier to clarity at 2 °C (~60 min). The clear solution was then dialyzed for 3 days against multiple changes of buffer. (3) Ultrasonication: SUV were prepared by sonication of up to 10 mg of lipid in 2 mL of PBS under nitrogen for 20 min at 2 °C (DOPC vesicles) or 45 °C (DPPC vesicles) using a titanium-tipped probe-type sonifier. The sonicate was then centrifuged (15000*g* for 30 min) to remove titanium fragments. In some experiments, "contaminating" LUV or multilamellar vesicles were removed by passing the preparation over a Bio-Gel A-15m column and collecting only the "included" vesicle peak.

Vesicle–Vesicle Exchange. Vesicle–vesicle exchange experiments were carried out essentially as described by Pagano et al. (1981a) except that the extent of lipid transfer was assessed by using Bio-Gel A-15m column chromatography. Briefly, fluorescent or radiolabeled donor SUV and acceptor LUV or donor LUV and acceptor SUV were prepared as described above. Donor and acceptor vesicles were then mixed at a 1/5 ratio (w/w), and the mixture was incubated for 30 min at room temperature or 37 °C. The mixed vesicle population (250 μL) was then separated according to size on a Bio-Gel A-15m column (49 × 0.9 cm) precalibrated with known standard vesicle preparations. Fractions were collected (~0.8 mL), and the presence of vesicles in the individual fractions was assessed by combined light-scatter, fluorescence, and radioactivity determinations.

Vesicle–Cell Incubation. Washed UV-2237 cells (10^7) were suspended in 1 mL of the indicated SUV preparations (1.0 mg of lipid/mL) and incubated for 1 h at 2 °C. The cells were then washed 4 times with PBS to remove nonadherent vesicles and were transferred to a new tube following each wash. Quantitative determination of the amount of lipid transferred to the cells was assessed by extracting the washed cells (suspended in several drops of PBS) with 3.8 mL of $CHCl_3$/MeOH/0.1 N HCl (1/2/0.8) for 1 h at 22 °C. Insoluble material was removed by centrifugation, and the solvent was partitioned by adding 1 mL of $CHCl_3$ and 1 mL of water, followed by vigorous vortexing and centrifugation. The organic phase was removed and dried under a stream of nitrogen. The lipid residue was then dissolved in 1 mL of ethanol. The ratios of radiolabeled to fluorescent lipids present in the extracts (as determined by scintillation and fluorescence spectrometry, respectively) were compared to that of the initial vesicle population extracted in an identical manner.

Fluorescence Measurements. The relative fluorescence of vesicles or vesicle-treated cells was determined in 1-mL samples at 22 °C. By use of crossed polarizers and 5-nm band-pass slits to minimize light scatter, the amount of C_6-NBD-PC, *N*-NBD-PE, and *N*-Rh-PE in the intact vesicles or various extracts was assessed by direct excitation at 470 nm (λ_{em} 525 nm) and 560 nm (λ_{em} 590 nm), respectively. The fluorescence of vesicles containing self-quenching concentrations of C_6-NBD-PC or *N*-NBD-PE (Schroit & Pagano, 1981) and of vesicles containing both the NBD and rhodamine analogues was quantified only in extracted or lysed samples (1% Triton X-100), which abrogates all energy transfer effects (Pagano et al., 1981a).

Results

Synthesis and Purification of ^{125}I-phenylpropionyl-PE. The complete sequence involved in the synthesis and purification of ^{125}I-phenylpropionyl-PE was assessed by thin-layer chromatography, as shown in Figure 2. Chromatography of ^{125}I-BHR that was solubilized in $CHCl_3$/MeOH (1/2) for 18 h at 2 °C revealed three major components (Figure 2, lane 1). Since this reagent is >95% pure, the appearance of additional spots following chromatography is probably a result of hydrolysis that occurred either during storage in $CHCl_3$/MeOH or upon chromatography in the water-containing solvent system. In any event, reacting ^{125}I-BHR in the presence of DPPE resulted in the formation of the iodinated product, ^{125}I-phenylpropionyl-PE (Figure 2, lane 2). When the reaction mixture was partitioned into aqueous and organic phases, some of the unreacted reagent partitioned into the aqueous phase (Figure 2, lane 3), whereas virtually all the product remained in the organic phase (Figure 2, lane 4). The product (R_f = 0.56) was isolated by scraping the autoradiographically identified area from a preparative thin-layer plate into $CHCl_3$/MeOH/H_2O (1/2/0.8). After the mixture was vigorously shaken, the gel was removed by centrifugation, and the solvent was partitioned by adding 1 volume each of $CHCl_3$ and H_2O. The organic phase was removed, dried under nitrogen, and further dried under high vacuum. It was then suspended in $CHCl_3$ and filtered to remove traces of silica gel. The final product was rechromatographed and found to be pure (Figure 2, lane 5). The product comigrated with DPPE derivatized with nonradioactive Bolton–Hunter reagent and was phosphate positive and ninhydrin negative (results not

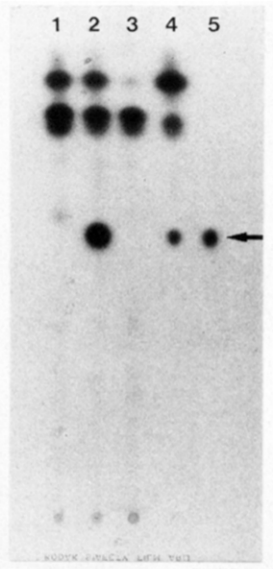

FIGURE 2: Thin-layer autoradiograph showing the various stages in the preparation of ^{125}I-phenylpropionyl-PE. Lane 1, ^{125}I-BHR; lane 2, unpurified reaction mixture; lanes 3 and 4, reaction products which partitioned in aqueous and organic phases, respectively; lane 5, purified ^{125}I-phenylpropionyl-PE. The arrow indicates the position of the product.

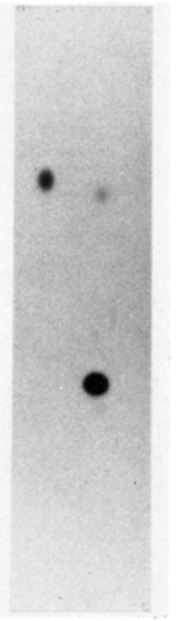

FIGURE 3: Thin-layer autoradiograph showing sensitivity of ^{125}I-phenylpropionyl-PE to phospholipase A_2. (Left) Purified ^{125}I-phenylpropionyl-PE; (right) phospholipase reaction product, assumed to be lyso-^{125}I-phenylpropionyl-PE.

shown). The product was stored at −70 °C in Pyrex glass in $CHCl_3$/MeOH (98/2). Typical yields of ^{125}I-phenylpropionyl-PE were in the range of 25–37% of the possible maximum, based on the amount of ^{125}I used.

Sensitivity of ^{125}I-phenylpropionyl-PE to Phospholipases. The purified ^{125}I-phenylpropionyl-PE was subjected to hydrolysis by phospholipases D (cabbage), C (*Clostridium perfringens*), and A_2 (hog pancreas) as described in detail by Kates (1972). The various procedures were carried out by using ^{125}I-phenylpropionyl-PE alone or ^{125}I-phenylpropionyl-PE mixed with milligram amounts of DOPC. Phospholipase A_2 treatment resulted in quantitative conversion of the material to the presumed lyso derivative, and no radiation was detected in the cleaved fatty acids, which migrate at the solvent front (Figure 3). On the other hand, ^{125}I-phenylpropionyl-PE was insensitive to phospholipases D and C, although added carrier lipid was quantitatively converted to the respective phosphatidic acid and diglyceride products. This lack of sensitivity may be caused by steric hindrance imposed by the relatively bulky polar group present in the derivatized lipid (see Discussion).

Stability of ^{125}I-phenylpropionyl-PE. Small amounts (estimated at <2%) of radiolabeled impurities became detectable (by overexposure of XR-5 film) after 6–7 weeks of storage at −70 °C. These impurities migrated faster than ^{125}I-phenylpropionyl-PE when analyzed by thin-layer chromatography ($R_f > 0.8$). They were also detectable in various vesicle preparations as dialyzable low molecular weight compounds that in undialyzed preparations eluted with the total included volume from Bio-Gel A-15m. Although these radiolytic impurities seemed to be water soluble, attempts to purify the product by solvent partitioning failed, and preparative thin-layer chromatography was the only effective means of repurification.

Formation of Vesicles Containing ^{125}I-phenylpropionyl-PE. ^{125}I-phenylpropionyl-PE was quantitatively incorporated into vesicles prepared by various techniques. Figure 4 shows Bio-Gel A-15m chromatography profiles of vesicles (composed of DOPC, ^{125}I-phenylpropionyl-PE, and *N*-NBD-PE) obtained by ethanol injection, detergent dialysis, and ultrasonication techniques. As can be seen, virtually all the radioactivity (^{125}I-phenylpropionyl-PE) eluted with the fluorescent marker (*N*-NBD-PE). Furthermore, additional radioactive peaks were absent, suggesting that all of the ^{125}I-phenylpropionyl-PE was incorporated into the vesicles. If radiolytic impurities were present (introduced by prolonged storage) in the initial ^{125}I-phenylpropionyl-PE preparation, a small "contaminating" peak (<2% of total radioactivity) would have been present in fractions 46–50 (data not shown).

^{125}I-phenylpropionyl-PE Does Not Transfer between Vesicles. When donor SUV containing ^{125}I-phenylpropionyl-PE were mixed with acceptor LUV containing the nonexchangeable lipid *N*-NBD-PE (Struck & Pagano, 1980), no transfer of ^{125}I-phenylpropionyl-PE from the donor to the acceptor vesicles is observed upon Bio-Gel A-15m chromatography (Figure 5). Similarly, when donor LUV containing ^{125}I-phenylpropionyl-PE and the readily exchangeable lipid C_6-NBD-PC (Struck & Pagano, 1980) were mixed with acceptor SUV containing the nonexchangeable lipid (*N*-Rh-PE (Struck et al., 1981), only C_6-NBD-PC was transferred to the acceptor vesicle population (Figure 6). Thus, ^{125}I-phenylpropionyl-PE can be classified as nonexchangeable regardless of whether a readily transferable lipid species is present in the same bilayer structure.

^{125}I-phenylpropionyl-PE Accurately Assesses Vesicle–Cell Interactions. For determination of whether the inclusion of ^{125}I-phenylpropionyl-PE in vesicles provides an accurate method for determining vesicle–cell interactions, two separate and independent assays were performed. These experiments are based on the observation that so-called solid[2] vesicles tend to adsorb to cell surfaces, whereas fluid vesicles demonstrate very little adsorption.

The results of a typical experiment using SUV composed of DPPC (solid vesicles) with trace amounts of ^{125}I-phenylpropionyl-PE and *N*-NBD-PE (a nonexchangeable lipid) incubated with UV-2237 cells for 1 h at 2 °C are shown in Table I. The washed vesicle-treated cells demonstrated essentially the same ratio of radiation to fluorescence as the initial vesicle

[2] The terms fluid and solid indicate whether the lipids are above or below their gel–liquid-crystalline phase transition temperature.

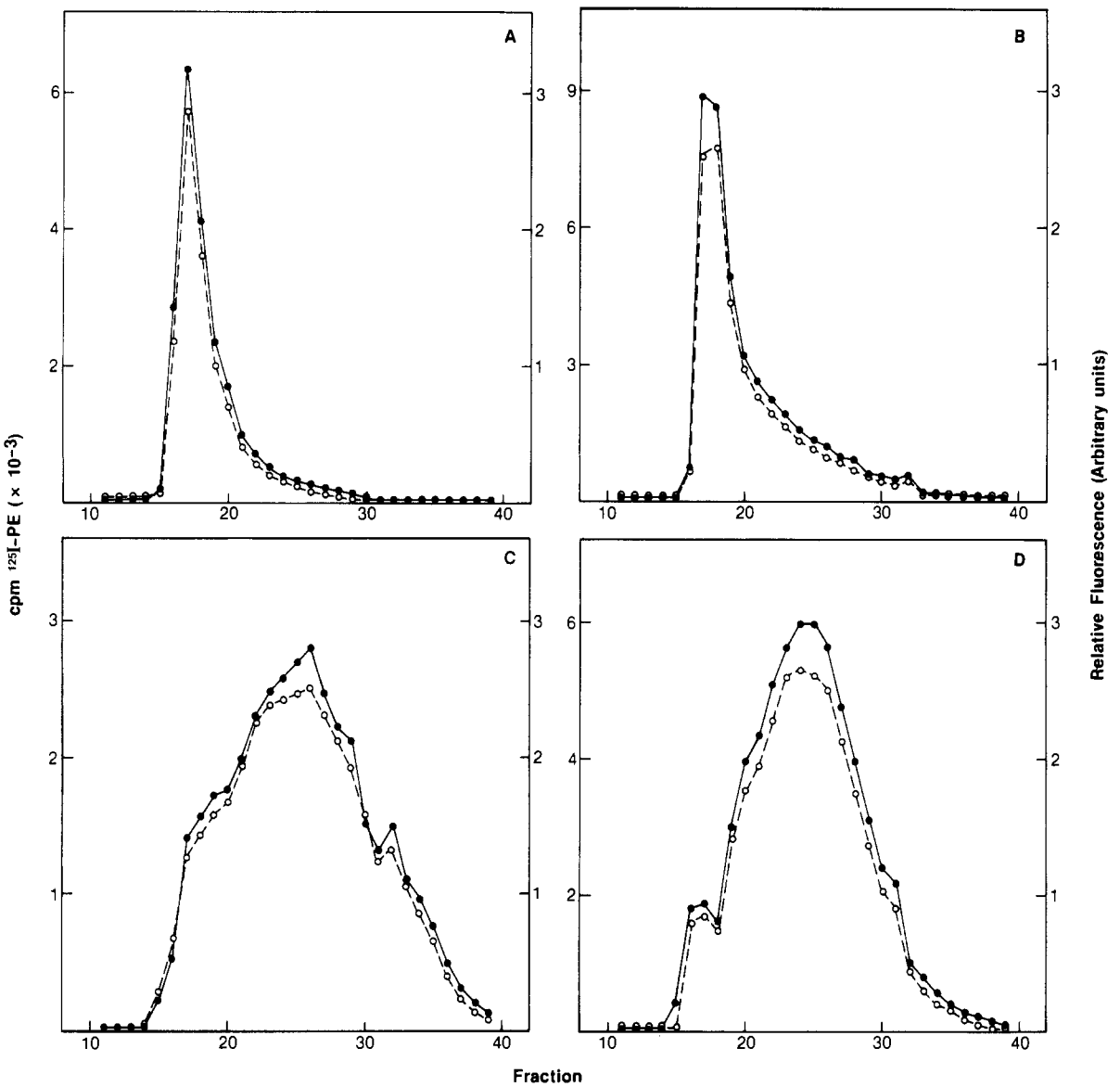

FIGURE 4: Bio-Gel A-15m chromatographic analysis of vesicles prepared by various procedures for incorporation of ^{125}I-phenylpropionyl-PE. (A) LUV prepared by ethanol injection; (B) LUV prepared by detergent dialysis; (C) SUV prepared by ethanol injection; (D) SUV prepared by ultrasonication. (●) ^{125}I-phenylpropionyl-PE; (○) *N*-NBD-PE.

population (~2% of the added vesicles became cell associated), suggesting that the vesicles adsorbed to the cells as intact structures. On the other hand, when SUV composed of DOPC (fluid vesicles) with trace amounts of C_6-NBD-PC (an exchangeable lipid), ^{125}I-phenylpropionyl-PE, and *N*-Rh-PE (a nonexchangeable lipid) are used as indicators of vesicle–cell adsorption (Struck et al., 1981), only NBD fluorescence was transferred to the cells in significant amounts (Table II). Although some ^{125}I-phenylpropionyl-PE was transferred to the cells, the ratio of radiation to rhodamine fluorescence remained constant, suggesting some degree of vesicle–cell adsorption (~0.07%) in this system. These results strongly suggest that ^{125}I-phenylpropionyl-PE can be used as an indicator of vesicle–cell interactions, in that it accurately monitors this process. Moreover, the presence of a selectively exchangeable lipid (C_6-NBD-PC) in the vesicle bilayer has no effect on the association of ^{125}I-phenylpropionyl-PE with the vesicle bilayer structure.

Table I: Analysis of Vesicle–Cell Adsorption with DPPC Vesicles Containing *N*-NBD-PE and ^{125}I-phenylpropionyl-PE[a]

	^{125}I-phenyl-propionyl-PE[b]	*N*-NBD-PE[b]	^{125}I-phenyl-propionyl-PE/*N*-NBD-PE
starting vesicles[c]	21 632	20.1	1076
vesicle-treated cells[d]	8 544	8.4	1017

[a] Vesicles were prepared by ultrasonication of DPPC (4 mg) with *N*-NBD-PE (100 μg) and ^{125}I-phenylpropionyl-PE (~1.5 μCi) at 45 °C. The sonicate was rapidly chilled, centrifuged (15000*g* for 30 min), and diluted to 1 mg of lipid/mL of vesicles. After extensive washings, the fraction of cell-associated fluorescence and radiation was determined as described in the text. [b] Radiation (cpm) and relative fluorescence (λ_{ex} 470 nm; λ_{em} 525 nm) measurements were carried out on extracted samples. [c] Analysis of a 50-μL aliquot. [d] Analysis of 10^7 cells.

Discussion

In this report we describe a method for the efficient synthesis of an iodinated lipid of known structure. The technique uses commerically available ^{125}I-BHR, which specifically acylates primary amino groups (Rudinger & Ruegg, 1973; Bolton & Hunter, 1973). Indeed, attempts to acylate both saturated and unsaturated phosphatidylcholines (DPPC and DOPC,

Table II: Analysis of Vesicle–Cell Interaction with Preferentially Nonadsorbing DOPC Vesicles Containing C_6-NBD-PC and ^{125}I-phenylpropionyl-PE (^{125}I-PE)[a]

	^{125}I-PE	*N*-Rh-PE[a]	C_6-NBD-PC[b]	^{125}I-PE/ *N*-Rh-PE	C_6-NBD-PC/ *N*-Rh-PE	C_6-NBD-PC/ ^{125}I-PE
starting vesicles[c]	37 567	31.4	40.3	1196	1.3	0.001
vesicle-treated cells[d]	529	0.4	2.2	1322	5.5	0.004

[a] Vesicles were prepared by ultrasonication of DOPC (4 mg) with C_6-NBD-PC (100 μg), Rh-PE (50 μg), and ^{125}I-phenylpropionyl-PE (~1.5 μCi) at 2 °C. 10^7 cells (UV-2237) were incubated for 1 h at 2 °C with 1 mL of vesicles (1 mg of lipid/mL). After extensive washings, the fraction of cell-associated fluorescence and radiation was determined. [b] Radiation (cpm) and relative fluorescence (NBD, λ_{ex} 470 and λ_{em} 525 nm; Rh, λ_{ex} 560 nm and λ_{em} 590 nm) measurements were carried out on extracted samples. [c] Analysis of a 50-μL aliquot. [d] Analysis of 10^7 cells.

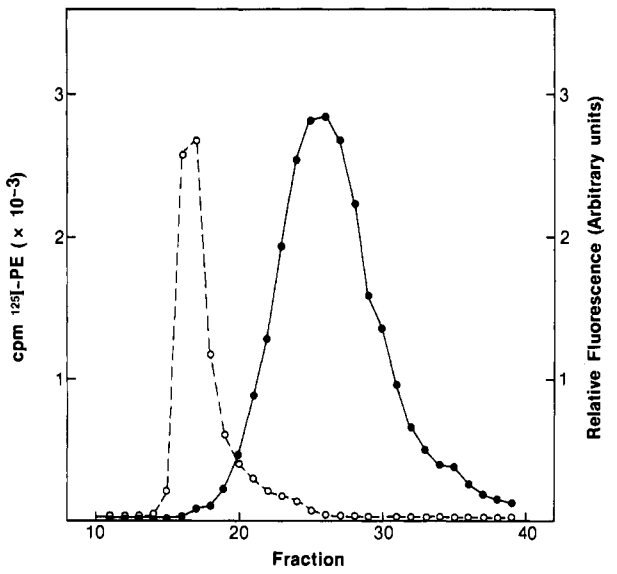

FIGURE 5: Lack of intervesicular transfer of ^{125}I-phenylpropionyl-PE between different vesicle populations. LUV were formed by ethanol injection from DOPC/NBD-PE (99/1) and SUV by ultrasonication from DOPC/^{125}I-phenylpropionyl-PE. Contaminating large vesicles were removed from the SUV preparation by prior gel filtration on Bio-Gel A-15m. Vesicles were mixed for 30 min at 22 °C prior to chromatography on Bio-Gel A-15m. (●) ^{125}I-phenylpropionyl-PE; (○) *N*-NBD-PE.

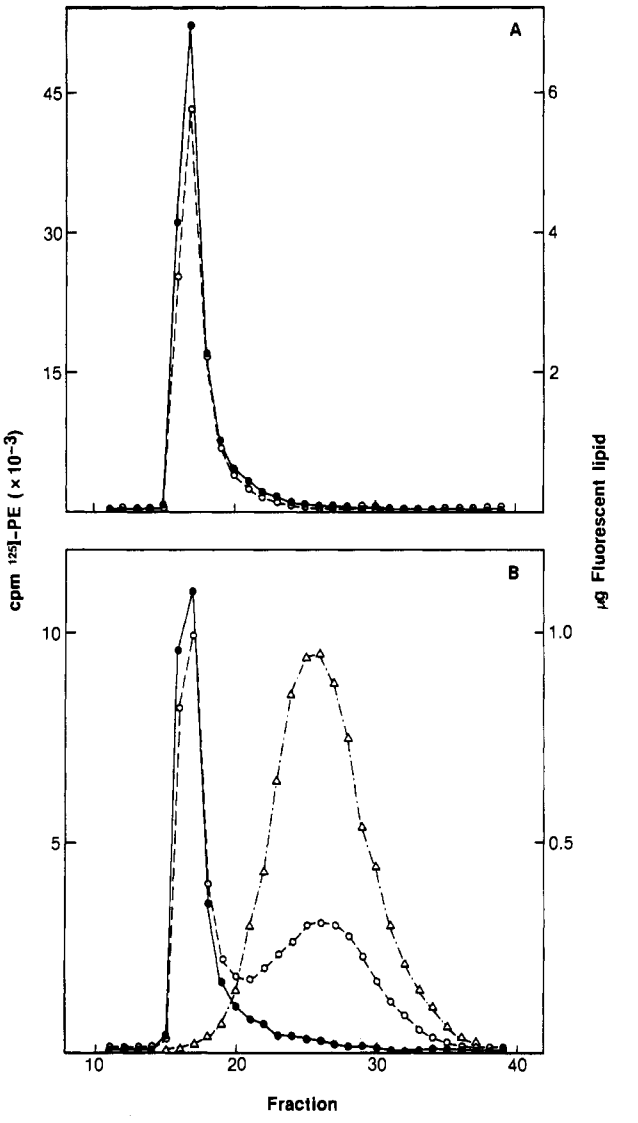

FIGURE 6: Lack of intervesicular transfer of ^{125}I-phenylpropionyl-PE in the presence of the readily transferable lipid C_6-NBD-PC. LUV were formed by ethanol injection from DOPC/C_6-NBD-PC/^{125}I-phenylpropionyl-PE (99/1/trace), and SUV were formed by ultrasonication of DOPC/*N*-Rh-PE (99/1). Bio-Gel A-15m chromatography of LUV alone (A) and a mixture of LUV and SUV after 30 min at 22 °C (B). (●) ^{125}I-phenylpropionyl-PE; (○) C_6-NBD-PC; (△) *N*-Rh-PE.

respectively) failed (data not shown), indicating that the process is highly specific. The product is obtained in high yield (~30% maximum theoretical yield) and has extremely high specific activity (~2000 Ci/mmol).

Previous methods for the preparation of iodinated lipids have been unsatisfactory because the products obtained were not readily identifiable and only very modest yields of pure material were obtained. Benenson et al. (1980) introduced a technique for labeling intact liposomes whereby ^{125}I is apparently introduced into phospholipid acyl chain double bonds using lactoperoxidase- and peroxide-generating systems. However, some investigators claim that this technique is nonspecific in that labeling occurs in the absence of any added enzyme (Tepperman & Campbell, 1979). In addition, it has been shown that acyl chain ^{125}I-labeled phospholipids are readily exchangeable with tissues under certain experimental conditions (Rabinowitz & Travares, 1977). In contrast, the iodinated lipid described here is obtained in consistently high yields and is essentially nonexchangeable. This finding is in agreement with the general observation that the addition of cyclical moieties to phosphatidylethanolamine abrogates lipid transfer in vesicle–cell (Struck & Pagano, 1980) and vesicle–vesicle (Pagano et al., 1981a) systems.

^{125}I-phenylpropionyl-PE was sensitive to phospholipase A_2; however, it was completely resistant to hydrolysis by phospholipases C and D. This lack of sensitivity probably results from steric hindrance imposed by the relatively large aromatic moiety present in the derivatized lipid. Similar lack of sensitivity has been observed for other N-acylated phosphatidylethanolamines such as NBD-PE and trinitrophenylated phosphatidylethanolamine (unpublished observations).

Preliminary experiments using ^{125}I-phenylpropionyl-PE in various biological assays have proved it to be an extremely useful vesicle tracer. Comparative assays between vesicles

labeled with ^{125}I-phenylpropionyl-PE and vesicles containing entrapped ^{125}I-labeled albumin have shown essentially identical uptake curves in macrophage phagocytosis assays and in in vivo tissue distribution studies (unpublished observations). The advantages of using ^{125}I-phenylpropionyl-PE in such systems are (1) removal of unincorporated tracer from vesicles is unnecessary since ^{125}I-phenylpropionyl-PE is completely inserted into the vesicle bilayer and (2) γ camera scintography techniques can be used for quantitation since ^{125}I-phenylpropionyl-PE is a strong γ emitter.

In addition, ^{125}I-phenylpropionyl-PE could be used in the rapidly evolving fields of liposome immunodiagnostics and liposome targeting (Leserman et al., 1980; Martin et al., 1981; Huang et al., 1981), methodologies which require high levels of sensitivity.

In conclusion, ^{125}I-phenylpropionyl-PE appears to be an accurate liposome marker, with a potential of becoming useful in a variety of in vitro and in vivo assays that require high levels of sensitivity unobtainable with most other lipid-labeling techniques.

Acknowledgments

The expert technical assistance of John Madsen is gratefully acknowledged.

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