Biochimica et Biophysica Acta, 778 (1984) 281–288
Elsevier

BBA 72320

PERYLENOYL- AND ANTHRYLVINYL-LABELED LIPIDS AS MEMBRANE PROBES

JULIAN G. MOLOTKOVSKY, YEFIM M. MANEVICH, VYACHESLAV I. BABAK and LEV D. BERGELSON

M.M. Shemyakin Institute of Bioorganic Chemistry, U.S.S.R. Academy of Sciences, ul. Vavilova 32, 117988, Moscow (U.S.S.R.)

(Received March 9th, 1984)
(Revised manuscript received July 2nd, 1984)

Key words: Perylenoyl-labeled lipid; Anthrylvinyl-labeled lipid; Fluorescent lipid; Model membrane; Fluorescence; NMR

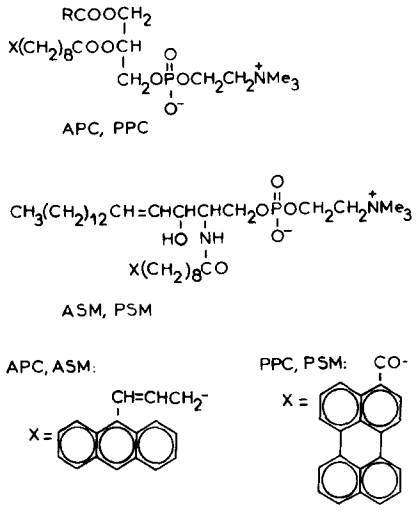
The properties of a new family of lipid-specific fluorescent probes, a fatty acid, a phosphatidylcholine and a sphingomyelin, bearing a 3-perylenoyl-labeled hydrophobic chain, are described. Perylenoyl-labeled lipids readily enter the lipid bilayer, the fluorophore being localized in the apolar region of the membrane. The perylenoyl fluorophore is characterized by a high quantum yield, its fluorescence parameters (λ_{ex} 446 nm, λ_{em} 479–545 nm) permit to apply it as an acceptor of excitation energy from the 9-anthrylvinyl fluorophore used earlier for phospholipid labeling (Molotkovsky, Jul. G., Manevich, Y.M., Gerasimova, E.N., Molotkovskaya, I.M., Polessky, V.A. and Bergelson, L.D. (1982) Eur. J. Biochem. 122, 573–579). The anthrylvinyl-labeled lipids were shown to be capable to report phase segregation between the corresponding prototype lipids in model systems. The combined use of anthrylvinyl- and perylenoyl-labeled lipids opens additional possibilities for investigation of lipid-lipid and lipid-protein interactions in artificial and biological membranes. Perylenoyl-labeled lipids appeared also to be useful as fluorescent dyes in cytological studies.

Introduction

Fluorescent probing is used widely in membrane studies (see, e.g., reviews in Refs. 1 and 2). However, most of the currently used non-polar or amphiphylic probes, such as 8-anilino-1-naphthalenesulfonate, 1,6-diphenyl-1,3,5-hexatriene, pyrene, cyanine dyes, differ significantly from the lipid components of biological membranes and consequently may strongly perturb the molecular organization of the region they are monitoring. Moreover, the position of such probes cannot be established with sufficient certainty. Thus, pyrene [3] and DPH [4] may reside in different apolar regions of the membrane assuming either a parallel or a perpendicular orientation of the probe to the bilayer surface. In plasma lipoproteins, DPH distributes between the inner core and the outer monolayer [5].

Application of non-lipid fluorescent probes to problems of domain organization is hampered further because the probes partition between fluid and solid domains of the membrane to an extent that is difficult to define. Moreover, the environment in which such foreign probes are localized may represent an atypical region in the membrane, or the probe may make its own lipid domain. Besides, the dynamic characteristics of non-lipid fluorescent probes differ, of course, significantly

Abbreviations: AA, 12-(9-anthryl)-11-*trans*-dodecenoic acid; APC, 1-acyl-2-[12-(9-anthryl)-11-*trans*-dodecenoyl]-*sn*-glycero-3-phosphocholine; ASM, *N*-[12-(9-anthryl)-11-*trans*-dodecenoyl]sphingosine-1-phosphocholine; dansyl, 5-dimethylamino-1-naphthalenesulfonyl; DPH, 1,6-diphenyl-1,3,5-hexatriene; NBD, 7-nitrobenz-2-oxa-1,3-diazol-4-yl; PA, 9-(3-perylenoyl)nonanoic acid; PPC, 1-acyl-2-[9-(3-perylenoyl)nonanoyl]-*sn*-glycero-3-phosphocholine; PSM, *N*-[9-(3-perylenoyl)nonanoyl]sphingosine-1-phosphocholine.



Scheme I.

from those of the membrane lipids and, hence, such probes cannot tell anything about differences in the behavior of various lipid classes within the membrane. Recently, these considerations stimulated a growing interest in lipid-specific fluorescent probes which themselves are modified natural lipids.

Earlier, we described the synthesis, properties and some applications of fluorescent phosphatidylcholine (APC) and sphingomyelin (ASM) carrying a 9-anthrylvinyl group attached to the end of one apolar chain (Scheme I) [6–9]. These anthrylvinyl-labeled lipids appeared to be effective tools for investigations of lipid-protein interactions, based on the study of excitation energy transfer from protein tryptophans to the anthrylvinyl fluorophore. At the same time, anthrylvinyl probes are, however, insensitive to environmental polarity changes and can provide only limited information about lipid-lipid interactions in membranes.

With the aim to close these gaps, we have accomplished the synthesis of another series of lipid-derived probes – phosphatidylcholine (PPC) and sphingomyelin (PSM) containing 9-(3-perylenoyl)nonanoic acid (PA) [10]. In the present paper, we describe the fluorescent properties of these perylenoyl-labeled lipids and discuss the possibilities of the use of both types of lipid probes in membrane studies.

Materials and Methods

Syntheses of anthrylvinyl- [6,7] and perylenoyl- [10] labeled lipids were described elsewhere. Probe-containing samples were protected from illumination, only weak incandescent light was allowed. Fluorescence spectra (uncorrected) were registered on Hitachi MPF-3 and 650-60 fluorimeters in thermostated quartz cuvettes (10 × 10 mm); bandwidths were: for emission spectra, 5 nm for both excitation and emission; for excitation spectra, 2 nm (excitation) and 20 nm (emission). Fluorescence anisotropy values were determined as described earlier [8]; fluorescence intensities were measured in excitation spectra, at 370 nm for anthrylvinyl probes (λ_{em} 430 nm), and at 450 nm for perylenoyl probes (λ_{em} 520 nm). Excited state lifetimes were measured on a FLM 4800 phase modulation fluorimeter.

Sonicated vesicles were prepared in 50 mM Tris-HCl (pH 7.4) as described earlier [7], and large unilamellar vesicles were prepared in the same buffer also as described earlier [11], lipid concentration being 1 mg/ml; the absorbance of the suspension did not exceed 0.2 in the interval 350–700 nm. Ethanol solutions of the probes (1–2 mg/ml) were added using a microsyringe to aliquots of the vesicle suspension with intense stirring and subsequent 3 h incubation; the ethanol concentration in the samples did not exceed 0.5%. Vesicles for NMP spectroscopy were prepared from dimyristoylphosphatidylcholine with 4% of myristic acid, the probe (PPC or PSM) was added to the starting lipid mixture, the molar lipid/probe ratio being 5 : 1 [8].

Results and Discussion

Chocie and characteristics of the labels

Conversion of natural lipids into fluorescent analogues inevitably requires introduction of some structural changes. Since it was our intention to obtain lipid-specific probes that would mimic the corresponding natural lipids, our choice of fluorescent labels was based on the consideration that introduction of the fluorophore into the fatty acid chain should not alter appreciably the apolar character of the chain and should not change the headgroup properties of the prototype lipid. These

requirements are largely met by polycyclic aromatic hydrocarbon residues. Although such residues are more rigid, bulkier and less hydrophobic than aliphatic hydrocarbons, they may be expected to induce less perturbances in membranes than most other fluorophores. Indeed, the anthrylvinyl-labeled phosphatidylcholine and sphingomyelin, APC and ASM, synthesized previously in this laboratory [6,7], proved to mimic closely the corresponding host phospholipids and not to cause large perturbations in the phospholipid packing at probe concentrations used in fluorescence studies [8]. Due to effective overlapping of the excitation spectrum of the anthrylvinyl group and the tryptophanyl emission spectrum, use of the anthrylvinyl-labeled lipids in excitation transfer measurements opened good possibilities for the investigation of lipid-protein interaction.

For extention of the same method to studies of lipid-lipid interactions, new lipid probes were required whose fluorophore could serve as an acceptor of excitation energy transfer from the anthrylvinyl group. The possibility to use the new fluorophore as an energy donor for the excitation of anthrylvinyl group was excluded because in that case its excitation and emission spectra would overlap with those of tryptophan, preventing application of the probes in the presence of proteins.

The search for such fluorophores led us to test the 3-perylenoyl group. Substances containing this group are known [12] but, to our knowledge, have never been used as fluorescent probes. We found that the 3-perylenoyl chromophore absorbs strongly in ultraviolet and visible regions; the broad band in the visible part of the spectrum (maximum 446 nm) overlaps effectively with the emission spectrum of the anthrylvinyl fluorophore (Fig. 1).

The position of the emission maximum of the 3-perylenoyl fluorophore depends strongly on the environmental polarity (Fig. 2) and varies from 479 nm in hexane to 545 nm in glycerol. Unlike perylene, the excitation and emission main maxima of which are very close each to another [13], the 3-perylenoyl group displays a considerable Stokes shift. Its quantum yield is rather high: 0.55 in ethanol and 0.65 in hexane (for PA methyl ester).

The emission spectra of 3-perylenoyl-labeled

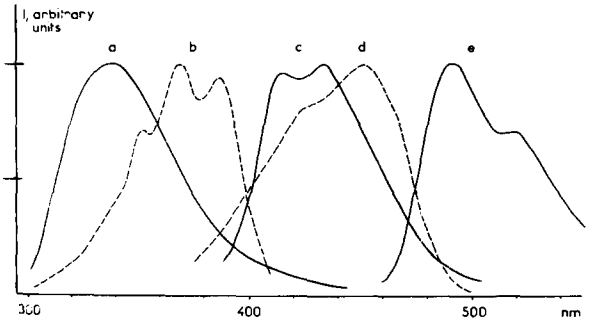


Fig. 1. Normalized spectra registered at 20°C: (a) emission of protein (human HDL_2) in 50 mM Tris-HCl (pH 7.4), λ_{ex} 294 nm; (b) excitation of AA, in dioxane, λ_{em} 430 nm; (c) emission of AA, in dioxane, λ_{ex} 370 nm; (d) excitation of PA, in dioxane, λ_{em} 520 nm; (e) emission of PA, in dioxane, λ_{em} 450 nm.

lipids in different solvents exhibit two maxima, in the 479–500 nm and 510–545 nm regions (Fig. 2). With increasing solvent polarity, the intensity of the first maximum decreases and the second maximum is shifted to longer wavelengths. Water quenching of the 3-perylenoyl fluorescence affects only the first maximum, the quenching follows the

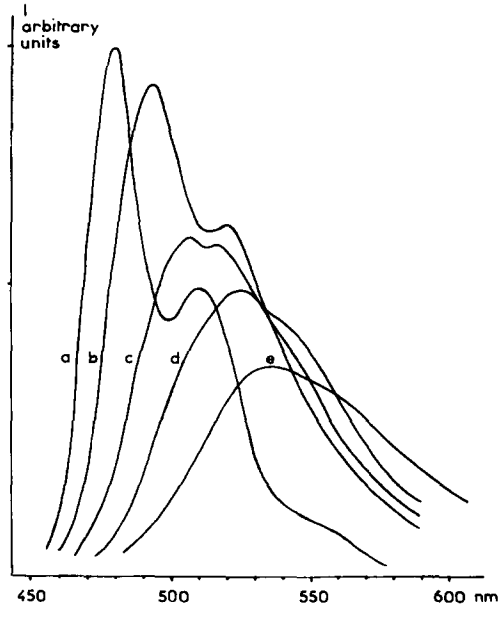


Fig. 2. Emission spectra of PA methyl ester registered at the same concentration; λ_{ex} 450 nm, temperature 22°C, in solvents: (a) hexane; (b) dioxane; (c) dimethylformamide; (d) isopropanol; (e) ethyleneglycol.

Stern-Volmer Law (Fig. 3). It is not excluded that the spectra result from the superposition of two or more species. In that case, the quenching could involve the loss of the fluorescence of a single species. Addition of water to dioxane solutions of PA methyl ester induces alterations in the emission spectrum similar to those produced by increasing the solvent polarity. No quenching occurred when water was added to an ethanolic solution of the same probe, only a small red shift of the emission maximum was recorded (7 nm for the change from 100 to 85%).

Behavior of the probes in model systems

Both anthrylvinyl- and perylenoyl-labeled phospholipids readily enter the bilayer. A part of the emission spectrum of PPC in egg phosphatidylcholine vesicles is shown in Fig. 4 (curve b); the inset shows the time-dependent increase of the fluorescence intensity after addition of the probe to the vesicles. PSM behaves similarly.

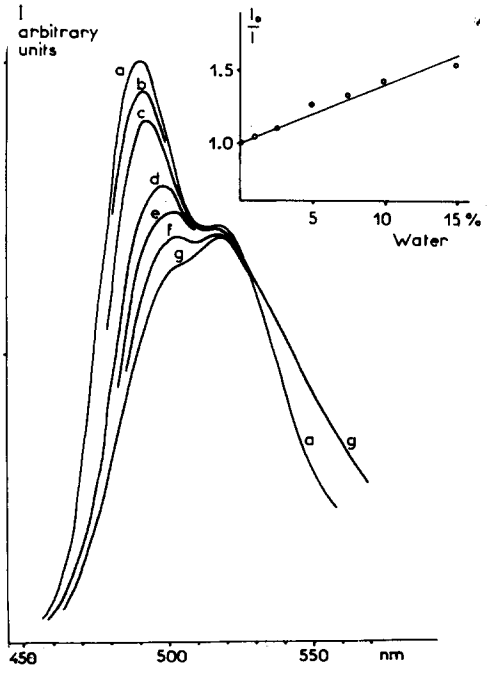


Fig. 3. Water quenching of fluorescence of PA methyl ester in dioxane (6 μg/ml), λ_{ex} 450 nm, temperature 20°C. Water concentration (%): (a)0; (b) 1.0; (c) 2.5; (d) 5.0; (e) 7.5; (f) 10; (g) 15. Inset: Stern-Volmer graph of the quenching; intensities of the first emission maximum (490–500 nm) were measured.

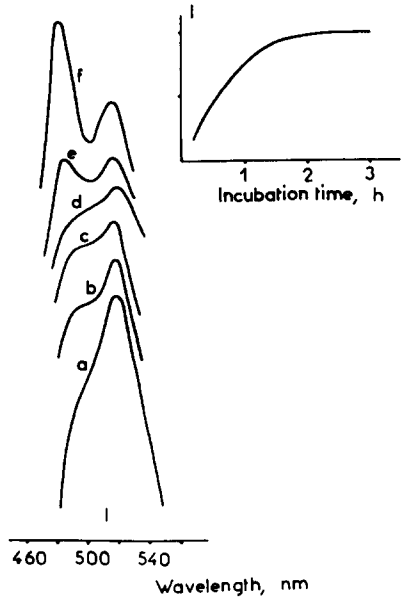


Fig. 4. Fluorescence maxima positions of PPC in vesicles made of: (a) dioleoylphosphatidylcholine; (b) egg phosphatidylcholine; (c) dimyristoylphosphatidylcholine; (d) egg phosphatidylcholine/bovine sphingomyelin (2:1, w/w); (e) egg phosphatidylcholine/bovine sphingomyelin/cholesterol (2:1:1, w/w); (f) dimyristoylphosphatidylcholine/cholesterol (2:1, w/w). Inset: intensity of PPC fluorescence in egg phosphatidylcholine vesicles as function of incubation time. λ_{ex} 450 nm, temperature 25°C.

For the anthrylvinyl probes APC and ASM, it was shown by NMR experiments that their fluorophores are localized in the central region of the bilayer and are oriented parallel to each other and perpendicular to the bilayer surface [8].

With the perylenoyl-labeled lipids, the situation is less clear. The similarity of the emission spectra of PA in low polar solvents and of PPC in phospholipid vesicles (cf. Figs. 3 and 4) suggests that in the bilayer, the 3-perylenoyl group is localized in the zone of low polarity corresponding to the fatty acid tail region [14]. This suggestion was confirmed by comparison of the NMR spectra of dimyristoylphosphatidylcholine vesicles prepared with and without the perylenoyl probes (Table I) (cf. Refs. 3 and 8).

The data of Table I show that the perylenoyl chromophore affects the fatty acid methylene proton resonances more (0.05–0.06 ppm shift) than it does those of the methyl groups (0.02 ppm shift).

TABLE I

NMR DATA FOR DIMYRISTOYLPHOSPHATIDYLCHOLINE VESICLES WITH INCORPORATED PROBES PPC AND PSM

The spectra were registered on a Varian SC 300 spectrometer; results are relative to acetate, low-field shifts are considered as negative; temperature 32°C.

Protons	Chemical shift (ppm)		
	Without probe	PPC added	PSM added
$C\underline{H}_3(CH_2)_{12}CO$	1.00	1.02	1.02
$CH_3(C\underline{H}_2)_{10}CH_2CH_2CO$	0.59	0.64	0.65
$N(CH_3)_3$	−1.35	−1.35	−1.35
$C\underline{H}_2N(CH_3)_3$	−1.79	−1.79	−[a]

[a] The value could not be determined because of CHNHCOR resonance overlapping.

It can be supposed on this basis that the perylenoyl fluorophore is localized in the region of those fatty acyl methylenes which are close to the glycerol moiety, but does not enter the polar region of the bilayer (the choline proton resonances are not affected).

However, an alternative explanation is also possible. It is known that aromatic systems impose not only diamagnetic (upfield) shift on resonances of neighboring protons which are not in the plane of the aromatic cycle, but also a paramagnetic (downfield) shift on resonances of those nearby protons which are in that plane. Thus, the fatty acyl methyl groups, being in the diamagnetic field of the nearest perylenoyl chromophores of their own bilayer leaflet, are, at the same time, in the paramagnetic field of perylenoyl groups of the opposite leaflet. The averaging of both effects should result in a smaller upfield shift of the methyl proton signals than of the methylene resonances which are less affected by chromophores of the opposite leaflet.

We observed a pronounced dependence of the perylenoyl emission spectrum on the vesicle lipid composition (Fig. 4). The emission spectrum of PPC in egg phosphatidylcholine vesicles (curve b) corresponds to that of PA methyl ester in solvents of medium polarity (85% dioxane; Fig. 3, curve g). Upon alteration of the vesicle lipid composition, the character of the spectra changes: in dioleoylphosphatidylcholine vesicles, the fluorophore environment appears to be more polar (curve a), whereas in dimyristoylphosphatidylcholine vesicles or vesicles made from a mixture of egg phosphatidylcholine/bovine sphingomyelin, the environmental polarity of the probe seems to be lower (curves c and d) than in egg phosphatidylcholine vesicles. Addition of cholesterol to the vesicles decreases the polarity of the probe environment (curves e and f). We suppose that these spectral differences are induced by differences in the water content of the fluorophore microenvironment. It is known that the hydrophobic region of phospholipid membranes contains some water, the dielectric constant of this zone being approx. 10 [14]. It has also been shown that the water permeability of the bilayer depends considerably on the lipid chain packing: increasing the number of double bonds leads to an increase of the water permeability, whereas addition of cholesterol results in a more dense arrangement of the chains and a concomitant water permeability decrease [15,16].

As can be seen from Fig. 4, the lipid composition-dependent changes correlate well with the water permeability changes that have been observed in vesicles of analogous composition [15,16]. However, whether the spectral differences are due to the water permeability of the vesicles or to the other factor, remains uncertain at the moment.

In order to evaluate how the probes reflect differences in the behavior of their prototype lipids, we studied the fluorescence anisotropy of anthrylvinyl- and perylenoyl-labeled phosphatidylcholines and sphingomyelins incorporated in vesicles of different lipid composition (see Table II).

TABLE II

FLUORESCENCE ANISOTROPY VALUES FOR ANTHRYLVINYL AND PERYLENOYL PROBES INCORPORATED IN EGG PHOSPHATIDYLCHOLINE VESICLES

S.D. ± 0.002.

Probe	Temperature (°C)	
	20	36.5
APC	0.054	0.038
ASM	0.064	0.046
PPC	0.168	0.125
PSM	0.173	0.129

The temperature plot of the fluorescence anisotropy of APC in large unilamellar vesicles shows a break near the phase transition temperature of the host lipid (23–24°C) (Fig. 5a) demonstrating that even at a relatively high concentration (1%), the probe does not form a separate phase. A less sharp break was observed with PPC (Fig. 5b). Probably, the perylenoyl group disturbs the bilayer to a higher extent than the anthrylvinyl group.

At the same time, the anisotropy values for the perylenoyl-labeled lipids are much higher than those of their anthrylvinyl counterparts (Fig. 5 and Table II). Judging from these data, the perylenoyl group must be more hindered in its motion than the smaller anthrylvinyl group (the measured excited state lifetimes of APC and PPC in egg phosphatidylcholine vesicles were 2.3 and 2.1 ns, respectively).

Anthrylvinyl → perylenoyl excitation energy transfer in membranes

The emission spectra of phosphatidylcholine vesicles doped with the probes 12-(9-anthryl)-11-*trans*-dodecenoic acid (AA) and PA (Fig. 6) or APC and PPC show effective excitation energy transfer from the antrhylvinyl to the perylenoyl fluorophore. That the significant decrease of anthrylvinyl fluorescence observed in these experiments is not due to direct light absorption by the perylenoyl probe, is shown by the fact that the anthrylvinyl regions (390–460 nm) of the emission

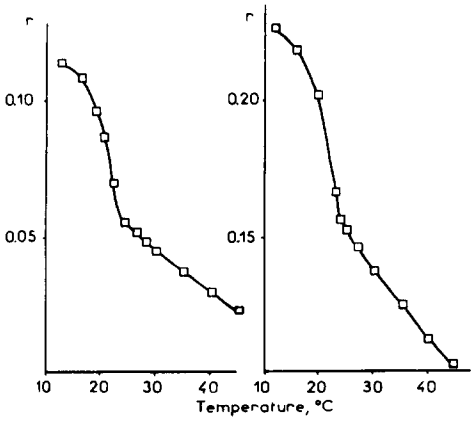


Fig. 5. Temperature dependence of fluorescence anisotropy of phospholipid probes in large unilamellar dimyristoylphosphatidylcholine vesicles, (a) APC, (b) PPC.

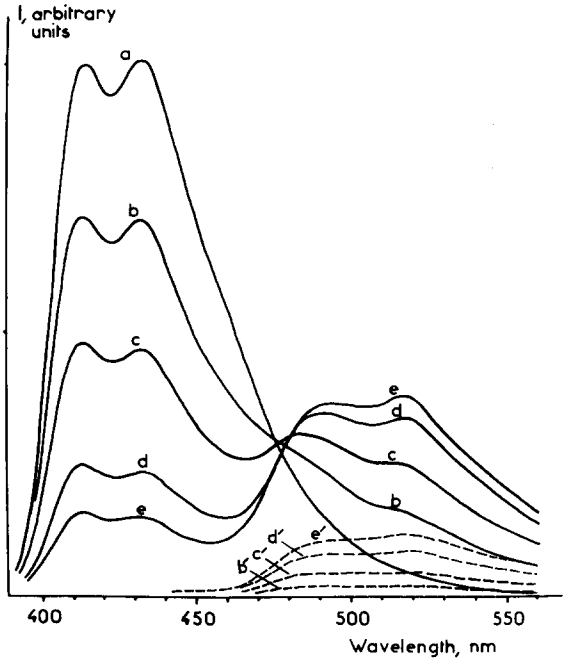


Fig. 6. Emission spectra of egg phosphatidylcholine vesicles with probes added: (a) 1 mol% AA; (b–e) 1 mol% AA + 0.2, 0.5, 1.0 and 1.5 mol% PA, respectively; (b′–e′) 0.2, 0.5, 1.0 and 1.5 mol% PA, respectively. λ_{ex} 370 nm, temperature 25°C.

spectra of APC-doped vesicles recorded immediately after dilution with the equal volume of PPC-doped vesicles or empty vesicles were identical (data not shown).

The effective overlap of the anthrylvinyl emission and perylenoyl excitation spectra (Fig. 1) opens new possibilities for studies of lipid-lipid interactions in membranes. Specifically, lipids bearing these labels permit to study intermembrane lipid transfer, avoiding separation of donor and acceptor membranes (cf. Ref. 17). This was demonstrated in experiments carried out with two populations of phospholipid vesicles, separately doped with an anthrylvinyl probe (APC or ASM) and with the perylenoyl probe PPC (Fig. 7). After mixing of equal volumes of both vesicle populations, the excitation energy transfer anthrylvinyl → perylenoyl increased steadily. For the pair APC-PPC, maximal energy transfer was reached nearly 2-times faster than for the pair ASM-PPC. This shows that the spontaneous intervesicular transfer of sphingomyelin proceeds much slower than that of phosphatidylcholine. During the ex-

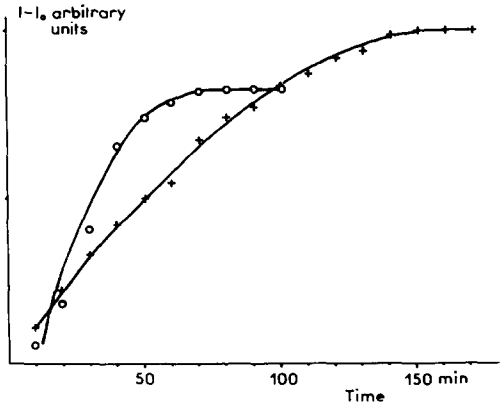


Fig. 7. Increase of excitation energy transfer induced by probe exchange between egg phosphatidylcholine/bovine sphingomyelin/cholesterol (2:1:1, w/w) vesicles, which were doped separately with APC and PPC (○) or ASM and PPC (+) (probe/lipid ratio, 1:100, w/w) and mixed. λ_{ex} 370 nm, temperature 36.5°C. Fluorescence intensities were taken at 520 nm immediately after mixing of vesicles (I_0) and through 10 min intervals (I).

periment, light scattering of the vesicle suspension did not change. Nevertheless, it is not excluded that some part of the energy transfer is due to vesicle fusion.

Perylenoyl-labeled lipids as fluorescent dyes

The rather strong light absorption of the perylenoyl fluorophore in the visible region (ε 23 500 $M^{-1} \cdot cm^{-1}$ at 446 nm) [10] and its high quantum yield allows the use of low concentrations of perylenoyl lipids for visualizing cells and other microscopic objects. The perylenoyl fluorophore emits in the 480–545 nm region which is convenient for work with natural objects, because the great majority of natural membrane components do not absorb or emit in that region.

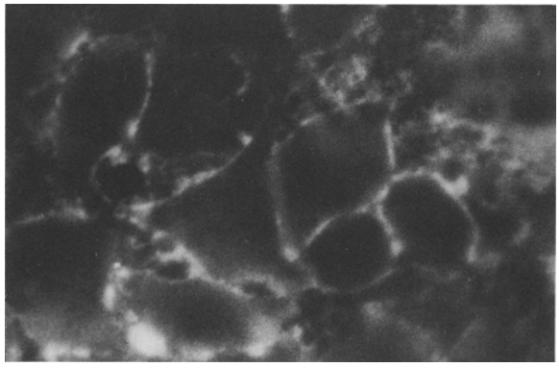


Fig. 8. Fluorescent micrograph (×375) of mouse L-fibroblasts treated with sonicated egg phosphatidylcholine vesicles, containing 1% PPC. Cells were incubated with vesicles in phosphate-buffered saline for 15 min at room temperature, and vesicles were washed away by the above saline.

These properties of the perylenoyl probes, together with their hydrophobicity and good stability, make them suitable dyes for fluorescent microscopy and automatic cell sorting. Preliminary experiments on cell labeling with perylenoyl probes gave promising results [18]. Fig. 8 shows fibroblasts stained with PPC.

Conclusions

A considerable number of fluorescent phospholipids with different labels in the apolar part of molecule have been described. These include parinaroyl phospholipids (see, e.g., Ref. 19), 3-pyrenyl- [20,21], 9-anthryl- [22], 2-anthryl- [23], *N*-dansyl- [24], *N*-NBD- [25], 9-carbazolyl- [26] and DPH- [27] labeled phospholipids. All these probes have their merits and disadvantages.

Although the 9-anthrylvinyl- and 3-perylenoyl-labeled lipids are also extrinsic probes and thus suffer from their specific shortcomings, they have some attractive features. Their fluorophores reside in the hydrophobic region of the bilayer, near the acyl methyl end groups; the positions of their excitation and emission maxima permit to use these probes in studies of lipid-protein (tryptophanyl → anthrylvinyl excitation energy transfer) and lipid-lipid (anthrylvinyl → perylenoyl transfer) interactions; both fluorophores show rather high quantum yields and fluorescence polarization values in membranes, and are of sufficient chemical and light stability. In addition, the anthrylvinyl-labeled lipids seem to produce minimal distortions of the lipid packing in membranes [8].

It has been suggested that the motion of a fluorescent molecule, e.g., DPH, may be significantly changed by its proximity to an integral protein, while that of the aliphatic lipid chains is not altered [28]. Of course, this may be true also for modified lipid molecules, such as those described in the present paper. It seems, however,

that differences (rather than the absolute values) of the fluorescent parameters of two probes having the same label but otherwise belonging to different membrane lipid classes, may provide reliable information about the peculiar behavior of the prototype lipids in the membrane. This was demonstrated convincingly by comparison of the fluorescence parameters of our probes introduced into high density lipoproteins (HDL). In human HDL, the fluorescence polarization and the excitation energy transfer of anthrylvinyl-labeled phosphatidylcholine and sphingomyelin (APC and ASM) were quite different despite the identity of their fluorophores [8]. Analogous large differences in the fluorescence polarization values were seen between perylenoyl-labeled phosphatidylcholine and sphingomyelin (PPC and PSM) in human HDL (not shown).

Hence, the immobilization of the probes by the apoproteins cannot be ascribed as entirely due to fluorophore-protein interactions. Since in homogeneous protein-free environment (egg phosphatidylcholine vesicles), the fluorescent analogues of phosphatidylcholine and sphingomyelin showed much smaller and much more similar polarization anisotropies, their different degrees of immobilization in HDL must reflect the different states of phosphatidylcholine and sphingomyelin in the lipoprotein globules.

Acknowledgements

The authors wish to thank Professor V.F. Bystrov and Dr. V.K. Kondakov for taking the NMR spectra, and Dr. L.B. Margolis for the micrograph of fluorescent-labeled L-fibroblasts.

References

1 Andersen, H.C. (1978) Annu. Rev. Biochem. 47, 359–383
2 Lakowicz, J.R. (1980) J. Biochem. Biophys. Methods 2, 91–119
3 Podo, F. and Blasie, J.K. (1977) Proc. Natl. Acad. Sci. USA 74, 1032–1036
4 Lentz, B.R., Barenholz, Y. and Thompson, T.E. (1976) Biochemistry 15, 4521–4528
5 Sklar, L.A., Doody, M.C., Gotto, A.M. and Pownall, H.J. (1980) Biochemistry 19, 1294–1301
6 Molotkovsky, Jul. G., Dmitriev, P.I., Nikulina, L.F. and Bergelson, L.D. (1979) Bioorgan. Khim. 5, 588–594
7 Molotkovsky, Jul. G., Manevich, Y.M., Dmitriev, P.I., Molotkovskaya, I.M. and Bergelson, L.D. (1981) Bioorgan. Khim. 7, 586–600
8 Molotkovsky, Jul. G., Manevich, Y.M., Gerasimova, E.N., Molotkovskaya, I.M., Polessky, V.A. and Bergelson, L.D. (1982) Eur. J. Biochem. 122, 573–479
9 Molotkovsky, Jul. G., Manevich, Y.M., Shvets, V.I., Smirnova, M.M., Krylova, V.N., Vorkunova, G.K., Bukrinskaya, A.G. and Bergelson, L.D. (1983) Voprosy Virusologii, 182–187
10 Molotkovsky, Jul. G. and Bergelson, L.D. (1982) Bioorgan. Khim. 8, 1256–1262
11 Szoka, F.J. and Papahadjopoulos, D. (1978) Proc. Natl. Acad. Sci. USA 75, 4194–4198
12 Gore, P.H. (1955) Chem. Revs. 55, 229–281
13 Shinitzky, M., Dianoux, A.-C., Gitler, C. and Weber, G. (1971) Biochemistry 10, 2106–2113
14 Waka, Y., Mataga, N. and Tanaka, F. (1980) Photochem. Photobiol. 32, 335–340
15 Graziani, Y. and Livne, A. (1972) J. Membrane Biol. 7, 275–284
16 Block, M.C., Van Deenen, L.L.M. and De Gier, J. (1977) Biochim. Biophys. Acta 464, 509–518
17 Pagano, R.E., Martin, O.C., Schroit, A.J. and Struck, D.K. (1981) Biochemistry 20, 4920–4927
18 Rudchenko, S.A., Molotkovsky, Jul. G., Orekhov, A.N. and Bergelson, L.D. (1983) Dokl. Akad. Nauk SSSR 269, 1487–1488
19 Sklar, L.A., Hudson, B.S. and Simoni, R.D. (1977) Biochemistry 16, 819–828
20 Galla, H.-J. and Hartmann, W. (1980) Chem. Phys. Lipids 27, 199–219
21 Pownall, H.J., Hickson, D., Gotto, A.M. and Massey, J.B. (1982) Biochim. Biophys. Acta 712, 169–176
22 Stoffel, W. and Michaelis, G. (1976) Hoppe-Seyler's Z. Physiol. Chem. 357, 7–19
23 De Boney, J. and Tocanne, J.-F. (1982) Chem. Phys. Lipids 32, 105–121
24 Huang, R.T.C. (1982) FEBS Lett. 145, 103–108
25 Nichols, J.W. and Pagano, R.E. (1982) Biochemistry 21, 1720–1726
26 Lakowicz, J.R. and Hogen, D. (1980) Chem. Phys. Lipids 26, 1–40
27 Morgan, C.G., Thomas, E.W., Moras, T.S. and Yianni, Y.P. (1982) Biochim. Biophys. Acta 692, 196–201
28 Chapman, D., Goni, F.M. and Pink, D.A. (1983) in Liposome Letters (Bangham, A.D., ed.), pp. 169–177, Academic Press, London