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New Diphenylhexatriene Derivatives as Fluorescent Membrane Probes: Partitioning Properties

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Three new diphenylhexatriene derivatives, two phospholipids and one single-chain amphiphilic molecule, have been synthesized and considered as probes for measuring membrane fluidity by fluorescence anisotropy. The possibility of using these probes to determine specifically fluidity of inner leaflets of cellular plasma membranes was inferred from their partitioning properties between gel and liquid crystalline phases of phospholipid vesicles of binary composition.

KEY WORDS: Diphenylhexatriene derivatives; partitioning properties; membrane fluidity.

INTRODUCTION

Fluorescence anisotropy is one of the most widely employed methods to measure membrane fluidity, with diphenylhexatriene (DPH) derivatives the most popular fluorescent probes because of their optimal photophysical properties. Particular interest has been devoted to cellular plasma membranes considered to be the locus of early responses of the cell to external stimuli, with possible fluidity effects. The composition and structure and, consequently, the fluidity of the two leaflets of plasma membranes differ significantly. Thus emerges the need of specific determinations of the fluidity of each leaflet.

In this work, two strategies were considered to probe selectively the inner leaflet of plasma membranes. First, we synthesized 1-palmitoyl-2-[3-(diphenylhexatrienyl)propanoyl]-*sn*-glycero-3-phosphoethanolamine (DPHpPE) with the hypothesis that such a PE derivative would localize in the PE-rich regions of plasma membranes, which, in most cases, are confirmed to be the inner leaflet. Second, two long-chain DPH derivatives were synthesized, namely, 1-palmitoyl-2-[21-(diphenylhexatrienyl)henicosanoyl]-*sn*-glycero-3-phosphocholine (LcDPHpPC) and 22-(diphenylhexatrienyl) docosyltrimethylammonium (LcTMA-DPH). We made the assumption that the long polymethylenic spacers will adopt a linear conformation and so cross the midpoint of the bilayer, ensuring a location of DPH moiety into the inner leaflet.

Partitioning properties of these probes between gel and liquid crystalline phases of lipid vesicles were measured by fluorescence anisotropy. Comparisons were made with two commercial probes, namely, DPHpPC and 3-(diphenylhexatrienyl) propyltrimethylammonium (TMAP-DPH). The data obtained allow us to check our starting hypothesis.

MATERIALS AND METHODS

The synthesis of the new DPH derivatives was described previously (1,2). Chemical structures are shown in Fig. 1. Multilamellar vesicles, fluorescently labeled, were obtained as published previously (2,3). The partition coefficients of the fluorescent probes, $K_{f/s}$, between

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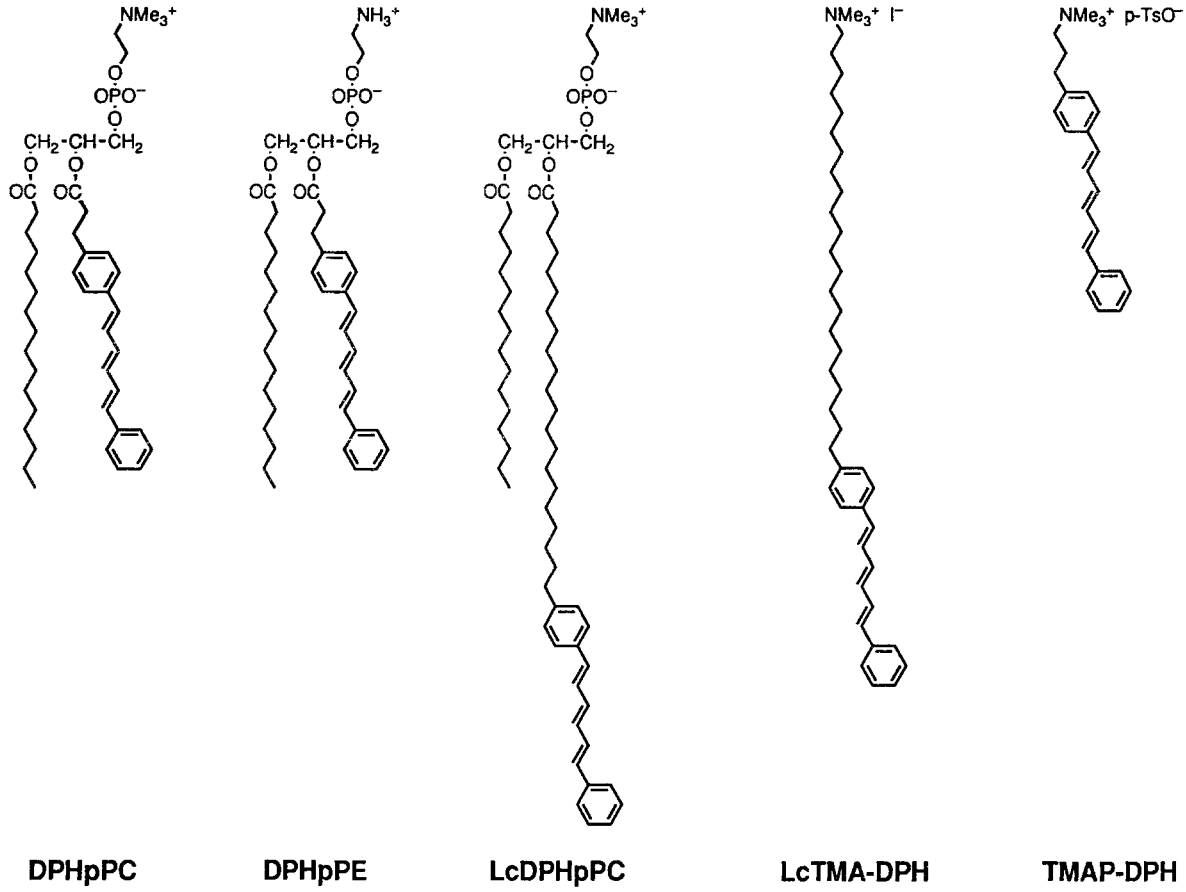

Fig. 1. Chemical structures of diphenylhexatriene derivatives.

the liquid-crystalline (fluid) and the gel (solid) phases were determined according to the method of Parente and Lentz (4), with our improvements (2,3). The experiments consist essentially in recording thermotropic profiles (Fig. 2) of the fluorescence anisotropy of the probes embedded in multilamellar vesicles of a binary composition, a high-melting and a low-melting phospholipid at varying ratios. The choice of the phospholipid pairs implies that corresponding phase diagrams are known.

RESULTS AND DISCUSSION

DPHpPE

Partition coefficients $K_{f/s}$ for DPHpPE and DPHpPC were obtained from lipid vesicles composed either exclusively of phosphatidylcholines, namely, dielaidoyl and disteraoyl PC, or of a mixture of PC and PE. In the latter condition, two systems were used, a high-melting PE with a low-melting PC, and vice versa (DEPC/DPPE and soybean PE/DPPC, respectively). $K_{f/s}$ data are given in Table I.

With DEPC/DSPC and DEPC/DPPE systems, DPHpPC shows a clear preferential partitioning for the fluid rather than the gel phase. The newly synthesized DPHpPE behaves differently. In the DEPC/DSPC system, it shows a small preferential partitioning for the gel phase, and in the DEPC/DPPE system, the preferential partitioning for the fluid phase is lowered. In the converse experiment, using low-melting PE and high-melting PC, both probes show a clear preferential partitioning for the gel phase (the PC phase). In fact, unsaturated PE, like soybean PE, may adopt a hexagonal H_{II} phase at high temperatures rather than a fluid lamellar phase. Thus, in this case, $K_{f/s}$ values would rather reflect a preference for the lamellar phase versus the hexagonal phase. What should be emphasized from these results is the fact that DPHpPE always seems to be "fleeing" from PE-rich regions. Never was a preferential parti-

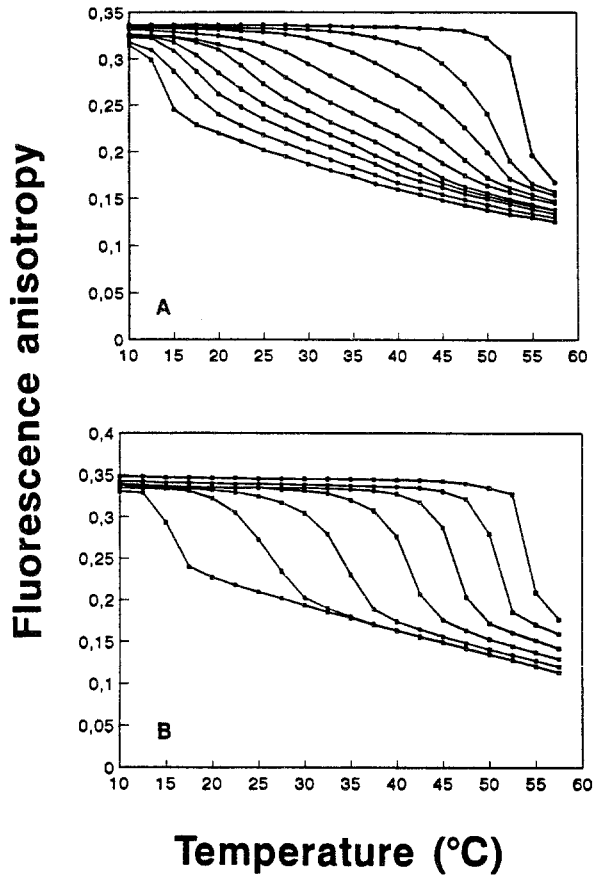

Fig. 2. Examples of thermotropic profiles of fluorescence anisotropy for DEPC/DSPC multilamellar vesicles. (A) TMAP-DPH and (B) LcTMA-DPH, with a phospholipid/probe ratio of 400. In both cases, the lower curve corresponds to 100% DEPC and the upper curve to 100% DSPC vesicles. In the A network, the intermediary curves range from 80 to 10% DEPC by steps of 10%. In the B network, the intermediary curves correspond to 90, 80, 60, 40, and 20% DEPC. Excitation wavelength, 350 nm; emission wavelength, 435 nm.

Table I. $K_{f/s}$ Data for DPHpPC and DPHpPE

Binary mixture	DPHpPC	DPHpPE
EPC/DSPC	2.6 ± 0.4	0.75 ± 0.2
DEPC/DPPE	2.8 ± 0.4	1.35 ± 0.5
Soybean PE/DPPC	0.5 ± 0.05	0.5 ± 0.05

tioning of this probe into a phase with the same PE polar head seen.

The Long-Chain Probes LcDPHpPC and LcTMA-DPH

Partition coefficients $K_{f/s}$ for LcDPHpPC and LcTMA-DPH, and their short-chain analogues, were determined using the DEPC/DSPC system. For the short-chain probes DPHpPC and TMAP-DPH, the $K_{f/s}$ was 2.6 ± 0.4 and 1.7 ± 0.3, respectively, indicating a preferential partitioning into the liquid crystalline phase. On the contrary, for the long-chain probes LcDPHpPC and LcTMA-DPH, the $K_{f/s}$ was 0.22 ± 0.11 and 0.12 ± 0.06, respectively, which means a strong partitioning into the gel phase. The reason for this particular behavior of long-chain probes may be explained by considering the interdigitation between the two leaflets and, more precisely, in the present case, the protrusion of the long polymethylenic chain among the acyl chains of the opposite leaflet of the bilayer. This assumption is supported by the work of Grant and colleagues (5,6) on similar systems. The better partitioning of long-chain species in a rigid medium appears to be a consequence of their interdigitation, favored by the better packing of acyl chains in a gel phase rather than in a liquid crystalline phase.

CONCLUSION

As DPHpPE never shows a preferential partitioning for PE-rich phases, this molecule cannot be considered as specific for any membrane leaflet enriched in PE domains. Concerning the long-chain probes LcDPHpPC and LcTMA-DPH, their strong partitioning for gel phases, explained by interdigitation of the long chains across the midpoint of the bilayer, allows the fluorophore to probe the inner leaflet, thus confirming our starting hypothesis.

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