Journal of Fluorescence, Vol. 3, No. 4, 1993

Influence of Lipid Composition and Membrane Curvature on Fluorescence and Solvent Relaxation Kinetics in Unilamellar Vesicles

R. Hutterer,[1] F. W. Schneider,[1] N. Pérez,[2] H. Ruf,[2] and M. Hof[3]

Received October 18, 1993

Time-resolved fluorescence on unilamellar vesicles shows that increasing amounts of anionic, natural lipid lead to a larger increase in polarity close to the headgroups than in the hydrophobic core of the bilayer. The region close to the headgroups is less polar in vesicles containing phosphatic acid rather than phosphatidylserine. A greater membrane curvature increases the mobility of the hydrated headgroups.

KEY WORDS: Lipid composition; membrane curvature; solvent relaxation; unilamellar vesicles; time-resolved fluorescence.

INTRODUCTION

Lipid composition and curvature are not only fundamental physicochemical parameters of membranes, but also regulating factors in enzymatic processes. In this work the effect of both lipid headgroup and vesicle size on the fluorescence decay of fluorophores and thus on membrane polarities is investigated. Furthermore, solvent relaxation times are determined to characterize the mobility of the hydrated headgroups.

MATERIALS AND METHODS

Phosphatidylcholine (PC) was isolated as described [1]. Phosphatidylserine (PS), phosphatic acid (PA), and dipalmitoylphosphatidylcholine (DPPC) were purchased from Fluka. The purity of lipids was checked by thin-layer chromatography. Small unilamellar vesicles (SUV) and large unilamellar vesicles (LUV) were prepared according to Refs. 2 and 3, respectively. Size distributions were determined precisely by dynamic light scattering, using the experimental setup, data collection, and data analysis described elsewhere [4]. The fluorophores 2- and 12-(9-anthroyloxy)stearic acid (2-AS, 12-AS), 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatrien (TMA),1,6-diphenyl-1,3,5-hexatriene (DPH), and 2-*p*-toluidinylnaphthalene-6-sulfonic acid (TNS) were obtained from Molecular Probes. Fluorophores dissolved in EtOH were added after vesicle preparation (lipid/fluorophore ratio, 400:1; lipid concentration, 1 mmol). The fluorescence decays were determined using single-photon counting equipment (Edinburgh Instruments 199 S). Data collection (λ_{ex} = 337 nm and λ_{em} = 430 nm, except for TNS) and data analysis were carried out as published [5]. Steady-state anisotropies were obtained using an Aminco Bowman II spectrometer.

RESULTS AND DISCUSSION

Influence of Natural, Anionic Lipids on Fluorescence Decay and Steady-State (ss) Anisotropies of 2-AS, 12-AS, and TMA in SUV

SUV composed of DPPC and different amounts of the natural, anionic lipids PS and PA were prepared.

[1] Institute of Physical Chemistry, University of Würzburg, D-97070 Würzburg, Marcusstr. 9/11, Germany.
[2] Max-Planck-Institute for Biophysics, D-60596 Frankfurt, Kennedy Allee 70, Germany.
[3] Institute of Physical Chemistry, Charles University, Cz-12840 Prague 2, Albertov 2030, Czech Republic.

1053-0509/93/1200-0257$07.00/0

The vesicles were labeled with 2-AS, 12-AS, and TMA, respectively. Excellent fits were obtained for all investigated systems using biexponential analysis. For PS/DPPC as well as for PA/DPPC vesicles, increasing amounts of anionic, natural lipid shorten the fluorescence lifetimes. This is shown for TMA in PS/DPPC vesicles by the average decay times [6] in Fig. 1. The effect is larger for 2-AS and TMA than for 12-AS as demonstrated in Table I. The decrease in the lifetimes of TMA (Fig. 2) and 2-AS (not shown) with increasing anionic, natural lipid content is significantly larger for PS than for PA. No significant differences for 12-AS between PS- and PA-containing vesicles were measured. Increasing amounts of anionic, natural lipid lead to a comparable decrease in the ss anisotropies for all dyes. Very similar results were obtained for PS and PA.

Increasing amounts of anionic, unsaturated lipid increase not only the fluidity, but also the polarity within the membrane. The increase in polarity with higher PS and PA contents is larger close to the interface (TMA, 2-AS) than in the hydrophobic core of the bilayer (12-AS). Since the anisotropies for PA/DPPC and PS/DPPC vesicles are comparable and the AS dyes are highly sensitive to polarity, the smaller reduction in the decay times for PA/DPPC systems (Fig. 2) indicates a lower polarity in the region close to the headgroups than in the PS/DPPC systems. This specific headgroup effect may be explained by strong intermolecular hydrogen bonds between PA headgroups and therefore closer headgroup packing. Thus the hydration of the headgroups may be lower for the PA- than the PS-containing vesicles, explaining the lower polarity in the PA systems.

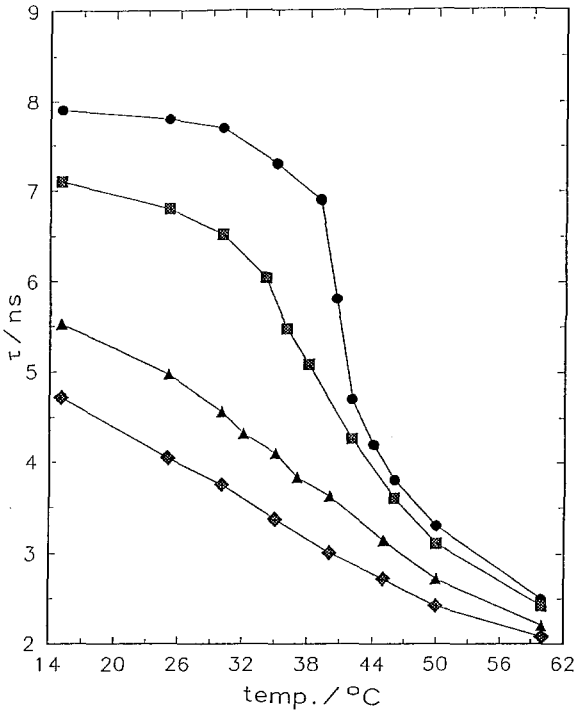

Fig. 1. Average decay times of TMA in PS/DPPC SUV as a function of PS content and temperature: (●) 100% DPPC; (▩) 80% DPPC/20% PS; (▲) 50% DPPC/50% PS; (◆) 20% DPPC/80% PS.

Table I. Percentage Decrease in Average Decay Times for 12-AS, 2-AS, and TMA in PS/DPPC SUV Due to Increasing PS Content (T=25°C)

	Percentage decrease		
	20% PS/ 80% DPPC	60% PS/ 40% DPPC	80% PS/ 20% DPPC
12-AS	−4	−6	−6
2-AS	−12	−18	−26
TMA	−12	−36	−48

[a]The reference was the decay time in 100% DPPC SUV.

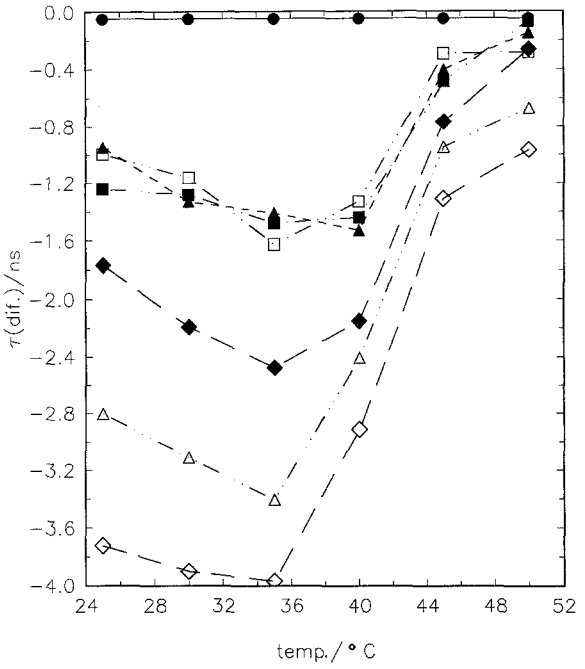

Fig. 2. Decrease in average decay times for TMA in PS/DPPC SUV (open symbols) and PA/DPPC SUV (filled symbols), respectively, due to increasing PS or PA content. The reference was the decay time in 100% DPPC SUV. (●) 100% DPPC; (▩) 80% DPPC/20% anionic lipid; (▲) 50% DPPC/50% anionic lipid; (◆) 20% DPPC/80% anionic lipid.

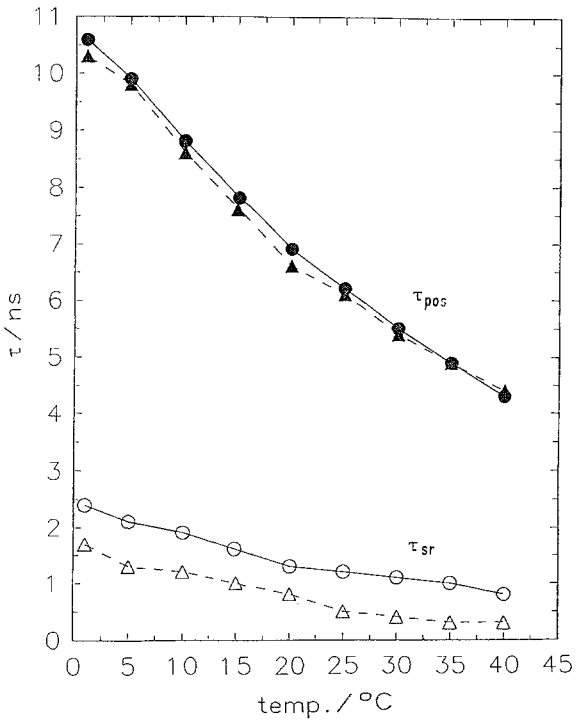

Fig. 3. Temperature dependence of the decay time of the mean relaxed state, τ_{pos}, of TNS in large (●) and small (▲) vesicles and of the solvent relaxation time, τ_{sr}, of TNS in large (○) and small (△) vesicles.

Influence of Membrane Curvature on the Decay Times of 12-AS and DPH and on the Solvent Relaxation Time of TNS

To investigate membrane curvature effects, two types of PC vesicles with a defined size distribution (SUV—average mass-weighted radius, r_m = 11 nm, distribution width Δr_m = 2.9 nm; LUV—r_m = 125 nm, Δr_m = 31 nm) were prepared.

The mobility of the hydrated headgroups was investigated by the solvent relaxation time τ_{sr} of TNS, which is adsorbed at the lipid–water interface region of membranes. The fluorescence decay was measured at the red edge of the emission spectrum (λ_{em} = 520 nm). It is well described by a biexponential decay law. Due to solvent relaxation one preexponential factor is negative. From the decay times τ_{pos} and τ_{neg} the solvent relaxation time τ_{sr} was calculated according to [7]

$$1/\tau_{neg} = (1/\tau_{pos}) + (1/\tau_{sr})$$

As shown in Fig. 3, τ_{pos}, which represents the decay time of a mean relaxed state [7], is independent of the vesicle size and decreases with increasing temperature. This indicates an identical TNS localization in systems with different membrane curvatures. However, τ_{sr} is up to 1 ns (corresponding to almost 100%) faster in SUV than in the larger vesicles. This may be explained by the lower packing density and hence higher mobility of the hydrated headgroups at the outer surface of the highly curved SUV membrane. The discussion above is focused on those TNS molecules that are adsorbed to the outer surface, since the negative charge of TNS prevents fast diffusion to the inner leaflet and the observed decay behavior remained constant within 24 h at 40°C. The fluorescence decays of DPH and 12-AS were biexponential and independent of the size of vesicles for the temperature range examined (1–50°C). Therefore membrane curvature does not affect the polarity deep inside the bilayer.

ACKNOWLEDGMENT

M.H. thanks the Fonds der Chemischen Industrie for support.

REFERENCES

1. W. S. Singleton, M. S. Gray, M. L. Brown, and J. C. White (1965) *J. Am. Oil Chem. Soc.* **42,** 53.
2. J. Brunner, P. Skrabal, and H. Hauser (1976) *Biochim. Biophys. Acta* **455,** 322.
3. L. T. Mimms, G. Zampighi, Y. Nozaki, C. Tanford, and J. A. Reynolds (1981) *Biochemistry* **20,** 833.
4. H. Ruf, E. Grell, and E. H. K. Stelzer (1992) *Eur. Biophys. J.* **21,** 21.
5. M. Hof, J. Schleicher, and F. W. Schneider (1989) *Ber. Bunsenges. Phys. Chem.* **93,** 1377.
6. J. R. Lakowicz (1983) *Principles of Fluorescence Spectroscopy*, Plenum Press, New York.
7. I. Gonzalo and T. Montoro (1985) *J. Phys. Chem.* **89,** 1608.