Journal of Fluorescence, Vol. 4, No. 4, 1994

Determination of the Partition Coefficients of the Nonionic Detergent $C_{12}E_7$ Between Lipid–Detergent Mixed Membranes and Water by Means of Laurdan Fluorescence Spectroscopy

Heiko Heerklotz,[1] Hans Binder,[1] and Gabriela Lantzsch[1]

Received October 18, 1993

The membrane–water partition coefficient of the detergent $C_{12}E_7$ between water and $C_{12}E_7$/POPC mixed membranes has been determined by means of steady-state fluorescence spectroscopy. The emission spectra of the fluorescent probe Laurdan were used as an indicator of membrane composition at different membrane concentrations in the sample. The partition coefficient expressed as the ratio of the mole fractions of the detergent in the membrane and water phases is about $6*10^5$ at low molar ratios of $C_{12}E_7$/POPC (R_e) and decreases rapidly with increasing R_e. The limiting detergent content of the lamellar phase ($R_e^* \approx 0.8$) is indicated by a minimum of $P(R_e)$.

KEY WORDS: POPC; nonionic detergent; $C_{12}E_7$; partition coefficient; Laurdan; solubilization.

INTRODUCTION

The interaction between lipid membranes and detergents is an object of interest for several fields of biophysics and biochemistry. On the addition of surfactants, biological membranes are solubilized to dissolve their ingredients [1]. Incorporation of suitable surfactant molecules into membranes can modify their fluidity, headgroup dipole density, and packing geometry. This is done, for instance, to study the origin of the "hydration force" between membrane surfaces [2].

The mixed heterogeneous system is characterized by partition of the components between the aggregates and the water phase. A general method for determining partition bases itself on a shift of any membrane composition-dependent parameter upon dilution of the system. Changes of the phase transition temperature (differential scanning calorimetry) and the point of starting solubilization (light scattering) [3] have been employed recently. The advantages of using a parameter determined by fluorescence spectroscopy are that a wide range of effective membrane composition is accessible and the experimental procedure is fast and convenient.

The fluorescence spectrum of dimethylaminonaphthalene (DAN) derivatives in lipid membranes is known to consist of two spectral fractions at 440 and 490 nm [4,5] resulting from a distribution of the probe molecules between two microenvironments [6,7]. The relation of their intensities is sensitive to the incorporation of surfactants into the membrane and can be conveniently expressed as "generalized polarization"(GP) [8].

Finding a continuous decrease in Laurdan GP upon addition of the surfactant, we conclude that equal GP values indicate equal local concentrations of surfactant in the membrane. Diluting a sample, the spectrum changes to that of one with a lower surfactant content, because some of the surfactant molecules ($C_{12}E_7$) leave

[1] Universität Leipzig, Fakultät für Physik und Geowissenschaften, Institut für Experimentelle Physik I, Abteilung Physik der Biomembranen, D-04103 Leipzig, Germany.

1053-0509/94/1200-0349$07.00/0

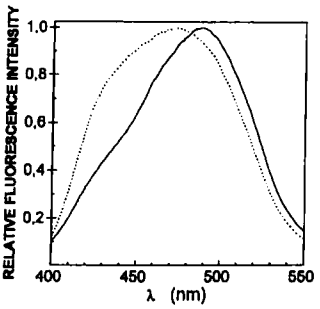

Fig. 1. Normalized emission spectra of Laurdan in aqueous dispersions of POPC (——) and POPC/$C_{12}E_7$ at a total molar fraction X_t=0.8 (····). The concentration of POPC was 0.5 m*M*.

the membrane to the added water. Comparing the effect of dilution with that of the total surfactant content in the sample allows calculation of the partition coefficient *P* and the real membrane concentration of surfactant corresponding to a given GP.

In this work the partition coefficient of the nonionic detergent heptaethyleneglycol-dodecyl ether $C_{12}E_7$ between water and lipid–surfactant mixed membrane vesicles has been investigated by Laurdan steady-state fluorescence spectroscopy. The determined partition coefficients describe the effective concentrations in the membrane. Its dependence on the surfactant content allows conclusions about the limiting ratio of additive per lipid for the lamellar phase [9].

EXPERIMENTAL

1-Palmitoyl-2-oleyl-*sn*-glycero-3-phosphatidylcholine (POPC) was purchased from Avanti Polar Lipids, heptaethyleneglycol-dodecyl ether [$C_{12}E_7$; i.e., CH_3–$C_{11}H_{22}$–(O–CH_2–CH_2)$_7$–OH] from Nikko Chemicals Ltd., Japan. The fluorescent probe 6-dodecanoyl-2-dimethylaminonaphthalene (Laurdan) was from Molecular Probes, Eugene, OR. All substances were used without further purification.

Stock solutions of all substances were prepared in spectroscopic-grade methanol and mixed in glass pistons. The solvent was removed under vacuum at room temperature. After the addition of purissimum water the samples were vortexed rapidly two times for 1 min each and left overnight under nitrogen at about 4°C.

The samples were found to consist predominantly of unilamellar vesicles. Ten samples containing fixed amounts of POPC (500 μ*M*) and Laurdan (1 per 250 lipids) and various amounts of $C_{12}E_7$ were prepared. Steady-state emission spectra were recorded at 25°C using quartz cuvettes on a Perkin Elmer LS50. The excitation wavelength was 337 nm.

After measuring the highest concentration the samples were added to water in five steps. In each dilution step the fluorescence spectrum was recorded after stirring for 10 min to equilibrate the system.

RESULTS AND DISCUSSION

The partition coefficient *P* is defined by [10]

$$P = X_e/X_w \tag{1}$$

where X_w and X_e are the molar fractions of surfactant monomers in the aqueous medium and in the aggregates, respectively.

$$X_e = D_e / (D_e + L)$$
$$X_w = D_w / (D_w + W) \tag{2}$$

with *L*, *D*, and *W* being the lipid, detergent, and water molar concentrations referred to the total volume. The index t is for total; the indices e, b, m, and w denote the fractions located in aggregates ("effective"), in bilayers, in micelles, and as monomers in water, respectively. In our case the water partial volume is nearly equal to the total sample volume, and we may use

$$X_w \approx D_w / W \tag{2a}$$

Introducing the effective molar ratio in the aggregates (bilayer or/and micelles),

$$R_e = D_e / L \tag{3}$$

we find

$$D_t = R_e * L + D_w \tag{4}$$

The generalized polarization (GP) values were calculated according to Parasassi et al. [8] using emission spectra uncorrected for detector response (Fig. 1):

$$GP = [I(440\text{ nm}) - I(490\text{ nm})] / [I(440\text{ nm}) + I(490\text{ nm})] \tag{5}$$

with the emission intensities (*I*) at fixed wavelengths. The GP values were plotted depending on the total surfactant molar fraction referred to the amphiphiles: $X_t = D_t/(D_t + L)$ (see Fig. 2). Generally we observed a continuous decrease in GP with increasing detergent content, which is suggested to be due to the increase in membrane fluidity.

The determination of the partition coefficient *P* is based on the single assumption that equal GP values indicate equal membrane compositions R_e. Cutting the

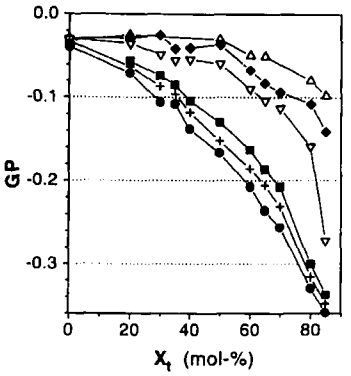

Fig. 2. Generalized polarization (GP) vs total molar fraction (X_t) of $C_{12}E_7$ in POPC/$C_{12}E_7$ aqueous dispersions for various lipid concentrations (L): 6 μM (△), 10 μM (♦), 20 μM (▽), 100 μM (■), 200 μM (+), and 500 μM (●).

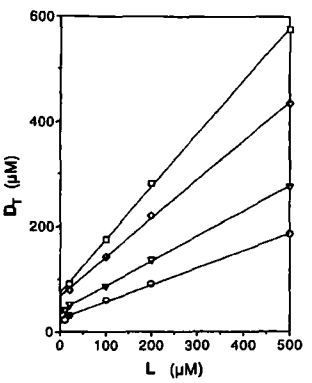

Fig. 3. Detergent concentration (D_w) vs lipid concentration (L) at constant GP: −0.09 (○), −0.12 (▽), −0.16 (◇), and −0.18 (□).

Table I. Effective Molar Ratio of $C_{12}E_7$/POPC (R_e) and Monomer Concentration of the Surfactant (D_w) Derived at Selected Generalized Polarization (GP) of Laurdan Fluorescence, Corresponding Partition Coefficients (P), and Standard Chemical Potentials ($\Delta\mu_0$).

GP	R_e	$D_w(\mu M)$	$P(10^5)$	$\Delta\mu_0$(kJ/mol)
−0.07	0.217 ± 0.005	17 ± 1	5.7 ± 0.5	32.9 ± 0.2
−0.09	0.323 ± 0.009	25 ± 2	5.3 ± 0.6	32.7 ± 0.3
−0.10	0.36 ± 0.01	30 ± 3	4.7 ± 0.6	32.4 ± 0.3
−0.12	0.473 ± 0.007	40 ± 2	4.4 ± 0.2	32.2 ± 0.1
−0.14	0.60 ± 0.01	53 ± 4	3.9 ± 0.3	31.9 ± 0.2
−0.16	0.74 ± 0.01	69 ± 4	3.4 ± 0.2	31.6 ± 0.2
−0.18	1.00 ± 0.02	75 ± 4	3.7 ± 0.2	31.8 ± 0.1
−0.21	1.68 ± 0.04	63 ± 12	5.5 ± 1.1	32.8 ± 0.5

curves for the various dilution steps at a fixed GP value, one finds interpolated X_t values for each of them. Thus a set of data pairs (L, D_t) is obtained after transforming X_t to D_t. Then Eq. (4) allows the calculation of R_e and D_w by a linear regression (Fig. 3). With (1), (2), and (3), we get the partition coefficient P:

$$P = \frac{R_e}{1 + R_e} * \frac{W + D_w}{D_w} \tag{6}$$

This approach is limited to the range of GP(R_e) (see Fig. 2) with a significant slope ($R_e > 0.2$) and a sufficient number of dilution steps reaching this GP (number of points in the linear fit) ($R_e < 1.6$).

The partition coefficient of the fluorophore Laurdan itself does not influence the method in binary aggregate–water equilibria for several reasons. Because relative changes of the spectra are analyzed, the probe concentration within the aggregates does not affect the GP. Laurdan monomers in aqueous solution do not contribute to the GP value because of the very low concentration, low quantum yield, and different fluorescence maximum wavelength. Correspondingly, we observe the GP of the pure lipid sample to remain constant upon dilution.

As mentioned above, the fit of Eq. (4) yields the effective ratio R_e in the aggregates and the corresponding aqueous detergent concentration D_w. According to Eq. (6) we get the partition coefficient P in dependence on X_e. It is related to the difference of the standard chemical potential upon transfer of detergent molecules from water into the aggregate [10]:

$$\Delta\mu^\circ = RT \ln P \tag{7}$$

The obtained data are presented in Table I.

For increasing effective ratios of detergent to lipid in the membrane (R_e), the partition P decreases continuously at $R_e < 0.8$, accompanied by an increasing monomer concentration D_w.

Assuming that $\Delta\mu^\circ$ at low R_e is due mainly to the detergent–lipid interaction and $\Delta\mu^\circ$ at higher R_e is dominated by the detergent–detergent interaction, we conclude the latter to be less favorable than the first, i.e., demixing due to preferable detergent–detergent interaction is thermodynamically prohibited.

In the average aggregate composition range $R_e \approx$ 0.8...2.3 the partition coefficient increases due to the formation of mixed micelles coexisting with mixed bilayers (solubilization), i.e., the fluorescence method measures an average partition coefficient (P) of the detergent between the aggregates and the water phases. This apparent P depends on the bilayer–water and micelle–water partition as well as on that of Laurdan between both types of aggregates. Inhomogeneous partition of the Laurdan will lead to deviations of the apparent D_w values from the real, which should be constant in the micelle–bilayer

coexistence range. Detailed interpretation of the data at $R_e > 0.8$ by means of a model considering the bilayer–micelle–water equilibrium of the components will be published elsewhere [11].

Edwards and Almgren [3] investigated the partition of $C_{12}E_8$ between water and egg yolk lecithin vesicles at $R_e = 0.62$ to be $P = 3.0*10^5$. This value is somewhat lower than that determined for $C_{12}E_7$ at $R_e = 0.6$ ($P=3.9*10^5$, see Table I), which is due to the change of the hydrophilic–lipophilic balance upon the addition of 1 hydrophilic oxyethylene unit.

The limiting ratio of the lamellar phase of $C_{12}E_7$/POPC ($R_e = 0.8$) is slightly higher than that for $C_{12}E_8$/EYPC ($R_e = 0.62$) obtained by means of light-scattering experiments [3]. This can be understood considering the larger headgroup of $C_{12}E_8$, which tends to introduce a higher curvature to the system.

CONCLUSIONS

The partition of a membrane fluidizing detergent between water and lipid bilayers can be investigated by fluorescence spectroscopy using Laurdan generalized polarization. The partition coefficients are of the order of magnitude of 10^5 and decrease rapidly with the surfactant contents in the bilayer.

The presented evaluation of the experimental data limits the method to the range of effective detergent contents lower than the limiting ratio of the lamellar phase, which is indicated by a sharp minimum of partition P.

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 294).

REFERENCES

1. C. Tanford and J. A. Reynolds (1976) *Biochim. Biophys. Acta* **457**, 133–170.
2. G. Klose, B. Kõnig, and S. Eisenblätter (1994) *J. Coll. Interf. Sci.* (in press).
3. K. Edwards and M. Almgren (1991) *J. Coll. Interf. Sci.* **147**(1), 1–21.
4. J. R. Lakowicz, D. R. Bevan, B. P. Maliwal, H. Cherek, and A. Balter (1983) *Biochemistry* **22**, 5714–5722.
5. G. Weber and F. Farris (1979) *J. Am. Chem. Soc.* **18**(14), 3075–3078.
6. T. Parasassi, G. De Stasio, G. Ravagnan, R. Rusch, and E. Gratton (1991) *Biophys. J.* **60**, 179–189.
7. P. L. Chong (1988) *Biochemistry* **27**, 399–404.
8. T. Parasassi, G. De Stasio, A. d'Ubaldo, and E. Gratton (1990) *Biophys. J.* **57**, 1179–1186.
9. D. Lichtenberg, R. J. Robson, and E. A. Dennis (1983) *Biochim. Biophys. Acta* **737**, 285–304.
10. C. Tanford (1973) *The Hydrophobic Effect,* Wiley & Sons, New York.
11. H. Heerklotz, H. Binder, G. Lantzsch, and G. Klose (1994) *Biochim. Biophys. Acta* (in press).