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Biochimica et Biophysica Acta 1369 (1998) 304–308

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Solubilization of planar bilayers with detergent

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Received 30 July 1997; revised 19 September 1997; accepted 24 September 1997

Abstract

The interaction of the nonionic detergent Triton X-100 with supported phosphatidylcholine planar lipid bilayers has been investigated by optically monitoring changes in the bilayer, using the technique of optical waveguide lightmode spectroscopy (OWLS). This technique has several advantages over the methods applied to the problem hitherto, including: high sensitivity; measurement in situ with good time resolution; the fact that the free detergent concentration is well-defined, and the lipid concentration in solution is zero; ease of studying the reversibility of the interaction; and the readiness with which absolute rather than effective amounts of detergent incorporated into the lipid can be determined. The main finding is that as the free Triton concentration increases, the detergent is first incorporated reversibly into the bilayer, then partly but never completely removes lipid, and finally (at or above the cmc) completely solubilizes the bilayer. The behaviour of the planar supported lipid bilayers is thus similar to that previously reported for lipid vesicles. © 1998 Elsevier Science B.V.

Keywords: Phospholipid; Detergent; Planar bilayer; Optical waveguide lightmode spectroscopy (OWLS)

1. Introduction

Supported lipid bilayers (i.e. lipid bilayers deposited on a planar solid substrate) are widely used as model biological membranes. They are extremely convenient for carrying out a variety of physical measurements, such as the determination of the lateral mobility of membrane constituents using fluorescence recovery after photobleaching (FRAP) [1], and the accurate determination of the kinetics of protein association with the membrane using optical waveguide lightmode spectroscopy (OWLS) [2–5]. In addition, they are also an excellent way to immobilize receptor proteins on the sensing pad of biosensors [6].

In these applications, it is often required to incorporate a protein into the lipid bilayer. Integral membrane proteins are usually extracted from their native environment using detergent [7], and even after purification, the protein remains associated with detergent; indeed this is usually the only state in which it is water soluble and can be conveniently handled.

The question whether the detergent has an adverse effect on the lipid membrane therefore arises. Our aim was to investigate the lipid–detergent interaction using optical waveguide lightmode spectroscopy (OWLS), which allows the opto-geometrical parameters of the membrane characterizing its structure to be investigated in situ (i.e. in the presence of detergent solutions and during rinsing with detergent-free buffer), and which is more convenient and accurate than ellipsometry.

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PII S0005-2736(97)00233-2

Previous work on lipid–detergent interactions (see reviews [8–10]) has used lipid bilayers in the form of vesicles [11–14,16–19], which have certain disadvantages for this kind of investigation. Apart from their intrinsic instability even in the absence of detergent, effects of the incorporation of detergent can only be estimated by indirect means (e.g. from changes in the turbidity of the solution). In particular the (incorporated detergent)/lipid ratio, one of the key parameters controlling the behaviour, cannot be determined very precisely, especially when multilamellar vesicles are used [1]. A further limitation of vesicles is that it is difficult to ascertain the reversibility of any interaction.

Using OWLS we have measured the effects of increasing concentrations of the nonionic detergent Triton X-100, widely used for membrane protein solubilization [7], on a planar phosphatidylcholine bilayer. At low concentrations, about half the critical micelle concentration (cmc), detergent is reversibly incorporated into the bilayer without adverse affect. At intermediate concentrations, there is a slow, irreversible solubilization of lipid. At high concentrations (above the cmc) the entire membrane is efficiently removed from the solid support.

2. Materials and methods

Synthetic palmitoyloleoylphosphatidylcholine (POPC) was purchased from Avanti Polar Lipids (Alabaster, Alabama) and used without further purification. Other chemicals, including Triton X-100, were analytical grade from Fluka (Buchs, Switzerland). The same buffer, 10 mM 4-(2-hydroxyethyl)-piperazine-1-ethane-sulphonic acid (Hepes)-NaOH, pH 7.3, was used throughout, i.e. for presoaking the optical waveguides, as the subphase during Langmuir–Blodgett deposition of the lipid bilayers, and for preparing the Triton X-100 solutions. The refractive index n_C of the buffer, and the refractive index increment $(dn/dc)_{det}$ of the Triton solution ($0.154\,cm^3/g$), were measured with an LI3 Rayleigh interferometer (Carl Zeiss, Jena, Germany). Planar optical $Si_{0.76}Ti_{0.24}O_2$ waveguides ('chips') incorporating a grating coupler (grating constant $\Lambda = 714.29$ nm) were obtained from Artificial Sensing Instruments, Zurich, Switzerland (type 1400). Before use the chips were equlibrated overnight in buffer solution.

2.1. Langmuir–Blodgett (LB) bilayer deposition

An appropriate amount of POPC was dissolved in 9:1 hexane:ethanol solution and spread on the surface of a laboratory-built Langmuir trough filled with buffer. After 5 min (the time needed for solvent evaporation) the lipid was slowly compressed to a surface pressure of 32 mN/m. The waveguide, lowered into the subphase prior to spreading the lipid, was then slowly (0.102 mm/s) raised vertically while keeping the surface pressure constant. This operation resulted in the deposition of a lipid monolayer on the surface of the waveguide. It was then lowered rapidly and horizontally through the floating monolayer into the subphase, resulting in a supported bilayer.

2.2. Optical waveguide lightmode spectroscopy (OWLS)

A small, round flow-through cuvette (diameter 10 mm, volume 23 mm^3) was sealed to the waveguide with an 'O'-ring such that it formed one wall of the cuvette. This ensures that the free detergent concentration (the thermodynamically relevant quantity) is constant and equal to the bulk dissolved Triton concentration. The assembly was then transferred to the goniometer head of an IOS-1 integrated optical scanner (Artificial Sensing Instruments, Zurich, Switzerland). In this instrument a monochromatic, polarized light beam (He–Ne laser, wavelength $\lambda = 632.82$ nm) is directed to the grating coupler area of the chip [20], and the angle α between the grating normal and the incident laser beam varied with microradian precision by a computer-driven stepping motor. At certain angles the light is coupled into the waveguide and can be detected with photodiodes positioned at the ends of the waveguide. The angles corresponding to the incoupling maxima are then used to calculate the effective refractive indices N of the transverse elec-

[1] Moreover, their high curvature is expected to result in a radius-dependent term in the expression describing the interaction energetics; the impossibility of preparing a truly monodisperse vesicle suspension complicates attempts to take this into account.

tric (TE) and transverse magnetic (TM) modes, according to the incoupling relation [21]:

$$N = n \sin\alpha + \ell\lambda/\Lambda \quad (1)$$

where n is the refractive index of air and ℓ is the diffraction order.

2.3. Sequence of measurements

First, pure buffer was allowed to flow through the cuvette in order to determine the parameters of the waveguiding film in the absence of an adlayer (n_F, the refractive index of the waveguiding film and d_F, the thickness of the film) from the measured N by solving the three-layer mode equations [21]. Subsequently the chip was removed from the IOS-1 instrument in order to deposit an LB bilayer, and then replaced. The lipids deposited on the surface form an adlayer A whose thickness d_A and mean refractive index n_A can be found by solving the four-layer mode equations [21]. To determine the amount L of lipid deposited on the surface we used the expression [22]:

$$L = d_A(n_A - n_C)/(\mathrm{d}n/\mathrm{d}c)_{lip}. \quad (2)$$

Here $(\mathrm{d}n/\mathrm{d}c)_{lip}$ is the refractive index increment for lipids, equal to $0.083\,cm^3/g$. Finally we allowed Triton solution to flow over the membrane. Throughout our experiments we used a flow rate of $0.5\,\mu l/s$, which was sufficient to maintain the dissolved Triton concentration in our measurement cell constant, whatever the degree of incorporation into the lipid bilayer. The mass of the lipid bilayer–Triton mixture was calculated according to (Eq. (2)), using $(\mathrm{d}n/\mathrm{d}c)_{lip}$. In region I (see Section 3), in which Triton is added to the bilayer and no lipid is removed, after subtracting the known pure lipid bilayer mass from the mixed Triton–lipid mass we multiplied the remainder by $(\mathrm{d}n/\mathrm{d}c)_{lip}/(\mathrm{d}n/\mathrm{d}c)_{det}$ in order to determine the mass of incorporated Triton. The Triton flow was always followed by pure buffer.

3. Results

Depending on the free Triton concentration c_T, we observed three types of behaviour, which we denote I, II, and III in order of increasing c_T (see Fig. 1).

I. $c_T < 0.19$ mM. Triton associates with the bilayer but can be completely removed by washing with pure buffer leaving the bilayer intact. The smallest Triton concentration giving a measurable signal was 80 μM. The adsorption curve reached a maximum value in 3–6 min. The maximum molar detergent/lipid ratio (D/L) was 0.31 (at 0.18 mM Triton solution). Fig. 2 shows the variation of D/L with c_T.

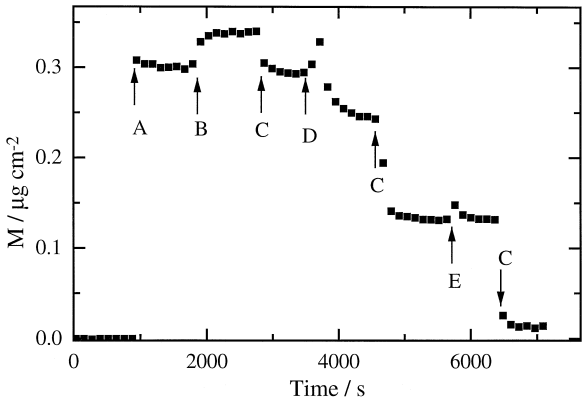

Fig. 1. Effect of different concentrations of Triton X-100 on a planar POPC bilayer. Arrows: A-bilayer deposition; B-0.1 mM Triton X-100; C-buffer; D-0.2 mM Triton X-100; E-1 mM Triton X-100.

II. $0.19 < c_T < 0.24$ mM. Triton starts to bind to the bilayer, but after reaching a certain value adsorption stops and removal of the bilayer is observed, which was faster with increasing Triton concentration. When pure buffer now flowed over the surface, a larger and more rapid decrease was observed. Adding detergent again to the solution resulted in a

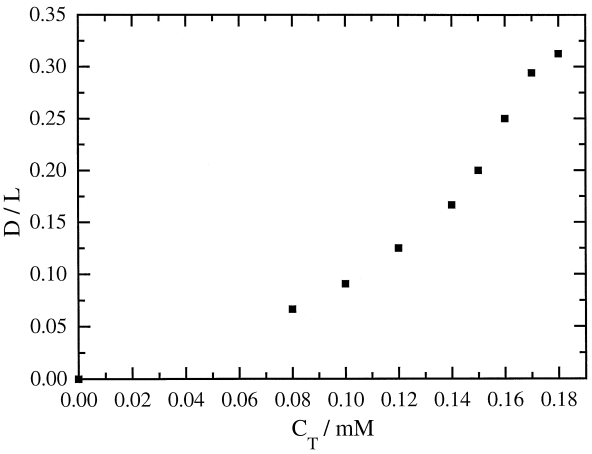

Fig. 2. Detergent/lipid ratio (D/L) in the bilayers versus the free Triton concentration c_T in the buffer at low concentrations (corresponding to arrow B in Fig. 1), calculated as described at the end of Section 2.

reversible increase in the adsorbed mass. In this concentration range it was never possible to remove the bilayer completely.

III. $c_T > 0.24$ mM. The lipid bilayer is immediately and completely removed. Following this an adlayer of Triton is adsorbed at the waveguide surface, but this monolayer can be completely removed by washing with pure buffer.

4. Discussion

Today it is generally accepted that, as originally proposed by Helenius and Simons [8], the solubilization of phospholipid bilayers (vesicles) by detergents occurs in three stages depending on the nature and concentration of the detergent: I, partitioning of the detergent between the aqueous phase and the bilayer; II, gradual solubilization of the bilayer, lipid vesicles saturated with detergent coexist with mixed micelles of lipid and detergent; III, complete solubilization of the bilayer, only mixed micelles exist. The proposal itself and all the supporting data are based on experiments using different vesicle systems, and no results have been hitherto obtained with planar bilayers (LB films). We can interpret the present result within the framework of the Helenius and Simon model, i.e. our regions I, II and III described in Section 3 appear to correspond to theirs.

4.1. Region I

Our experiments clearly show the existence of a critical Triton concentration in the buffer (0.18 mM), up to which the bilayer becomes fully saturated with Triton, but otherwise remains intact. The value of the critical concentration is in good agreement with the results of Paternostre et al. [16] [2]. The detergent/lipid ratio D/L is close to the R_e values reported in the literature [15,18,19] [3].

Since the Triton–lipid association is completely reversible we can determine a partition coefficient K_T, defined as

$$K_T = \frac{D/L}{c_T}. \quad (3)$$

At saturation (Fig. 2) $K = 1.77\ \mathrm{mM}^{-1}$, practically the same value as that obtained by Partearroyo et al. [18].

4.2. Region II

Our second stage is more difficult to compare with the vesicle literature, since our measurement gives no information about any lipid–detergent micelles which may be formed. The big decrease occurring when the solution is switched back to pure buffer after detergent (Fig. 1, arrow C following arrow D) can be interpreted in the following way: the detergent partially solubilizes the bilayer leaving some detergent–lipid micelles still bound to the surface, which are then washed away by the buffer.

4.3. Region III

Complete solubilization of the bilayer occurs at the reported cmc of the Triton (0.24 mM [8]).

5. Conclusions

The present methods (OWLS) is able to measure the incorporation of Triton into lipid bilayers more directly than before. Results show that planar bilayers behave in a similar way to vesicles. The detergent is already incorporated into the bilayer below the cmc, and above a critical concentration partial solubilization of the bilayer takes place. The bilayer is completely solubilized only at concentrations equal to or above the cmc.

[2] In the vesicle experiments, the solubilization is analyzed according to $D_{tot} = c_T + R_e[\mathrm{lipid}]$, where D_{tot} is the total detergent concentration and R_e is defined in [3]. In our system, however, the lipid concentration [lipid] = 0, since all the lipids are attached to the waveguide surface. Moreover, the amount of detergent incorporated in the lipid bilayer is always much smaller than that present in the volume of the cuvette. Hence we can put $D_{tot} = c_T$.

[3] Lichtenberg [15] suggested a method for calculating the (incorporated detergent)/lipid ratios from series of data in which both lipid and detergent concentrations are varied. His procedure enables an effective (incorporated detergent)/lipid ratio R_e to be defined and determined.

Acknowledgements

This work formed part of the COST project ''Preparation and characterization of model biological interfaces'' within Action D5 ''Chemistry of surfaces and Interfaces''. We thank the Swiss COST office for their encouragement, and the Federal Office of Education and Science for financial support.

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