STUDIA UNIVERSITATIS BABES-BOLYAI, CHEMIA, XLIV, 1(2),2000

SPECTROPHOTOMETRIC AND RECTANGULAR DIFFUSION STUDY OF SOLUBILIZING EGG YOLK LECITHIN LIPOSOMES WITH DETERGENTS UNDER TITRIMETRIC AND EQUILIBRIUM CONDITION

ZITA FAZAKAS[1], ŞTEFAN HOBAI[1] and JÁNOS ZSAKÓ[2]

1. *Department of Biochemistry, University of Medicine and Pharmacy, 38 G.Marinescu st.,Tg.Mures, Romania.*
2. *Department of Physical Chemistry, Babes-Bolyai University,* 11 *Arany János st.,Cluj, Romania.*

ABSTRACT. The interaction of the liposomes with detergents helps to understand the solubilization of biological membranes for protein separation and reconstitution.

After evaporation of an organic solvent from a solution of egg yolk lecithin, the lipid was hydrated and sonicated resulting a SUV suspension. The suspension was titrated with detergent solutions (Triton X-100, sodium deoxycholate) and the optical transmissions and diffusions were recorded. At the same time these optical values were measured for samples at equilibrium. The differences between these values were interpreted in terms of thermodynamics.

INTRODUCTION

Liposomes are models of cellular membrane, due to lipid arrangement in their membrane and their electrochemical properties. They can be used as drug vectors with controlable pharmacokinetics.They are biodegradables, having a small intrinsic toxicity and a reduced antigenic potential.

This study concerning the interaction of phospholipid liposomes with non-ionic and ionic detergents is determined by the fact that biological membrane solubilization for separation or membrane protein reconstitution is achieved by this procedure [1,2].

It integrates in actual interest in founding some procedures for isolation of membrane proteins or their integration in phospholipid anulus into membranes.
The solubilization of vesicle membranes is frequently studied by measuring turbidity, optical diffusion and absorption, as well as fluorescebce [3-9].

I. Materials

1. Devices: Rotary evaporator Buchi type 350. Sonicator with titanium probe type Ultrasonics A180G.Spectrophotometer Specol with zv amplifier and FR optical diffusion system and photocells. Microtitrator TiMi. Recorders K-200. Automatic microburette Radelkis OP-930.

2. Substances: Egg yolk lecithine, SIGMA type X-E (cat.no.P5394/1996) purified by us by neutral alumina column chromatography, verified by thin layer chromatography (silicagel). The mobile phase was a mixture $CHCl_3$:CH_3OH:H_2O (65:30:4,vol). The identification of the phospholipid was made with iodine and shows a single spot. Sodium deoxycholate (DOCNa)(Merck). TRITON X-100 (TX-100) (Sigma). Tris(hydroxymethyl)aminomethane-HCl (TRIS-Cl) (Austranal).
3. Solvents: $CHCl_3$ purified by distilation on P_2O_5. CH_3OH redistiled. Solvent mixture $CHCl_3$:CH_3OH (9:1,vol).
4. Solutions: Buffer TRIS-Cl, 0.05M, pH=7.2. DOCNa 20 mM in buffer TRIS-Cl. TX-100 20 mM in buffer TRIS-Cl.

II. Methods

A. Liposome preparation

The suspension of small unilamellar vesicles (SUV) was prepared by sonication of a suspension of multilamellar vesicles (MLV) obtained by film hydration [10].

1 ml lecithin solution in a solvent mixture, 18.6 mmol, was evaporated under methane current for 15 minutes, at 52 °C. The lipid film formed on the wall of a 100 ml flask attached to the rotary evaporator, was dried at a low pressure ($p<0.01$ Torr) for 4 hours at room temperature. Taking into account the mass of the lipid film and phosphate content of it (mineralized with nitroperchloric mixture,and assayed by Briggs method), we determined the mean molecular weight of the lecithin as being 766 Da.

We mention that this molecular weight is almost identical with the value of the lecithin supplied by Avanti Polar Lipids Inc. (USA).

The lipid film was hydrated at the room temperature with 16.5 ml buffer TRIS-Cl, under methane, for 20 minutes in the flask.

Taking into account that transition temperature of the lecithin is between -15° C and -7°C, all the operations took place over the shown temperature interval. Aliquots of 4 ml from MLV suspension were sonicated for 30 minutes, with some pauses for cooling. The ultrasound generator was adjusted at the power level 2, tuning 3.

After sonication, the aliquotes containing SUV were centrifugated for 20 min at 3000 rpm, in order to remove particles of titanium released from the sample.

B. Optical measurements

Optical transmission was measured on 1 cm path and diffusion measurements were made on a perpedicular direction on the incident beam, using the TiMi microtitrator, the transmitted and diffused light being recepted by identical photocells, conected to K-200 recorders.

B1. Titration curves

1.5 ml SUV suspension was introduced from microburette into a cuvette with X-Y transparence, in the microtitrator. The detergent solution (20 mM) was added into the cuvette, under agitation produced by a magnetic stirrer with constant angular speed. The rate of microtitration was 4 μl/sec.

The recording of the optical transmissions (T) and diffusions (D) were made with two photocells, connected to the two recorders.

The titration curve of optical transmission had a sygmoidal shape (fig.1).

The diffusion curves had a hyperbolic shape between the initial point and the upper end of the hyperbola there was an increase of optical diffusion, wich had a maximum, situated about at the point which corresponds to the molar ratio phospholipid/detergent = 1.

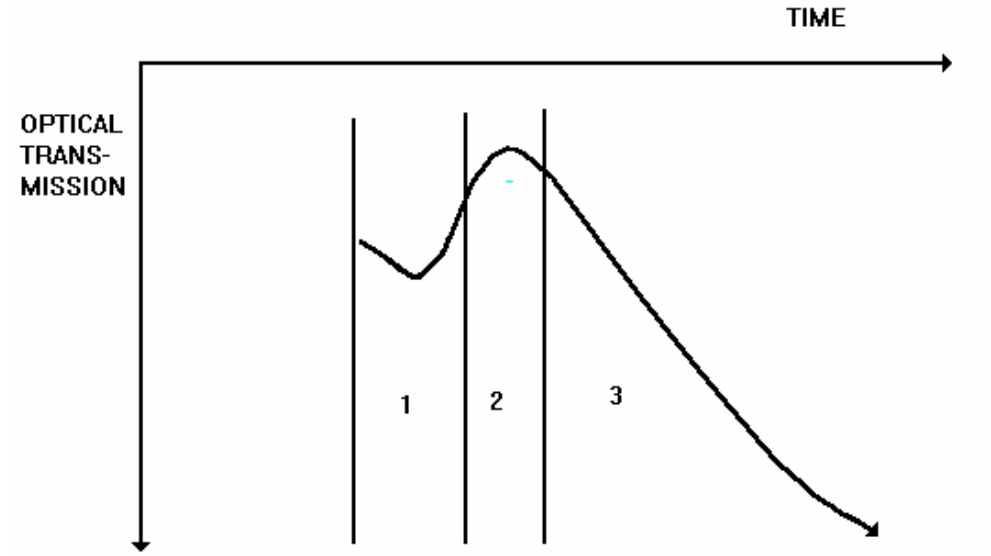

Figure 1. Optical transmission during the titration.

B2. Transmission and diffusion measurements under equilibrium conditions

Aliquotes of 1.5 ml SUV were treated with different volumes of detergent solution and after 2 hours were measured the optical transmissions and diffusions still under agitation.

RESULTS

It were taken into account some detergent volumes, added under dynamic (titrimetric) and equilibrium conditions, and for the resulted solutions, there were measured their percentage transmissions (T%) and diffusions (D%).

For transmission measurement the compensator was calibrated so that the maximum of recording width to be between 0-100% optical transmission.

For optical diffusion the compensator was calibrated so that the difference between abscissa of the start and the abscissa towards reach the recorder pen at end of titration to be between 0-100%.

In Table 1 appear the values of percentage transmissions of samples titrated with TX-100 and of those being under equilibrium conditions.

Table 1.

Percentage transmissions (T%) of the suspensions treated with TX-100.

Detergent total conc.(mM)	0.52	1.01	1.48	1.93	2.35	2.76
Dynamic system (T%)	28.50	29.00	38.00	50.00	70.00	89.50
Equilibrium system (T%)	29.50	34.75	42.25	54.25	86.00	96.75

In Table 2 appear the values of percentage diffusions of samples titrated with TX-100 and of those being under equilibrium conditions.

Tabel 2

Percentage diffusions (D%) of the suspensions treated with TX-100.

Detergent total conc.(mM)	0.52	1.01	1.48	1.93	2.35	2.76
Dynamic system (D%)	104.60	85.90	47.90	24.30	10.60	3.50
Equilibrium system (D%)	98.90	84.50	47.90	29.20	8.50	2.50

In Table 3 appear the same data as in Table 1, using DOCNa instead of TX-100 and the differences $\Delta\nu$, expressed in μmol, necessary to equalize the transmission of the titrated samples with equilibrium ones.

Table. 3

Percentage transmission (T%) of the suspensions treated with DOCNa

Detergent total conc.(mM)	0.52	1.01	1.48	1.93	2.35	2.76
Dynamic system (T%)	-	36.50	-	50.00	80.00	-
Equilibrium system (T%)	-	41.50	-	60.25	84.75	-
$\Delta\nu$ (μmol)	-	0.100	-	0.205	0.095	-

In Table 4 appear the same data as in Table 2 using DOCNa instead of TX-100 and the values necessary to equalize in the hystograms the diffusions under the two conditions.

Table 4

Percentage diffusions of the suspensions treated with DOCNa.

Detergent total conc.(mM)	0.52	1.01	1.48	1.93	2.35	2.76
Dynamic system (D%)	-	87.30	-	42.90	16.90	-
Equilibrium system (D%)	-	59.90	-	36.90	18.70	-
$\Delta\nu$ (μmol)	-	0.548	-	0.12	-0.036	-

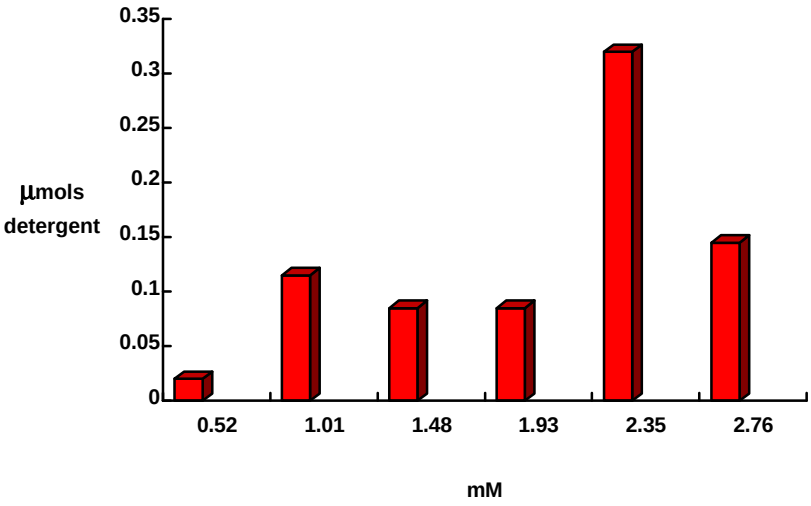

Figure 2. The values of the differences ($\Delta\nu$) for titration with TX-100 and for equilibrium conditions.

($\Delta\nu$) which equalize transmission of titrated samples with of those in equilibrium conditions, calculated at some total detergent concentrations. This Figure corresponds to data from Table 1.

In Figure 3 there are the hystograms of the detergent (TX-100) amounts ($\Delta\nu$) which equalize diffusions of titrated samples with of those being under equilibrium conditions, calculated at some total detergent concentrations.
This Figure corresponds to data from Table 2.

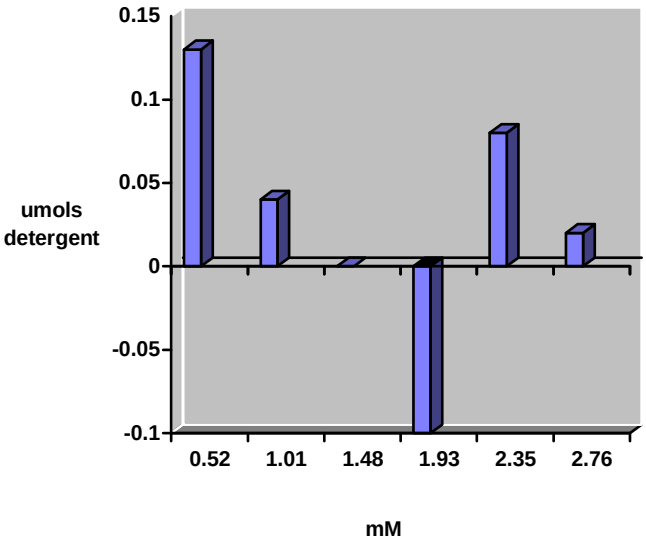

Figure 3. The values of the differences ($\Delta\nu$) for titration with TX-100 and for equilibrium conditions.

DISCUSSIONS AND CONCLUSIONS

Experimental data indicate that the transmissions of the samples in equilibrium conditions are higher than those titrated in the concentration interval 0-2.76 mM. This fact indicates that the solubilization process with TX-100 has a determined kinetics on the whole area of detergent concentration, the measurements being made at total detergent concentrations higher than his CMC. This difference reaches a maximum at the molar ratio lipid:detergent=1:2.37. It is situated at total detergent concentration of 2.35 mM (tab.1). The difference of the transmissions can be compensate by addition at the sample, which is in dynamic conditions, of 0.135 μmol of TX-100. The transmission of samples titrated with DOCNa (tab.3) evoluates similar with ones titrated with TX-100. The difference between the two evolutions consists in the fact that the maximum of the values of $\Delta\nu$ for titration with DOCNa, is not situated at molar ratio corresponding to TX-100, but at ratio 1:1.89 (phosfolipid-detergent), that is at a total concentration of detergent smaller than in the case shown at titration with TX-100.

The diffusions in dynamic conditions are higher than under equilibrium conditions in the concentration interval 0.52-1.01 mM TX-100 (tab.2). At 1.48 mM detergent, diffusions are identical in both conditions. At 1.93 mM the diffusion under equilibrium conditions is higher than under dynamic conditions. Between 2.35-2.76 mM there is the same situation as in the interval 0.52-1.01 mM TX-100.

There is a difference between transmission and diffusion evolution, that is not on the whole interval of detergent concentration, solubilization under dynamic conditions remains after solubilization under equilibrium conditions. As well, at 1.93 mM TX-100 the apparent solubilizant effect of detergent it is more increased in dynamic system, than in equilibrium system. Taking into account that vesicles population is heterogeneous from the dimensional point of view, just a part of them being SUV, it is to be expected a difference between the transmission variation and the diffusion variation with the increase of detergent concentration.

The interaction of liposomes with anionic and ionic detergents, graphically represented by the relationship T=f(time) (fig.1), has two stages:

I.Passive tritonization

Under both conditions (dynamic and equilibrium ones), the solubilization process [11] starts with the charging of the vesicles with detergent (portion 1 on the transmission curve), called "vesicular charging". This charging produces the increase of the vesicles volume, therefore a decrease of transmission and an increase of diffusion. On the portion 2 begins the transition: *liposomes (charged)* ---> *mixed micelles* and the charging continuously of a part of liposomes, which maintains the transmission approximately constant. This portion contains the point at wich molar ratio lipid-detergent =1:1.

II. Active tritonization

Under both conditions, in the following stage it is produced the accelerate transformation *liposomes---> mixed micelles*, which determines the rapid increase of transmission and decrease of diffusion. Finally, the mixed micelles grow richer with detergent molecules, tending towards "pure micelles".

It was found that at 2.35 mM TX-100, which is a solubilizing concentration, the differences between optical values for dynamic and equilibrium conditions reach a maximum. This is explained by taking into account that, in spite of the pronounced increase of the entropy in the course of solubilization, this process has a greater enthalpy than the process of liposome charging with detergent molecules at sub-solubilizing concentrations.

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