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DETERMINATION OF SURFACE POTENTIAL IN LIPOSOMES

MARTA S. FERNÁNDEZ

Departamento de Bioquímica, Centro de Investigación y de Estudios Avanzados del I.P.N., P.O. Box 14-740, Mexico 14, D.F. (Mexico)

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The ability of the fluorescent pH indicator 4-heptadecylumbelliferone to detect the electrical potential at the surface of negatively charged liposomes, was investigated. The vesicles were prepared from mixtures of egg lecithin and dicetyl phosphate at different molar ratios in NaCl solutions of various concentrations. It has been found that the dependence of the experimental surface potential on the proportion of charged lipid in the vesicles and on the salt concentration in the aqueous phase, was very similar to the predictions of the Gouy-Chapman equation as calculated by assuming a reasonable value for the mean molecular area of the lamellar lipids. In view of the good correlation obtained between the experimental and theoretical results, it is concluded that 4-heptadecylumbelliferone is quantitatively sensitive to changes in double-layer potential at the surface of lipid vesicles.

Introduction

Natural membranes bear net surface charge due to the presence of ionized groups in their lipids and proteins [1]. The separation existing between these fixed charges and the free ions dissolved in the aqueous phase surrounding the membrane, gives rise to an interfacial electric field which determines a surface potential [2].

A large number of biological phenomena, ranging from cell-cell adhesiveness and fusion to membrane permeability, are influenced by the surface potential [1,3]. As a consequence, many attempts have been made to measure this parameter [4–7].

In recent years a method using the lipoid pH indicator 4-heptadecylumbelliferone has proved useful for surface potential estimations of simple structures such as monolayers and micelles [8,9]. The present work represents a further step towards the use of the indicator method in natural membranes. It describes an investigation of the ability of 4-heptadecylumbelliferone to sense the surface potential of liposomes, a model system closely related to biological membranes. The measurements of surface potential were obtained through studies of the dissociation behavior of the dye bound to negatively charged lipid vesicles prepared from mixtures of egg lecithin and dicetyl phosphate at different molar ratios in media containing various salt concentrations.

The liposome surface potentials were also estimated theoretically according to the Gouy-Chapman equation. A comparison of the experimental and theoretical results is presented.

A preliminary account of this work has been presented elsewhere [10].

Characteristics of the acid-base indicator

The interfacial pK_0 characterising the dissociation of an acid base indicator located at a neutral surface, can be obtained by measuring its dissociation degree (α) and the bulk pH (pH_b) of the solution surrounding the membrane [9]:

$$pK_0 = pH_b - \log(\alpha/(1-\alpha)) \quad (1)$$

If the surface carries net charge, the apparent pK

of the indicator will be shifted with respect to the value at the neutral interface [9]:

$$pK_{ch} = pK_0 - (\psi F/2.3RT) \quad (2)$$

where pK_{ch} is the apparent pK of the indicator at the charged interface; ψ is the surface potential; R, F and T are the gas constant, Faraday constant and absolute temperature, respectively.
Rearranging:

$$\psi = -(pK_{ch} - pK_0) \cdot 2.3RT/F \quad (3)$$

The surface potential can be obtained by introducing in Eqn. 3 the values of pK_{ch} and pK_o as determined through the use of an appropriate indicator [9].

The acid-base indicator employed in this work is the dye 4-heptadecylumbelliferone (4-heptadecyl-7-hydroxycoumarin) which is insoluble in water but soluble in media of lower polarity. This property makes possible a complete incorporation of the dye into membranes in contrast to the water soluble, chainless umbelliferone [9,11] which is only partially incorporated. It can be presumed that the amphipathic character of 4-heptadecylumbelliferone leads to an orientation of the dye when located at membranes: The long alkyl chain penetrating the hydrophobic region and the polar, fluorescent moiety, pointing towards the lipid-water interface [12].

4-Heptadecylumbelliferone can be used as an acid base indicator because only the basic form (resulting from the dissociation of the hydroxyl group) fluoresces at 452 nm when excited at 377 nm, the fluorescence being proportional to the dissociation degree. Therefore, the pK can be obtained from fluorescence and pH measurements.

By studying the dissociation of 4-heptadecylumbelliferone in charged and neutral liposomes, the surface potential of the charged vesicles can be determined.

Experimental

Procedure for the determination of liposome surface potentials

Unilamellar lipid vesicles were prepared by the injection method [13,14] in aqueous solutions of NaCl from mixtures of zwitterionic egg lecithin and negatively charged dicetyl phosphate at different molar ratios. The indicator was added to the lipid mixture dissolved in ethanol prior to preparing the liposomes. The ratio of indicator molecules to lipid molecules was 1 : 1,500.

Titration of the indicator incorporated to liposomes was performed in the pH range 5.50–12.50, by adding small aliquots of a concentrated aqueous solution of NaOH to the lipid dispersion. After each addition, the bulk pH and the fluorescence of the sample were recorded. For the determination of bulk pH, a Radiometer pH meter Model 22 equipped with a combination electrode (glass/Ag-AgCl) was used. Fluorescence measurements were made with an Aminco Bowman spectrophotofluorometer by exciting at 377 nm and detecting the emission at 452 nm (uncorrected wavelengths). Excitation and emission slit widths equivalent to 5.5 nm bandpass were used. The temperature was kept constant at 295 K.

To determine the dissociation degree (α) of the indicator at different pH values, the fluorescence measured at each pH was divided by the fluorescence at high pH (12.50) where all the dye is dissociated. In this way, curves of dissociation degree as a function of bulk pH, were obtained. The apparent pK values were taken from these curves, as the pH values corresponding to $\alpha = 0.50$, according to the Henderson-Hasselbach equation [9].

This procedure was applied to negatively charged liposomes made of different molar ratios of lecithin: dicetyl phosphate, and to neutral, pure egg lecithin liposomes, in media containing various concentrations of NaCl. The apparent pK values thus obtained were introduced in Eqn. 3 to calculate the surface potentials.

Materials

Egg yolk lecithin purchased from Sigma Chemical Co. was purified by column chromatography on alumina [15]; thereafter its purity was checked by thin layer chromatography. The purified lecithin was quantified by determining its phosphorous content [16]. Dicetyl phosphate from ICN Pharmaceuticals Inc. was used as received. The indicator 4-heptadecylumbelliferone was a gift from P. Fromherz. All other reagents were analytical grade. Glass redistilled water was used throughout.

Results and Discussion

The circles in Fig. 1 represent the experimental values of surface potential for vesicles of egg lecithin-dicetyl phosphate in NaCl solutions. It can be seen that the magnitude of the potential increases with the concentration of dicetyl phosphate in the vesicles. The potentials are also dependent on the salt concentration in the aqueous phase, the values at 0.010 M NaCl being more negative than those at 0.100 M salt.

The screening effect of electrolytes on surface potential is best appreciated in Fig. 2 where the triangles indicate the experimental values obtained for lecithin vesicles containing a constant proportion of dicetyl phosphate (8 mol%) in NaCl solutions of variable concentration. It can be observed that the magnitude of the potential becomes smaller as the salt concentration increases.

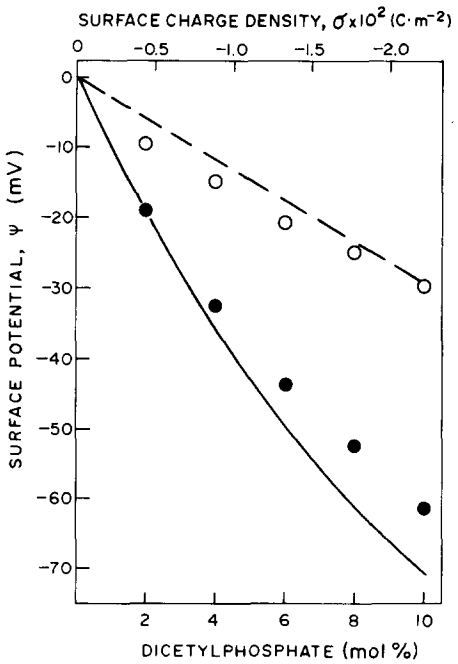

Fig. 1. Surface potential of egg lecithin-dicetyl phosphate liposomes in aqueous solution of NaCl as a function of the molar percentage of dicetyl phosphate in the vesicles. The circles represent the experimental surface potentials obtained with the indicator 4-heptadecylumbelliferone at two different NaCl concentrations: 0.010 M (•) and 0.100 M (○). The lines are the theoretical potentials computed according to the Gouy-Chapman equation using the surface charge densities shown in the top scale; these charge densitites were estimated for vesicles containing the dicetyl phosphate concentrations specified in the bottom scale by taking a value of 0.72 nm^2 for the lipid mean molecular area; salt concentrations were 0.010 M (———) and 0.100 M (– – –); T, 295 K.

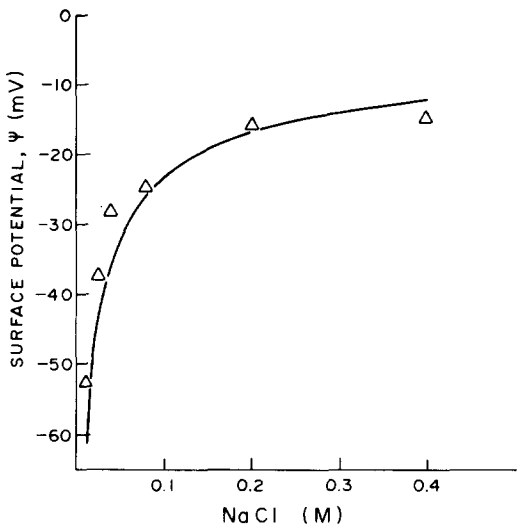

Fig. 2. Surface potential of egg lecithin-dicetyl phosphate liposomes as a function of the concentration of NaCl in the aqueous phase. The concentration of dicetyl phosphate in the vesicles was kept constant at 8 mol%. The triangles represent the experimental surface potentials obtained with the indicator 4-heptadecylumbelliferone. The line shows the dependence of the calculated theoretical Gouy-Chapman potentials on salt concentration. The surface charge density of the vesicles was estimated to be $-1.78 \cdot 10^{-2}$ C · m^{-2} by assuming a lipid mean molecular area of 0.72 nm^2. T, 295 K.

For the sake of comparison, the Gouy-Chapman theory of the diffuse double layer [1,2] was also used to estimate liposome electrical potentials. Although this theory was developed for plane surfaces and large errors are involved if its predictions on the spatial variation of the potential are extrapolated to curved interfaces, very small discrepancies are found it only the potential at the surface is considered [4]. This was actually the theoretical liposome potential needed in the present study since the comparison was to be made with the experimental results obtained by use of an indicator strongly bound to the liposome membrane [9].

The electrical potential at the surface (ψ) is given by the following equation, according to the Gouy-Chapman theory:

$$\psi = \frac{2kT}{e} \cdot \sinh^{-1}\left(\frac{\sigma e}{\sqrt{8\epsilon_r \epsilon_0 ckT}}\right) \qquad (4)$$

k being the Boltzman constant, T the absolute temperature, σ the surface charge density, e the electronic charge, ϵ_r the aqueous dielectric constant, ϵ_o the permittivity of free space and c the concentration of uni-univalent electrolyte. The use of this equation required a previous estimation of the liposome surface charge densities which was carried out using the expression:

$$\sigma = \frac{-Pe}{100A_m} \quad (5)$$

where p is the molar percentage of dicetyl phosphate in the egg lecithin-dicetyl phosphate liposomes, e the electronic charge and A_m the lipid mean molecular area which in all cases was taken as 0.72 nm^2. This value has been found for egg lecithin lamellar phases in excess water at 24°C by X-ray diffraction [17]. The approximation of taking a constant A_m was necessary since to the author's knowledge there are no reported data for the mean molecular areas of egg lecithin-dicetyl phosphate in lamellae. It was also assumed that the propotion of charged lipid is the same in both the outer and inner layers of the vesicles [4].

The results of the Gouy-Chapman potential computations are shown by the lines in Figs. 1 and 2. It can be observed that, in general, there is good agreement between the experimental and theoretical values, the largest deviations appearing for vesicles containing the higher proportions of the charged lipid in media of low salt concentrations. Nevertheless, the deviations are smaller than 10 mV.

The small discrepancies found between the experimental and Gouy-Chapman potentials may be due in part to the approximation of taking a constant value for A_m which implies to disregard the possible influences of salt concentration and ratio of dicetyl phosphate to lecithin on the lateral packing of the lipid lamellae. It is evident from examination of Eqns. 4 and 5 that if the assumed A_m were larger than the actual value, the magnitude of the theoretical potential would be underestimated whereas if an A_m smaller than the real mean molecular area were chosen, the magnitude of the theoretical potential would be overestimated. More precise theoretical predictions of ψ would be obtained if the actual mean molecular areas for lamellar egg lecithin-dicetyl phosphate mixtures in NaCl solutions at the concentrations used in this work, were available. It should be mentioned, however, that some of the deviations could also arise from the adsorption of sodium to the negative charges of the vesicles [18].

The good correlation obtained between the experimental and theoretical estimations of surface potential indicates that 4-heptadecylumbelliferone is quantitatively sensitive through its dissociation behaviour, to changes in double layer potential at the surface of negatively charged liposomes.

It could be expected that the indicator method should also prove useful in sensing surface potentials of natural membranes.

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References

1 McLaughlin, S. (1977) in Current Topics in Membrane and Transport (Bronner, F. and Kleinzeller, A., eds.), Vol. 9, pp. 71–144, Academic Press, New York
2 Aveyard, R. and Haydon, D.A. (1973) An Introduction to the Principles of Surface Chemistry, pp. 40–47, Cambridge University Press, London and New York
3 Prieve, D.C. and Ruckenstein, E. (1976) J. Theor. Biol. 56, 205–228
4 Castle, J.D. and Hubbell, W.L. (1976) Biochemistry 15, 4818–4831
5 Gaffney, B.J. and Mich, R.J. (1976) J. Am. Chem. Soc. 98, 3044–3045
6 Barton, P.G. (1968) J. Biol. Chem. 243, 3884–3890
7 Haydon, D.A. and Myers, V.B. (1973) Biochim. Biophys. Acta 307, 429–443
8 Fromherz, P. and Masters, B. (1974) Biochim. Biophys. Acta 356, 270–275
9 Fernández, M.S. and Fromherz, P. (1977) J. Phys. Chem. 81, 1755–1761
10 Fernández, M.S. (1979) 11th Int. Congr. Biochem. Toronto, Abstr. 05-2-R70
11 Montal, M. and Gitler, C. (1973) Bioenergetics 4, 363–382
12 Fromherz, P. (1973) Biochim. Biophys. Acta 323, 326–334
13 Batzri, S. and Korn, E.D. (1973) Biochim. Biophys. Acta 298, 1015–1019
14 Kremer, J.M.H., Esker, M.W., Pathmamanoharan, C. and Wiersema, P.H. (1977) Biochemistry 16, 3932–3935
15 Singleton, W.S., Gray, M.S., Brown, M. and White, J.L. (1965) J. Am. Oil Chem. Soc. 42, 53–56
16 Bartlett, G.R. (1958) J. Biol. Chem. 234, 466–468
17 Small, D.M. (1967) J. Lipid Res. 8, 551–557
18 Eisenberg, M., Gresalfi, T., Riccio, T. and McLaughlin, S. (1979) Biochemistry 18, 5213–5223