Biochimica et Biophysica Acta, 436 (1976) 301–306

BBA 77338

AN IMPROVED METHOD FOR THE DESCRIPTION OF NON-ELECTROLYTE PERMEATION THROUGH LIPOSOMES, BASED ON IRREVERSIBLE THERMODYNAMICS

E. J. J. VAN ZOELEN, M. C. BLOK and J. DE GIER

Laboratory of Biochemistry, State University of Utrecht, Transitorium III, Padualaan 8, De Uithof, Utrecht (The Netherlands)

(Received January 13th, 1976)

SUMMARY

In this paper experimental conditions are described to measure the three irreversible thermodynamic parameters for the nonelectrolyte permeation through liposomes.

The values for the reflection coefficient σ, measured by this method, differ significantly from those found previously.

INTRODUCTION

Attempts have been made to correlate the permeability properties of membranes with their chemical composition [1–4]. A method frequently used is measuring the initial swelling rate of cells suspended in an isotonic solution containing a permeable nonelectrolyte [1–3]. This approach has been applied to both model membrane systems (liposomes; refs. 1 and 2) and biological membranes (*Acholeplasma laidlawii*; ref. 3). This initial swelling rate proved to be dependent on the lipid composition of the cell membrane and showed an exponential increase with rising temperature. From this behaviour a value for the energy barrier of polyalcohols was found that depended only on the nature of the permeant and not on the lipid composition or cholesterol content of the cell membrane [2, 3].

Hill and Cohen [5] criticized this way of measuring permeabilities by stating that the initial swelling rate will depend on both the permeability coefficient (ω) of the permeant and on the reflection coefficient (σ) of the system, and hence that the values of the energy barriers will be an amalgam of the temperature dependencies of these two parameters. Based on the irreversible thermodynamic formulas of Kedem and Katchalsky [6] Hill and Cohen described a method for independent measurement of ω and σ, and in a recent paper Cohen [7] listed the corrected values of the energy barriers by measuring the temperature dependency of the permeability coefficient.

This paper will show that the values of σ as measured according to the method of Hill and Cohen, are incorrect because the equations used do not correspond to their experimental conditions.

A modified method will be described to determine σ for the non-electrolyte permeation through liposomes, and also to determine relative values for the other two irreversible thermodynamic parameters, ω and L_p (filtration coefficient).

MATERIALS AND METHODS

Liposomes of egg lecithin/egg phosphatidic acid 96 : 4 were prepared [1] in 20 mM glucose (unless stated otherwise) and dialysed overnight at 4 °C against the same solution. Volume changes were measured spectrophotometrically, using the linear relationship between the volume change and the change in the reciprocal of the absorbance at 450 nm [8]. A thermostatted Vitatron MPS type equipped with a stirrer was used throughout the experiments. The final lipid concentration was 0.2–0.25 μmol/ml.

RESULTS AND DISCUSSION

The irreversible thermodynamic considerations of Kedem and Katchalsky [6] describe the solute and water flow through the membrane of a selectively permeable cell by the following equations:

$$\frac{dV}{dt} = -AL_pRT(\Delta c_i + \sigma \Delta c_s) \tag{1}$$

$$\frac{dn_s}{dt} = A\omega RT\Delta c_s + \frac{dV}{dt}(1-\sigma)\frac{\Delta c_s}{\Delta \ln c_s} \tag{2}$$

in which V is the volume and A the outer area of the swelling cell, c_s is the concentration of the permeable species, and c_i of the impermeable ones. $\Delta c = c^o - c^i$, the difference between the outside and the inside concentration, while n_s is the number of permeant molecules inside the cell, and σ the "impermeable" fraction of the solute.

Hill and Cohen [5] based their analysis on the experimental finding that liposomes, suspended in sufficiently hypertonic media containing a permeable solute, will first shrink, then pass through a minimum volume and subsequently start swelling with a linear volume change with time. Using the condition that $d^2V/dt^2 = 0$ they derived from Eqn. 1 that this linear volume change is given by

$$\frac{dV}{dt} = \sigma \frac{dn_s}{dt}(c_i^i + \sigma c_s^i)^{-1} \tag{3}$$

Our criticism is directed against their interpretation of Eqn. 2. Hill and Cohen assumed that at the minimum volume the concentration of the permeant inside the liposomes is still so much smaller than the outside concentration that the second term in Eqn. 2 can be ignored. However, this assumption is not valid under the conditions used by Hill and Cohen [5]. This is illustrated by comparison of the osmotic behaviour of liposomes suspended in hypertonic solutions of glucose, glycerol and erythritol, as given in Fig. 1. Both the size of the minimum volume and the time required to reach this volume largely depend on the velocity of passage of the permeant relative to that of water. An approximation of the concentration of the permeant inside the liposomes at the minimum volume can be made in the following way.

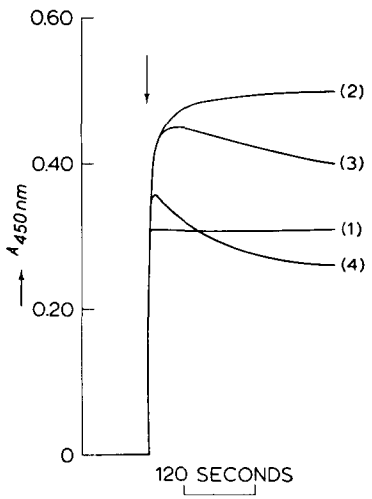

Fig. 1. Shrinkage of liposomes in hypertonic solutions of various nonelectrolytes. Liposomes, prepared in 20 mM glucose, were suspended in 20 mM glucose (1), 100 mM glucose (2), 100 mM glycerol (3) and 100 mM erythritol (4) respectively. Temperature, 30 °C. The change in $A_{450\,nm}$ was measured as described in Materials and Methods.

Knowing the linear relationship between $1/A_{450\,nm}$ and $1/c$ at equilibrium [8], where c is the glucose concentration in which the liposomes are treated osmotically, one can relate the absorbance at the minimum volume to the inside concentration of glucose (c_i^i) at this point, neglecting small changes in the refraction indices. With a known value of σ and using the condition that $\Delta c_i + \sigma \Delta c_s = 0$, now a value for the inside concentration of the permeant at the minimum volume can also be obtained. Doing this for the example given in Fig. 1 and using the σ values found in this paper (see Table I) one can calculate that c_s^i at the minimum volume is about 40 mM for both glycerol and erythritol at 30 °C, whereas these values are 55 mM and 10 mM respectively at 15 °C. Using the σ values obtained by Hill and Cohen, large negative values for the inside erythritol concentration at the minimum volume were found at both temperatures.

To check the σ values given by Hill and Cohen we used a modification of the

TABLE I

VALUES FOR σ AND ω FOUND ACCORDING TO EQN. 4 (METHOD I) AND EQNS. 8 AND 9 (METHOD II). LIPOSOMES WERE PREPARED IN 20, 40 OR 100 mM GLUCOSE

	Temperature 30 °C			Temperature 15 °C		
	σ glycerol	σ erythritol	ω glycerol / ω erythritol	σ glycerol	σ erythritol	ω glycerol / ω erythritol
Method I	0.48±0.03	0.96±0.03		0.73±0.03	0.98±0.04	
Method II	0.53±0.03	0.96±0.04	11.3±0.3	0.68±0.08	–	26.1±1.5
Hill and Cohen (ref. 5)	–	0.38±0.06		–	0.66±0.2	

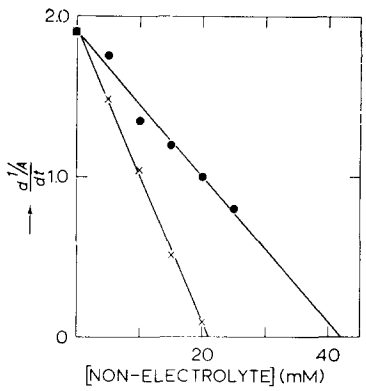

Fig. 2. The initial swelling rate of liposomes as a function of the concentration of the permeable nonelectrolyte in the outside medium. Liposomes, prepared in 20 mM glucose, were suspended in glycerol (●–●) and erythritol (×–×). Temperature, 30 °C. The swelling was measured by following the changes in $A_{450\,nm}$.

so-called "zero time method" [9]. Liposomes, made in 20 mM glucose (c_t), were suspended in solutions of the permeable solute of different concentrations (c_s^o), and the initial swelling rate $(dV/dt)_{t=0}$ was measured.
Eqn. 1 shows that under these conditions:

$$\left(\frac{dV}{dt}\right)_{t=0} = AL_pRT(c_t - \sigma c_s^o) \tag{4}$$

By plotting $(dV/dt)_{t=0}$ against different values for c_s^o, (Method I), a linearity is obtained yielding a value for σ (see Fig. 2).

Since in general the value of A is unknown and an additional light-scattering parameter is involved, only a relative value for the filtration coefficient L_p is found. The values of σ obtained in this way differ significantly from the values obtained by Hill and Cohen (see values listed in Table I).

Knowing the reflection coefficient of the system, the experimental conditions for measuring ω were chosen as follows: Liposomes were suspended in several hypertonic solutions containing such concentrations of the permeable non-electrolyte that

$c_i^o + \sigma c_s^o = c_i^i \; (= c_t)$.

In this way it was arranged that the minimum volume with $dV/dt = 0$ is reached at zero time. Since under these conditions $c_s^i \ll c_s^o$ now the second term in Eqn. 2 can be ignored. Furthermore, it was found that as long as the applied gradient of the permeant is steep enough, there is still a linear increase of volume with time, so Eqn. 3 remains valid. Combining Eqns. 2 and 3, and using the condition that $c_s^i \ll c_s^o$, this linear volume change v is given by

$$v = A\omega RT\sigma c_s^o (c_i^i + \sigma c_s^i)^{-1} \tag{5}$$

From Eqn. 1 it can be seen that $c_i^i + \sigma c_s^i = c_i^o + \sigma c_s^o + (v/AL_pRT)$, so

$$v\left(c_i^o + \sigma c_s^o + \frac{v}{AL_pRT}\right) = A\omega RT\sigma c_s^o \tag{6}$$

Since $AL_pRT(c_i^o+\sigma c_s^o)$ would be the swelling rate when the liposomes are suspended in pure water (see Eqn. 4), $v \ll AL_pRT(c_i^o+\sigma c_s^o)$. We can, therefore, make the approximation that

$$1+\frac{v}{AL_pRT(c_i^o+\sigma c_s^o)} \approx 1\Big/\left(1-\frac{v}{AL_pRT(c_i^o+\sigma c_s^o)}\right)$$

Combining with $c_i^o+\sigma c_s^o = c_t$, it follows:

$$v\left(\frac{c_i^o+\sigma c_s^o}{A\omega RT\sigma c_s^o}+\frac{1}{AL_pRTc_t}\right) = 1 \tag{7}$$

From Eqn. 7 we can derive:

$$\frac{1}{v} = \frac{1}{A\omega RT\sigma}\frac{c_i^o}{c_s^o}+\frac{1}{A\omega RT}+\frac{1}{AL_pRTc_t} \tag{8}$$

and:

$$\frac{1}{v} = \frac{1}{A\omega RT\sigma}\frac{c_t}{c_s^o}+\frac{1}{AL_pRTc_t} \tag{9}$$

Thus, by plotting from the same set of data $1/v$ against c_i^o/c_s^o and $1/v$ against c_t/c_s^o (Method II), two straight lines are obtained yielding a value for σ and relative values for L_p and ω. This is illustrated graphically in Fig. 3. Values for σ and ω found accord-

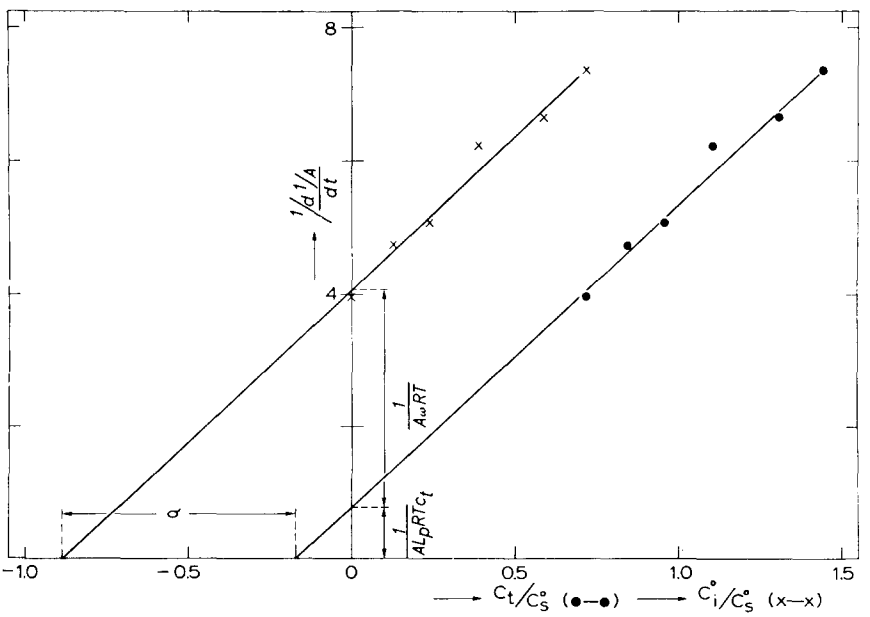

Fig. 3. Graphical illustration of the method for determining the three irreversible thermodynamic parameters σ, ω and L_p. Liposomes were prepared in 100 mM glucose and suspended in glycerol solutions such that $c_i^o+\sigma c_s^o = c_t$. Temperature, 15 °C. Volume changes were measured by following the changes in $A_{450\,nm}$.

ing to this method are listed in Table I. From these two linearities another set of values for σ and L_p can be obtained, which gives an internal check of the values found by the two methods. The approximation in the above derivation is such, that for solutes with a σ value down to 0.5, the error in the values for the three parameters is within 5 %.

Furthermore, it can be seen that the swelling rate of liposomes, suspended in a solution of the permeable nonelectrolyte with a concentration $c_s^o = c_t/\sigma$, gives a good (relative) value for the permeability coefficient ω, provided that a correction is made for the term $1/AL_pRTc_t$ (found according to Method I).

Finally it was checked that the value of σ found by Method II, was independent of the inside concentration of the impermeable solute, by doing experiments with liposomes prepared in 20, 40 or 100 mM glucose, respectively.

Since the linearity of the volume changes with time is best with steep gradients of the permeable solute, most reliable results were obtained with liposomes prepared in 100 mM glucose.

The above method will make it possible to describe the temperature dependency of the nonelectrolyte permeation through liposomes in a much better way than those used previously.

ACKNOWLEDGEMENTS

The present investigations were carried out under the auspices of the Netherlands Foundation for Chemical Research and the Netherlands Foundation for Biophysics and with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

REFERENCES

1 de Gier, J., Mandersloot, J. G. and van Deenen, L. L. M. (1968) Biochim. Biophys. Acta 150, 666–675
2 de Gier, J., Mandersloot, J. G., Hupkes, J. V., McElhaney, R. N. and van Beek, W. P. (1971) Biochim. Biophys. Acta 233, 610–618
3 McElhaney, R. N., de Gier, J. and van der Neut-Kok, E. C. M. (1973) Biochim. Biophys. Acta 298, 500–512
4 van Deenen, L. L. M. and de Gier, J. (1974) The Red Blood Cell, 2nd edn., Chapter 4, Academic Press New York
5 Hill, M. W. and Cohen, B. E. (1972) Biochim Biophys. Acta 290, 403–407
6 Kedem, O. and Katchalsky, A. (1958) Biochim. Biophys. Acta 27, 229–246
7 Cohen, B. E. (1975) J. Membrane Biol. 20, 205–234
8 Bangham, A. D., de Gier, J. and Greville, G. D. (1967) Chem. Phys. Lipids 1, 225–246
9 Lelievre, J. and Rich, G. T. (1973) Biochim. Biophys. Acta 298, 15–26