Biochimica et Biophysica Acta, 468 (1977) 238—244

BBA 77751

PERMEABILITY OF IODIDE IN MULTILAMELLAR LIPOSOMES MODELED BY TWO COMPARTMENTS AND A RESERVOIR

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(Received November 18th, 1976)

Summary

A previously published rate law for the diffusion of iodide from multilamellar egg phosphatidylcholine liposomes (Schullery, S.E. (1975) Chem. Phys. Lipids 14, 49—58) is fitted to the relatively simple mathematical model of two compartments in series with a reservoir. All of the inner liposome compartments are assumed to behave as effectively one compartment in series with the liposome's outermost compartment. Based on this model, reasonable values are calculated for the fraction of the total solution trapped by liposomes which is in the outermost liposome compartment, 17%, and the permeability coefficient of iodide against isotonic, mixed iodide-chloride solution, $2 \cdot 10^{-9}$ cm/s.

In a previous paper [1] the experimental rate law was determined for the diffusion of iodide trapped in phosphatidylcholine (PC) liposomes (multilayers) into isotonic chloride solution surrounding the liposomes. The experiment was to prepare liposomes in 0.1 M KI, exchange the iodide external to the liposomes by passage over a Dowex 1-Cl^- column, and then monitor the release of iodide with an iodide specific electrode. The following rate law indicative of two distinct first-order processes was found to fit the data over a 90-min time period:

$$(I^-) = 4.9 \cdot 10^{-4} - 4.2 \cdot 10^{-5}\, e^{-(0.08)t} - 4.1 \cdot 10^{-4}\, e^{-(0.0031)t} \quad (1)$$

where units of molarity and minutes are used. The infinite time iodide concentration, $4.9 \cdot 10^{-4}$ M, was determined by addition of Triton X-100 to the dispersion. The simplest mechanisms consistant with this rate law are, (a) parallel paths for iodide release from two classes of liposomes, for instance normal versus structurally defective liposomes, (b) series paths for iodide release thru the successive concentric compartments of the liposomes. Since the faster process was shown to be sensitive to pH, cholesterol, and Tris buffer, the series pathway mechanism seemed more likely. It was suggested that the inner liposome compartments were behaving in effect as one compartment in series

with the outermost liposome compartment which in turn lost iodide irreversibly to the surrounding medium.

In this paper boundary conditions and approximations appropriate to the liposome system are utilized to obtain the solution to the differential equation describing permeation from liposomes into a reservoir. A rate constant, k, and the volume of the outermost liposome compartments, V_y, are among the parameters in the theoretical rate law. By comparing corresponding terms in the theoretical and experimental rate laws, k and V_y are evaluated. Since the total system of equations is overdetermined, independent values of V_y (or k) can be calculated, which, by virtue of their reasonable magnitude and internal consistency, lend strong support to the validity of the model. A similar model for the tracer exchange upon 'soaking out' of the intra and extracellular fluids of tissue has been treated by Huxley [2], however, the constants in the rate law for appearance of permeant in the reservoir were not analyzed in terms of the parameters of the system. Johnson and Bangham [3] also solved the equivalent model employing approximations based on the relative volumes of sonicated vesicles in a dialysis bag in a reservoir. The general solution for steady-state tracer exchange between two compartments in series with a reservoir has been presented by Kotyk and Janacek [4].

The model is illustrated in Fig. 1. X represents all of the liposome compartments except for the outermost one; Y represents the outermost liposome compartment; Z is the solution surrounding the liposomes. Molar concentrations of iodide in X, Y, and Z are represented by x, y, and z respectively. A_{xy} and A_{yz} are the areas of the inner and outer membranes bounding the outermost liposome compartment. Due to the large concentration gradient (approx. 10^4 M at $t = 0$) across A_{yz}, back diffusion from Z can be neglected. The differential equations describing the compartmental concentration changes then are:

$$dx/dt = -(k_{xy}A_{xy}/V_x)x + (k_{yx}A_{xy}/V_x)y \tag{2a}$$

$$dy/dt = (k_{xy}A_{xy}/V_y)x - (k_{yx}A_{xy}/V_y)y - (k_{yz}A_{yz}/V_y)y \tag{2b}$$

$$dz/dt = (k_{yz}A_{yz}/V_z)y \tag{2c}$$

Three approximations are now made in order to reduce these equations to a more tractable form. First we assume that the two membrane areas, A_{xy} and A_{yz}, are approximately equal. This is reasonable for large, unsonicated liposomes. The second and third assumptions are that the volumes, V_x and V_y, are constant, and that the various rate constants are approximately the same. These last two assumptions are equivalent to the steady state or quasi-equilibrium assumption commonly made in tracer analysis [4]. Since the ion exchange column exchanged iodide one-for-one with chloride, there was no osmotic flux to change the liposome volumes. In addition however, the constant volume assumption requires that the net transfer of iodide out of the liposomes be countered by exchange uptake of an anion of similar partial molar volume, chloride or conceivably hydroxide. (The solution was buffered at pH 8.0 with 0.01 M Tris HCl.) The equality of the three rate constants is a non-trivial assumption since the mix of chloride and iodide, against which iodide diffusion occurs, is different in each compartment. A rigorous justification of these

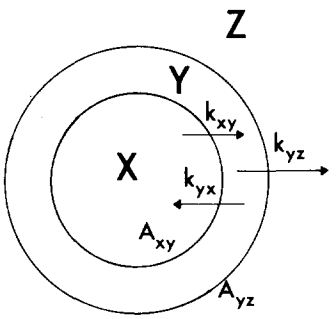

Fig. 1. The multilamellar liposome is modeled as two compartments in series with a reservoir. Compartment X represents all of the aqueous channels inside the liposome except for the outermost shell. Y represents the outermost liposome channel. Z is the solution surrounding the liposomes. The areas of the bilayers bounding the outermost channel are A_{xy} and A_{yz}. The rate constants k_{xy}, k_{yx} and k_{yz} are equal to the permeability coefficients times the areas of the surfaces.

assumptions requires an understanding of the mechanism(s) of halide ion permeation. Unfortunately this information is still unsettled. A series of two recent papers by Toyoshima and Thompson provide an extensive discussion of the possibilities [5]. They found that in both semispherical bilayers and sonicated vesicles chloride diffusion was markedly slower against nitrate than chloride and cite this as "strong evidence for the coupling of inward and outward chloride fluxes." Nicholls and Miller also report [6] that chloride is released from liposomes at a slower rate against iodide or nitrate than against chloride. On the other hand, Bangham et al. [7] found that chloride was exchanged from multilamellar liposomes more rapidly against iodide than chloride, and Hauser et al. [8] report that chloride diffused from sonicated vesicles somewhat faster against nitrate than chloride "indicating that exchange diffusion is not important." It should be noted that in the work analyzed here an alkaline pH was maintained to eliminate the reported transport of iodide as the I_3^- complex [9]. A satisfactory justification of the assumptions of constant volume and identical rate constants clearly depends on future experimental work as well as on the predictive success of the model presented in this paper. Granting these approximations Eqns. 2a–c may be simplified as Eqns. 3a–c.

$$dx/dt = -(k/V_x)x + (k/V_x)y \tag{3a}$$

$$dy/dt = (k/V_y)x - (2k/V_y)y \tag{3b}$$

$$dz/dt = (k/V_z)y \tag{3c}$$

Such a system of linear homogeneous equations with constant coefficients has as a general solution a set of equations of the following form [10]:

$$x = A_1e^{\lambda_1 t} + A_2e^{\lambda_2 t} + A_3e^{\lambda_3 t} \tag{4a}$$

$$y = B_1e^{\lambda_1 t} + B_2e^{\lambda_2 t} + B_3e^{\lambda_3 t} \tag{4b}$$

$$z = C_1e^{\lambda_1 t} + C_2e^{\lambda_2 t} + C_3e^{\lambda_3 t} \tag{4c}$$

We desire to evaluate the constants in Eqn. 4c in terms of the parameters of the liposome system and then compare these theoretical quantities with their experimental counterparts in Eqn. 1.

The characteristic equation associated with Eqns. 3a—c is:

$$\begin{vmatrix} -k/V_x - \lambda & k/V_x & 0 \\ k/V_y & -2k/V_y - \lambda & 0 \\ 0 & k/V_z & -\lambda \end{vmatrix} = 0 \tag{5}$$

Solution of the corresponding cubic equation in λ yields the following three roots:

$$\lambda_1 = 0 \tag{6a}$$

$$\lambda_2 = -k[1/2V_x + 1/V_y + 1/2(1/V_x^2 + 4/V_y^2)^{1/2}] \tag{6b}$$

$$\lambda_3 = -k[1/2V_x + 1/V_y - 1/2(1/V_x^2 + 4/V_y^2)^{1/2}] \tag{6c}$$

The fact that the model postulates no z dependence in Eqns. 2a—c results in $\lambda_1 = 0$ and thus produces the double exponential behavior consistent with experiment. Both exponents are seen to be directly proportional to k but to have a more complicated dependence on V_x and V_y.

We will not attempt to evaluate the constant coefficients in Eqns. 4a and 4b since they are experimentally inaccessible in the liposome system. The coefficients C_1, C_2 and C_3 however can be evaluated using the following experimental boundary conditions.

$$\text{At } t = \infty,\ z = 4.9 \cdot 10^{-4}\ \text{M} \tag{7a}$$

$$\text{At } t = 0,\ z = 3.8 \cdot 10^{-5}\ \text{M} \tag{7b}$$

$$\text{At } t = 0,\ dz/dt = 5.5 \cdot 10^{-6}\ \text{M/min} \tag{7c}$$

These values furnish the following independent relationships respectively.

$$C_1 = 4.9 \cdot 10^{-4}\ \text{M} \tag{8a}$$

$$C_1 + C_2 + C_3 = 3.8 \cdot 10^{-5}\ \text{M} \tag{8b}$$

$$C_2\lambda_2 + C_3\lambda_3 = 5.5 \cdot 10^{-6}\ \text{M/min} \tag{8c}$$

Eqns. 8a—c are solved for the values below.

$$C_1 = 4.9 \cdot 10^{-4}\ \text{M} \tag{9a}$$

$$C_2 = -4.47 \cdot 10^{-5}\ \text{M} - C_3 \tag{9b}$$

$$C_3 = \frac{5.5 \cdot 10^{-6}\ \text{M/min} + (4.47 \cdot 10^{-4}\ \text{M})\lambda_2}{\lambda_3 - \lambda_2} \tag{9c}$$

Eqns. 6b, 6c, 9b and 9c give λ_2, λ_3, C_2 and C_3 as functions of k, V_x and V_y. If these expressions for λ_2, λ_3, C_2 and C_3 are each equated to their experimental counterparts in Eqn. 1 we obtain eqns. 10a—c expressing k in terms of V_x and V_y.

$$\text{From } \lambda_2,\ k = 0.08[1/2V_x + 1/V_y + 1/2(1/V_x^2 + 4/V_y^2)^{1/2}]^{-1} \tag{10a}$$

$$\text{From } \lambda_3,\ k = 0.0031[1/2V_x + 1/V_y - 1/2(1/V_x^2 + 4/V_y^2)^{1/2}]^{-1} \tag{10b}$$

From C_2 or C_3,

$$k = 5.5 \cdot 10^{-6}[2.24 \cdot 10^{-4}/V_x + 4.47 \cdot 10^{-4}/V_y - 1.85 \cdot 10^{-4}(1/V_x^2 + 4/V_y^2)^{1/2}]^{-1} \tag{10c}$$

It is a credit to the validity of the model that the equations for C_2 and C_3 (9b and 9c) lead to the same expression for k within experimental error. An equivalent demonstration of this agreement is the equality (within 1%) that obtains when the experimental C_2 and C_3 are substituted into Eqn. 9b.

If V_x is treated to a first approximation as a known quantity, Eqns. 10a, 10b, 10c can be equated pair-wise and solved for three values of V_y. For this purpose V_x was approximated by $(V_x + V_y)$, which could be determined since the total iodide released at $t = \infty$ was initially contained at a 0.10 M concentration in V_x and V_y. $(V_x + V_y)$ was calculated to be $3.5 \cdot 10^{-5}$ l. Once an approximate value of V_y was thus obtained, it was used to calculate an improved V_x, and the calculation was then reiterated until V_y converged. The following values for V_y were obtained.

From 10a and 10b, $V_y = 5 \cdot 10^{-6}\ l$

From 10b and 10c, $V_y = 3.5 \cdot 10^{-6}\ l$

From 10a and 10c, $V_y = 9 \cdot 10^{-6}\ l$

The average value with estimated limits of error is $V_y = (6 \pm 3) \cdot 10^{-6}$ l. Using the average value of V_y, k is calculated to be $2 \cdot 10^{-7}$ l/min under these experimental conditions [1].

The reasonably close agreement among the values of V_y strongly supports the applicability of the model for iodide diffusion from multilamellar liposomes. Furthermore, although an absolute check is impossible, the magnitude of V_y is quite reasonable. The ratio of V_y to the total trapped solution in the liposomes $(V_x + V_y)$ is 17% which agrees with the values of 17—20% estimated by Bangham et al. [11]. Using literature values for the dimensions of the lipid bilayer [12], the aqueous channel [13,14], and an aqueous core presumed equivalent to a sonication-limit vesicle [12], the average number of shells necessary to produce a ratio of $V_y/(V_x + V_y)$ equal to 0.17 was calculated to be 15. This is quite a reasonable number based on numerous published electron microscopic studies of liposomes (e.g. ref. 15). The calculated dependence of $V_y/(V_x + V_y)$ on the number of shells beyond the liposome core is shown in Fig. 2 for up to 25 shells.

Only a very approximate comparison of the calculated k with literature values is possible. If the surface area of the dispersion is assumed to be 200 $cm^2/\mu mol$ PC [11], a total surface area of 2200 cm^2 is calculated for our system. Dividing this into k gives a permeability coefficient of $2 \cdot 10^{-9}$ cm/s. This number has a high uncertainty in light of recent ion-binding experiments (unpublished) in our laboratory which imply tremendous variation in the effective surface area of liposome dispersions due to aggregation. The aggregation is sensitive to dilution, ion binding and incorporation of charged amphipaths into the bilayers. Unfortunately literature values for the iodide permeability coefficient in PC liposomes are not available, however, the value obtained is at least of a reasonable magnitude when compared to the range of chloride permeability coefficients reported for related systems. Hauser et al. [8] report a chloride permeability coefficient of $5.5 \cdot 10^{-11}$ cm/s at pH 5.5 and room temperature from egg PC vesicles which had experienced about 8% chemical degradation during sonication. Under similar experimental conditions

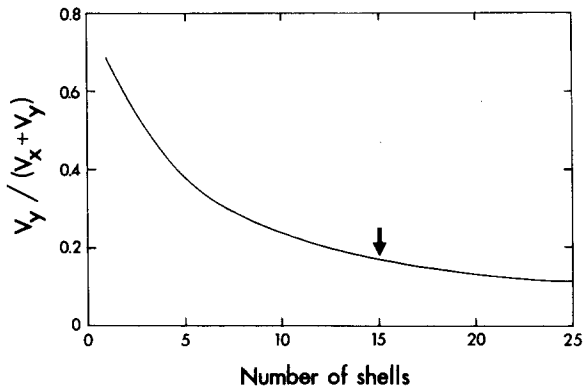

Fig. 2. The fraction of liposome-trapped solution contained in the outermost channel was calculated for varying numbers of bilayers using the following equation:

$$\frac{V_y}{V_x + V_y} = \frac{(4/3)\pi([N(a+b)+c]^3 - [N(a+b)+c-a]^3)}{(4/3)\pi c^3 + (4/3)\pi \sum_{n_i=1}^{N} ([n_i(a+b)+c]^3 - [n_i(a+b)+c-a]^3)}$$

where a is the thickness of the aqueous channels, b is the thickness of the bilayers, and c is the radius of the aqueous core of the liposomes. n_i is the i'th bilayer beyond the core and N is the outermost bilayer. Liposomes were assumed to be spherical. The following values from the literature (see text) were used: a = 30 Å, and b = 40 Å, and c = 100 Å. The arrow indicates the average number of bilayers (beyond the bilayer which bounds the aqueous liposome core) which corresponds to the value of 0.17 reported for $V_y/(V_x + V_y)$ in this paper.

Toyoshima and Thompson [5] found chloride's permeability coefficient to be $1.13 \cdot 10^{-10}$ cm/s. These workers also noted the presence of oxidation products due to sonication. We should note that the sonication process has been reported to affect both ionic permeability [16] and osmotic permeability [17]. The chloride permeability coefficient in single bilayer membrane systems has been found to be $6.8 \cdot 10^{-8}$ cm/s for diphytanoylphosphatidylcholine [5] and $2.4 \cdot 10^{-7}$ cm/s for an egg PC bilayer still containing some of the organic solvent from which it was formed [18].

In conclusion, the simple model of two compartments in series with a reservoir seems to describe the diffusion of iodide from multilamellar PC liposomes remarkably well. If additional experiments reveal that the model applies to other permeants as well, the long standing problem of presumed complex, multi-compartment kinetics [19] will be largely eliminated as an obstacle to the use of liposomes in membrane permeability studies.

Acknowledgements

The author gratefully acknowledges support from a Cottrell Research Grant. Acknowledgement is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for the partial support of this research. Also the author thanks Professor Hartmut Hoft of the E.M.U. Mathematics Department for a critical reading of the mathematical analysis.

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