MASS-ACTION FORMULATIONS OF MONOVALENT AND DIVALENT CATION ADSORPTION BY PHOSPHOLIPID MEMBRANES

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ABSTRACT An extension of a previous treatment (Cohen, J. A., and M. Cohen, 1981, *Biophys. J.*, 36:623–651) is presented for the adsorption of monovalent and divalent cations by single-component phospholipid membranes, where monovalent cations adsorb with a cation/phospholipid stoichiometry of 1:1 and divalent cations adsorb with stoichiometries of 1:1 and 1:2. Previously the 1:1 and 1:2 binding of divalent cations were assumed to occur by independent, parallel pathways. Here a serial adsorption scheme is considered in which 1:2 binding occurs via reaction of 1:1-bound complexes with adjacent unoccupied phospholipids. This two-dimensional lattice reaction is shown to obey a law of mass action, and the mass-action equilibrium constant is used to parameterize the adsorption isotherm. This isotherm is shown to be mathematically equivalent to the previous isotherm, although the two formulations differ in the dependence of 1:2 binding on the 1:1 association constant.

INTRODUCTION

In a previous paper (1) we discussed the adsorption of monovalent and divalent cations by single-component, charged phospholipid membranes. We derived an adsorption isotherm for the case in which monovalent cations bind to the membrane with a cation/phospholipid stoichiometry of 1:1, whereas divalent cations bind with stoichiometries of 1:1 and/or 1:2. Using statistical mechanics we showed that one can calculate the average equilibrium number of adsorbed monovalent cations ($\overline{N}_M$), 1:1-bound divalent cations ($\overline{N}_{D1}$), and 1:2-bound divalent cations ($\overline{N}_{D2}$) in terms of the single-particle partition functions of these cations both in solution and on the membrane. In the statistical-mechanical treatment one considers only the final equilibrium state of the system; it is not necessary to mention the dynamic processes or kinetic pathways by which the adsorption occurs.

To present our results more conventionally, we showed that our isotherms can also be expressed in terms of mass-action equilibrium association constants. One can presume the adsorption to occur by the following reactions:

$$M + X \rightleftharpoons MX \tag{1a}$$

$$D + X \rightleftharpoons DX \tag{1b}$$

$$D + X_2 \rightleftharpoons DX_2, \tag{1c}$$

where M and D refer to monovalent cations and divalent cations in solution, X is an unoccupied membrane binding site, X_2 is a pair of adjacent X-sites, and MX, DX, and DX_2 are the respective adsorbed complexes on the membrane. The mass-action equilibrium association constants of the reactions of Eqs. 1a–c are, by definition,

$$K_M \equiv \frac{(MX)}{[M](X)} = \frac{\overline{N}_M}{[M]\overline{N}_X} \tag{2a}$$

$$K_{D1} \equiv \frac{(DX)}{[D](X)} = \frac{\overline{N}_{D1}}{[D]\overline{N}_X} \tag{2b}$$

$$K_{D2} \equiv \frac{(DX_2)}{[D](X_2)} = \frac{\overline{N}_{D2}}{[D]\overline{N}_{X_2}}, \tag{2c}$$

where ( ) denotes a surface density or concentration on the membrane, [ ] denotes a volume concentration in the aqueous solution at the membrane-water interface, $\overline{N}_X$ is the average number of X sites on the membrane at equilibrium, $\overline{N}_{X_2}$ is the average number of X_2 pairs on the membrane at equilibrium, and $\overline{N}_M$, $\overline{N}_{D1}$, and $\overline{N}_{D2}$ have been defined above. Eqs. 2a–c are readily understood in terms of kinetic theory. For example, the rate of the left-to-right reaction of Eq. 1c, which proceeds via collisions between divalent cations in solution and X_2 pairs on the membrane, is proportional to the product $[D]\overline{N}_{X_2}$, whereas the rate of the right-to-left reaction is proportional to $\overline{N}_{D2}$. Equating the two rates at equilibrium, we obtain Eq. 2c, where K_{D2} depends only on temperature.

In reference 1 we used statistical mechanics to show that the interfacial reactions of Eqs. 1a–c obey laws of mass action if ion-ion interactions in solution and on the membrane are neglected.[1] That is, K_M, K_{D1}, and K_{D2} were shown to be constants, independent of ion concentrations in solution or on the membrane. These constants are simply expressed in terms of the cation single-particle partition functions and the volume of the aqueous phase. The solution to the adsorption problem can therefore be couched in terms of the paramters K_M, K_{D1}, and K_{D2}, which was done in Eqs. 51 and 52 of reference 1.

We now consider the possibility of adsorption schemes other than that of Eqs. 1a–c. In particular, it has been proposed (A. J. Murphy, personal communication) that a DX_2 complex could also be formed by reaction of a 1:1-bound complex (DX) with an adjacent unoccupied site (X) on the membrane. In this scheme, formation of a 1:2-bound complex requires prior formation of a 1:1-bound complex. In this case Eqs. 1a–c would be replaced by

$$M + X \rightleftharpoons MX \tag{3a}$$

$$D + X \rightleftharpoons DX \tag{3b}$$

$$DX\cdot X \rightleftharpoons DX_2, \tag{3c}$$

where $DX\cdot X$ refers to a pair of neighboring sites comprised of a DX complex and an adjacent unoccupied X site.

Eq. 3c represents a two-dimensional lattice reaction. The left-to-right reaction rate is proportional to $\bar{N}_{D1,0}$ and the right-to-left reaction rate is proportional to $\bar{N}_{D2}$, where $\bar{N}_{D1,0}$ is the average number of $DX\cdot X$ pairs on the membrane at equilibrium, and $\bar{N}_{D2}$ has been defined above. Therefore, at equilibrium we expect the ratio $\bar{N}_{D2}/\bar{N}_{D1,0}$ to be a function only of temperature and not of the number of divalent or monovalent cations in solution or on the membrane. That is, we expect

$$\frac{\bar{N}_{D2}}{\bar{N}_{D1,0}} = \frac{(DX_2)}{(DX\cdot X)} \equiv K'_{D2}, \tag{4}$$

where the mass-action equilibrium constant K'_{D2} depends only on temperature. The purpose of this communication is to show that Eq. 4 is true and can be deduced from Eqs. 2a–c with the identification $K'_{D2} = K_{D2}/2K_{D1}$.

THEORY

In the Appendix we derive Eq. 4 from Eqs. 2a–c in a mathematically rigorous way using a number of results from reference 1. Here, however, we present an elementary argument that displays the basic idea more clearly.

To facilitate visualization we refer to a 1:1-bound divalent cation DX as a standing dimer on the membrane, and a 1:2-bound divalent cation DX_2 as a lying dimer (i.e., we visualize the divalent cations as rods that cover two sites when lying down and one site when standing on end). A monovalent cation is called a monomer and an unoccupied site X is called a vacancy. Here we treat the competitive case in which a membrane site may bind a monomer or one end of a dimer, but not both simultaneously. Extension to the noncompetitive case is straightforward.

Suppose we consider configurations of the membrane in which there are N_{D2} lying dimers, N_{D1} standing dimers, N_M monomers, and N_X vacancies. We note that $N_X = N - 2N_{D2} - N_{D1} - N_M$, where N is the total number of sites on the membrane. If we specify the positions of the lying dimers, there are still $(N - 2N_{D2})!/(N_{D1})!(N_M)!(N_X)!$ different configurations corresponding to the various ways of placing the standing dimers and monomers on the available sites. All of these configurations are equally probable if there are no interactions among the adsorbed molecules. If we focus our attention on a particular site that is not covered by a lying dimer, the fraction of the configurations in which this site is occupied by a standing dimer is $N_{D1}/(N - 2N_{D2})$; i.e., this is the conditional probability of finding a standing dimer on the site, given that the lying dimers are in the specified positions. Similarly, the conditional probabilities of finding this site vacant or occupied by a monomer are $N_X/(N - 2N_{D2})$ and $N_M/(N - 2N_{D2})$, respectively. If we focus our attention on a particular *pair* of sites that are not covered by lying dimers, the fraction of configurations in which both sites are empty is

$$\left(\frac{N_X}{N - 2N_{D2}}\right)\left(\frac{N_X - 1}{N - 2N_{D2} - 1}\right). \tag{5}$$

Neglecting "1" compared with N_X and $N - 2N_{D2}$, we can say that the conditional probability (given that the lying dimers are in the specified positions) of finding both sites vacant is

$$\left(\frac{N_X}{N - 2N_{D2}}\right)^2. \tag{6}$$

Similarly, the conditional probability that one site is vacant and one site is occupied by a standing dimer is

$$2\left(\frac{N_X}{N - 2N_{D2}}\right)\left(\frac{N_{D1}}{N - 2N_{D2}}\right). \tag{7}$$

(The factor of two arises because there are two ways of choosing the occupied site.)

Let us call a site a Y site if it is not covered by a lying dimer, i.e., if it is vacant or is occupied by a standing dimer or by a monomer. We also introduce the terminology Y_2 to denote a pair of adjacent Y sites and N_{Y_2} to denote the number of such pairs on the membrane. If the standing dimers and monomers were removed from the membrane,

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[1] Including electrostatic interactions does not invalidate a mass-action law, provided that the interactions are described by a mean field approximation, as in Gouy-Chapman theory. Because of the long-range character of electrostatic interactions, a mean field treatment is quite accurate.

leaving the lying dimers where they are, then the number of locations (pairs of adjacent vacant sites) where an additional lying dimer could be placed is N_{Y_2}. The probability that a Y_2 pair consists of a vacancy and a standing dimer is given by Eq. 7. Thus, for a given arrangement of the lying dimers, the average number (averaged over the configurations of the standing dimers and monomers) of pairs consisting of a vacancy adjacent to a standing dimer is

$$N_{D1,0} = 2\left(\frac{N_X}{N - 2N_{D2}}\right)\left(\frac{N_{D1}}{N - 2N_{D2}}\right)N_{Y_2}. \qquad (8)$$

Similarly, the average number of pairs of adjacent vacancies is

$$N_{X_2} = \left(\frac{N_X}{N - 2N_{D2}}\right)^2 N_{Y_2}. \qquad (9)$$

On general grounds, because the membrane has many sites, the probability distribution for any of the quantities under discussion (e.g., N_{D1}, N_{D2}, N_X, N_{X_2}, N_{Y_2}) is peaked very sharply (when measured in units of its average value) about a most likely value, which can be identified with the average value when N is large.[2] Thus Eqs. 8 and 9 are true when the quantities involved are replaced by their average values at thermal equilibrium. Hence,

$$\overline{N}_{D1,0} = 2\left(\frac{\overline{N}_X}{N - 2\overline{N}_{D2}}\right)\left(\frac{\overline{N}_{D1}}{N - 2\overline{N}_{D2}}\right)\overline{N}_{Y_2} \qquad (10)$$

$$\overline{N}_{X_2} = \left(\frac{\overline{N}_X}{N - 2\overline{N}_{D2}}\right)^2 \overline{N}_{Y_2}. \qquad (11)$$

Dividing Eq. 11 by Eq. 10, we get

$$\frac{\overline{N}_{X_2}}{\overline{N}_{D1,0}} = \frac{\overline{N}_X}{2\overline{N}_{D1}}. \qquad (12)$$

From the mass-action laws of Eqs. 2b and c, the right side of Eq. 12 is

$$\frac{K_{D2}}{2K_{D1}}\,\frac{\overline{N}_{X_2}}{\overline{N}_{D2}}.$$

Thus,

$$\frac{\overline{N}_{D2}}{\overline{N}_{D1,0}} \equiv K'_{D2} = \frac{K_{D2}}{2K_{D1}}, \qquad (13)$$

which is the desired result.

Because K'_{D2} is independent of monovalent- and divalent-cation concentrations in solution and on the membrane, we have verified that K'_{D2} is a constant depending only on temperature and that the reaction of Eq. 3c obeys a law of mass action. Therefore, K'_{D2} can be used as a parameter of the problem. If one chooses to formulate the adsorption isotherm in terms of Eqs. 3a–c, the result is given by Eqs. 51 in reference 1 with K_{D2} replaced by $2K'_{D2}K_{D1}$.

DISCUSSION

We have shown that the monovalent- and divalent-cation adsorption problem can be formulated either in terms of a parallel kinetic scheme (Eqs. 1a–c) or a serial kinetic scheme (Eqs. 3a–c). In the former case the adsorption isotherms are parameterized in terms of K_M, K_{D1}, and K_{D2}, whereas in the latter case they are parameterized in terms of K_M, K_{D1}, and K'_{D2}, where $K'_{D2} = K_{D2}/2K_{D1}$. Formally, there is no difference between the two cases because each involves three independent constants. In fact equilibrium statistical mechanics requires that the two formulations be equivalent. The adsorption isotherms have the same functional dependence on [M] and [D] (or C^+ and C^{++} in reference 1) irrespective of which parameterization is used.

A conceptual difference exists, however, if one considers the physical meaning of the association constants. For the parallel case, the 1:2-bound charge density (or lattice coverage) is a decreasing function of K_{D1} (with K_{D2} held constant), as a result of competition between 1:1-bound and 1:2-bound divalent cations. For the serial case, except at very high lattice coverages, the 1:2-bound charge density is an increasing function of K_{D1} (with K'_{D2} held constant), as a result of the dependence of 1:2 complexation on prior 1:1 complexation. In order to distinguish experimentally between the two adsorption pathways, time-resolved studies of the adsorption process would be necessary.

APPENDIX

Here we derive Eq. 13 using the formalism of reference 1. For clarity we initially delete monovalent-cation binding from this discussion. We wish to evaluate K'_{D2}, hence, we must first find $\overline{N}_{D1,0}$.

First, we note that $\overline{N}_{D1,0}$ (the average number of standing dimer-vacancy pairs on the membrane lattice) is just $\overline{N}_{D1}$ multiplied by the average number of vacancy pairs created when one standing dimer is removed from the lattice. The reason is that removal of a standing dimer from the lattice converts all DX·X pairs involving that dimer into X_2 pairs. Analogous to reference 1, we define $g_N(N_{D1}, N_{D2})$ as the number of ways of placing N_{D1} indistinguishable standing dimers and N_{D2} indistinguishable lying dimers on a q-coordinated lattice of N sites. (Here standing dimers have the same role as monomers in reference 1.) The average number of vacancy pairs in the presence of $\overline{N}_{D1}$ standing and $\overline{N}_{D2}$ lying dimers is

$$\overline{N}_{X_2} = (\overline{N}_{D2} + 1)\,\frac{g_N(\overline{N}_{D1}, \overline{N}_{D2} + 1)}{g_N(\overline{N}_{D1}, \overline{N}_{D2})}, \qquad (A1)$$

which is analogous to Eq. 34 of reference 1. The number of vacancy pairs created by removing one standing dimer is the number of vacancy pairs existing in the presence of $\overline{N}_{D1} - 1$ standing and $\overline{N}_{D2}$ lying dimers, less that existing in the presence of $\overline{N}_{D1}$ standing and $\overline{N}_{D2}$ lying dimers. Hence,

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[2] Our final result, Eq. 13, is true even when the membrane does not have many sites, but in this case a more careful discussion of the meaning of the averages is necessary.

by using Eq. A1,

$$\bar{N}_{\mathrm{D1,0}} = \bar{N}_{\mathrm{D1}} \left[\frac{(\bar{N}_{\mathrm{D2}} + 1)\, g_N(\bar{N}_{\mathrm{D1}} - 1, \bar{N}_{\mathrm{D2}} + 1)}{g_N(\bar{N}_{\mathrm{D1}} - 1, \bar{N}_{\mathrm{D2}})} - \frac{(\bar{N}_{\mathrm{D2}} + 1)\, g_N(\bar{N}_{\mathrm{D1}}, \bar{N}_{\mathrm{D2}} + 1)}{g_N(\bar{N}_{\mathrm{D1}}, \bar{N}_{\mathrm{D2}})} \right]. \tag{A2}$$

By Eq. 33 of reference 1:

$$g_N(\bar{N}_{\mathrm{D1}}, \bar{N}_{\mathrm{D2}}) = g_N(\bar{N}_{\mathrm{D2}}) \binom{N - 2\bar{N}_{\mathrm{D2}}}{\bar{N}_{\mathrm{D1}}}, \tag{A3}$$

where $g_N(\bar{N}_{\mathrm{D2}})$ is the number of ways of placing $\bar{N}_{\mathrm{D2}}$ indistinguishable lying dimers on the lattice in the absence of standing dimers, and the second factor is the binomial coefficient, which is the number of ways of placing standing dimers on the remaining vacancies. Therefore, Eq. A2 reduces to

$$\bar{N}_{\mathrm{D1,0}} = \frac{2\,\bar{N}_{\mathrm{D1}}\bar{N}_{\mathrm{D2}}\bar{N}_{\mathrm{X}}}{(N - 2\bar{N}_{\mathrm{D2}})^2} \frac{g_N(\bar{N}_{\mathrm{D2}} + 1)}{g_N(\bar{N}_{\mathrm{D2}})}, \tag{A4}$$

where we have used Eq. A3, $\bar{N}_{\mathrm{D2}} \gg 1$, and $\bar{N}_{\mathrm{X}} = N - 2\bar{N}_{\mathrm{D2}} - \bar{N}_{\mathrm{D1}}$. But by Eqs. 31 and 43b of reference 1,

$$\frac{g_N(\bar{N}_{\mathrm{D2}} + 1)}{g_N(\bar{N}_{\mathrm{D2}})} = \frac{1}{z}, \tag{A5}$$

where

$$z = \frac{K_{\mathrm{D2}}[\mathrm{D}]}{(1 + K_{\mathrm{D1}}[\mathrm{D}])^2}. \tag{A6}$$

From Eq. 40 of reference 1,

$$\frac{\bar{N}_{\mathrm{D1}}}{N} = \frac{K_{\mathrm{D1}}[\mathrm{D}]}{1 + K_{\mathrm{D1}}[\mathrm{D}]} \left(1 - \frac{2\bar{N}_{\mathrm{D2}}}{N}\right), \tag{A7}$$

which merely states that the standing dimers bind, via a Langmuir adsorption isotherm, to the sites left vacant by the lying dimers. Using Eqs. 2b and A7, we find from Eqs. A4–A6 that

$$\bar{N}_{\mathrm{D1,0}} = \frac{2\bar{N}_{\mathrm{D2}}\, K_{\mathrm{D1}}}{K_{\mathrm{D2}}}. \tag{A8}$$

Thus,

$$K'_{\mathrm{D2}} \equiv \frac{\bar{N}_{\mathrm{D2}}}{\bar{N}_{\mathrm{D1,0}}} = \frac{K_{\mathrm{D2}}}{2\, K_{\mathrm{D1}}}. \tag{A9}$$

Eq. A9 is identical to Eq. 13. When monovalent-cation binding is included, it is readily shown that Eq. A9 remains unchanged.

REFERENCES

1. Cohen, J. A., and M. Cohen. 1981. Adsorption of monovalent and divalent cations by phospholipid membranes. The monomer-dimer problem. *Biophys. J.* 36:623–651.