ADSORPTION OF MONOVALENT AND DIVALENT CATIONS BY PHOSPHOLIPID MEMBRANES

THE MONOMER-DIMER PROBLEM

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ABSTRACT A generalization of the Stern theory is derived to treat the simultaneous adsorption of monovalent cations and divalent cations by single-component phospholipid membranes, where the ion:phospholipid binding stoichiometries are 1:1 for the monovalent cations and 1:1 and/or 1:2 for the divalent cations. This study treats both the situation in which the monovalent and divalent cations compete for membrane binding sites and that in which they do not compete. The general formalism of the screening/binding problem is reviewed, and it is shown how the adsorption problem can be isolated from the electrostatics. The statistical mechanics of mixed 1:1- and 1:2-stoichiometric adsorption (the monomer-dimer problem) is treated, and the problem of simultaneous 1:1 and 1:2 binding is solved. A simple expression for this solution, given in the Bethe approximation, is combined with the electrostatics to yield an adsorption isotherm encompassing both 1:1 monovalent-cation, and 1:1 and 1:2 divalent-cation, binding to charged membranes. A comparison with the simplified treatment of previous authors is made and the significance of their assumptions clarified in light of the present result. The present and previous treatments are plotted for a representative case of Na^+ and Ca^{++} binding to a phosphatidylserine membrane. Criteria are established to permit unambiguous experimental testing of the present vs. previous treatments.

INTRODUCTION

The interaction of divalent cations, particularly calcium, with phospholipid membranes has been a subject of interest for some time (McLaughlin et al., 1981, and references therein). Such interest arises from the well-known regulatory role played by calcium in many biological phenomena, as well as from the ability of calcium to induce aggregation and fusion in phospholipid vesicular dispersions (Papahadjopoulos et al., 1979). The phospholipid membrane-binding properties of Ca^{++} vs. those of other divalent cations have also been examined (Nir et al., 1978; McLaughlin et al., 1978; McLaughlin et al., 1981; Lau et al., 1981). It has become clear in such investigations that, particularly for the case of membranes that are electrically charged, electrostatic forces exert an important influence on the ion-membrane interaction (McLaughlin, 1977). In its simplest form, such interaction may be considered in two parts, involving long-range Coulombic and short-range interfacial "binding" (or adsorption) forces. The former have been treated successfully in terms of the Gouy-Chapman theory of the diffuse double layer (Gouy, 1910; Chapman, 1913), whereas the latter generally are treated in terms of a Langmuir adsorption isotherm (Langmuir, 1918), in which the strength of the binding (or adsorption) interaction is expressed by a phenomenological association constant. The connection between the electrostatics and the binding lies in use of the interfacial value of the aqueous ion concentration in the adsorption isotherm. This interfacial value is determined from the ion concentration in the bulk aqueous phase by a Boltzmann relation involving the membrane electrostatic surface potential. The surface potential, in turn, is affected both by ions in the aqueous phase (screening) and ions that are bound or adsorbed by the membrane (alteration of surface-charge density). The "total" adsorption isotherm, giving the adsorbed ion density vs. bulk aqueous ion concentration, including the electrostatic effects, is known as the Stern adsorption isotherm (Stern, 1924).[1] The Stern theory, as outlined above, has been reviewed by McLaughlin (1977). Excellent treatments of these topics are also given by Adamson (1976), Aveyard and Haydon (1973), and Davies and Rideal (1963).

Recently, evidence has been mounting to show that monovalent cations, as well as divalent cations, can bind to phospholipid membranes. Such effects have been observed particularly for highly charged phosphatidylserine (PS) membranes (Nir et al., 1978; Newton et al., 1978; Kurland et al., 1979; Eisenberg et al., 1979; Ohki and Kurland, 1981). The existence of monovalent-cation binding significantly affects the binding of divalent cations to the membrane, with regard to the shape of the divalent-cation adsorption isotherm as well as to the inferred values of the divalent-cation association constants. For example, neglect of the (then unknown) binding of Na^+ to PS phospholipids has resulted in reports of $Ca \cdot PS$ association constants that are far too low (McLaughlin et al., 1971; Ohki and Sauve, 1978).

The situation in which both monovalent cations and divalent cations bind to phospholipid membranes is one of considerable theoretical complexity. It is clear that, in order experimentally to determine meaningful divalent-cation:membrane association constants when monovalent cations are present, the manner in which monovalent cations interfere with the divalent-cation binding must be ascertained. This problem is biologically important, since divalent-cation phenomena in vivo invariably occur in the presence of considerable background concentrations of Na^+ and/or K^+.

The statistical mechanics of the monovalent- and divalent-cation adsorption problem depends strongly on the stoichiometry of association of each type of cation with the membrane phospholipids. If both monovalent and divalent cations bind with ion:phospholipid stoichiometries of 1:1, then the Stern theory can be generalized in a simple manner to yield the total adsorption isotherm (McLaughlin et al., 1981). If the divalent cations bind with a 1:2 stoichiometry, as has often been assumed in the past (McLaughlin et al., 1971; Nir et al., 1978; Bentz, 1981; Ohki and Kurland, 1981), the appropriate generalization of the Stern theory is more complicated. In previous approaches to this problem, both excluding monovalent-cation binding (McLaughlin et al., 1971) and including it (Nir et al., 1978; Bentz and Nir, 1980; Bentz, 1981; Ohki and Kurland, 1981), the statistical mechanics of the adsorption process has not been treated properly.[2] In this paper we use statistical mechanics to solve the

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[1] More precisely, this should be called the "modified" Stern isotherm as the finite size of the ions in solution is ignored (McLaughlin, 1977).

[2] Kolber and van Breemen (1981) and Kolber (personal communication) have treated the case of 1:1 monovalent-cation and 1:2 divalent-cation binding to membranes of smooth muscle cells, where the binding sites are assumed to occur as isolated pairs associated with membrane proteins. Their result is equivalent to the $q = 1$ case of our treatment (cf. Eq. 48).

general case of simultaneous 1:2 binding of divalent cations, 1:1 binding of divalent cations, and 1:1 binding of monovalent cations to single-component phospholipid membranes.[3]

First we review the general formalism of the screening/binding problem. Then we show how the nonelectrostatic part of this formalism can be isolated, permitting consideration of the adsorption problem independent of electrostatics. The statistics of mixed 1:1- and 1:2-stoichiometric adsorption (the "monomer-dimer problem"[4]) is treated, and the problem of simultaneous 1:1 and 1:2 binding is solved. A simple, tractable expression for the solution is displayed in the Bethe approximation, which is compared to the exact solution. The Bethe expression is then combined with the electrostatics to produce a generalization of the Stern theory which encompasses both 1:1 monovalent-cation, and 1:1 and 1:2 divalent-cation, binding to charged membranes. A comparison with the simplified treatment of previous authors is made, and the significance of their assumptions clarified in light of the present result. Isotherms from the present treatment, as well as from previous ones, are then plotted for a representative case of Na^+ and Ca^{++} binding to a PS membrane, including the cases of competitive and noncompetitive Na^+ vs. Ca^{++} binding. Finally, criteria are established to permit unambiguous experimental testing of the present vs. previous treatments.

THE GENERAL PROBLEM

We begin by considering the membrane surface-charge density which is assumed, in the spirit of the Gouy-Chapman theory, to be uniformly smeared over the plane of the membrane-water interface. This surface-charge density has two components: the charge density intrinsically associated with the membrane phospholipids ($\sigma_{\text{intrinsic}}$) and the charge density resulting from ions reversibly bound or adsorbed to the membrane surface (σ_{adsorbed}). We define the net (or total) surface-charge density as

$$\sigma_{\text{net}} \equiv \sigma_{\text{intrinsic}} + \sigma_{\text{adsorbed}}. \tag{1}$$

The charge density σ_{net} is related to the bulk concentrations of ions in solution and to the electrostatic potential Ψ_0 at the membrane surface (relative to that in the bulk solution) by the Grahame equation (Grahame, 1947), which takes the form

$$\sigma_{\text{net}} = f_G(C^+, C^{++}, \Psi_0) \tag{2}$$

when only monovalent and divalent salts are present, at bulk concentrations C^+ and C^{++},

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[3] Related problems have been studied in the context of immunology. Dembo and Goldstein (1978), Perelson (1979), and Perelson and DeLisi (1980) discuss the binding of divalent and monovalent antigens to receptor sites on cell surfaces. The immunological receptor sites are assumed divalent or multivalent; therefore, large complexes of cross-linked receptors can be formed. The formation of these complexes is analyzed by rate equations which ignore correlations induced by the finite size of the antigens and receptors. In the present work the receptor sites are monovalent. Our emphasis is on the geometrical effects of the competition for sites on the lattice, and on the electrostatic effects associated with the adsorbed charge. We thank Dr. Alan Perelson for calling our attention to the work of the Los Alamos group.

[4] The terms "monomer" and "dimer" refer to molecules that bind to the membrane with molecule:phospholipid stoichiometries of 1:1 and 1:2, respectively.

respectively. For the case of 1:1 and 2:1 electrolytes (e.g., NaCl and $MgCl_2$),

$$f_G = \frac{\Psi_0}{|\Psi_0|} A' \left\{ C^+ \left(\exp\frac{e\Psi_0}{kT} + \exp\frac{-e\Psi_0}{kT} - 2 \right) + C^{++} \left(2\exp\frac{e\Psi_0}{kT} + \exp\frac{-2e\Psi_0}{kT} - 3 \right) \right\}^{1/2}, \tag{3a}$$

while for the case of 1:1 and 2:2 electrolytes (e.g., NaCl and $MgSO_4$),

$$f_G = \frac{\Psi_0}{|\Psi_0|} A' \left\{ C^+ \left(\exp\frac{e\Psi_0}{kT} + \exp\frac{-e\Psi_0}{kT} - 2 \right) + C^{++} \left(\exp\frac{2e\Psi_0}{kT} + \exp\frac{-2e\Psi_0}{kT} - 2 \right) \right\}^{1/2}, \tag{3b}$$

where $A' = (2\,\epsilon\epsilon_0 kT)^{1/2}$; ϵ is the dielectric constant of the aqueous solution; ϵ_0 is the permittivity of free space; k is Boltzmann's constant; T is the absolute temperature; e is the magnitude of the electron charge, and the positive square root is assumed. Eqs. 3 follow directly from Poisson's equation, which relates electrostatic potential to charge density in the aqueous phase, and from the Boltzmann distribution, which relates aqueous ion concentration to local electrostatic potential.

The term $\sigma_{\text{intrinsic}}$ in Eq. 1 is simply a constant, which is zero in the case of a neutral membrane, or is $-e$ · (anionic phospholipid density) in the case of a membrane comprised of anionic phospholipids, assuming one negative charge per phospholipid. For the case of a PS membrane at neutral pH, we take (Nir et al., 1978; McLaughlin et al., 1981)

$$\sigma_{\text{intrinsic}} = \text{constant} = -(\ell)' \approx -1/70\, e/\text{Å}^2, \tag{4}$$

where $(\ell)' \equiv e(\ell)$, and (ℓ) is the phospholipid density.[5]

The term σ_{adsorbed} is given by an adsorption isotherm whose exact functional form will be discussed later. We note now, however, that it must depend on the following variables:

$$\sigma_{\text{adsorbed}} = f_A(C_0^+, C_0^{++}, K_M, K_{D1}, K_{D2}), \tag{5}$$

where f_A is the adsorption-isotherm function; K_M, K_{D1}, and K_{D2} are the 1:1 monovalent-cation, and 1:1 and 1:2 divalent-cation, membrane association constants, respectively. C_0^+ and C_0^{++} are aqueous monovalent- and divalent-cation concentrations. (We limit this discussion to binding of a single monovalent-cation and a single divalent-cation species. Generalization to the binding of other ions, including anions, is straightforward.) In accordance with the Stern theory, the aqueous ion concentrations used in Eq. 5 must be those existing at the membrane-water interface, which differ from those in bulk solution if the membrane surface is charged. The Boltzmann distribution gives

$$C_0^+ = C^+ \exp\left(\frac{-e\Psi_0}{kT}\right) \tag{6a}$$

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[5] If the membrane phospholipids contain titratable surface groups, a Henderson-Hasselbalch expression involving the intrinsic pK_a's of such groups and the interfacial pH (bulk pH + $e\Psi_0/2.303kT$) is easily inserted here, giving $\sigma_{\text{intrinsic}}$ as a function of the bulk pH and Ψ_0. In addition, if the membrane condenses or expands as a function of screening and/or binding, such effects may be included via insertion of a functional dependence of (ℓ) on the screening and binding parameters (if such dependence is known) into this and later equations (cf. Eqs. 51 and 52). For the purpose of this paper, however, we simply use Eq. 4.

and

$$C_0^{++} = C^{++} \exp\left(\frac{-2e\Psi_0}{kT}\right). \tag{6b}$$

Eq. 5 may thus be written

$$\sigma_{\text{adsorbed}} = f_A(C^+, C^{++}, \Psi_0, K_M, K_{D1}, K_{D2}), \tag{7}$$

where it is understood that once a functional form for the adsorption isotherm (Eq. 5) has been chosen, Eq. 7 is readily obtained by use of Eq. 6 *a* and *b*.

Eq. 1 can now be restated in the form

$$f_G(C^+, C^{++}, \Psi_0) - \text{const.} - f_A(C^+, C^{++}, \Psi_0, K_M, K_{D1}, K_{D2}) = 0, \tag{8}$$

where the constant term is that appearing in Eq. 4. This is an implicit equation for Ψ_0 in terms of C^+, C^{++}, K_M, K_{D1}, and K_{D2} which can be solved numerically for any given values of these parameters. All information regarding the adsorption problem, within the framework of this treatment, is contained in these solutions for Ψ_0. For example, the adsorbed charge as a function of any parameter (say C^{++}) can be found from Eq. 7 once the appropriate values of Ψ_0 have been determined from Eq. 8.

The problem is now completely defined once the functional form of the adsorption isotherm, Eq. 5, is chosen. If $f_A = 0$, then Eq. 8 is the Grahame equation. If f_A is a Langmuir isotherm, then Eq. 8 becomes the Stern equation. If a different isotherm is used, Eq. 8 may be regarded as a generalization of the Stern equation. For our purposes, Eq. 8 tells us how to insert the electrostatics (at the level of the Gouy-Chapman theory) once an adsorption isotherm (Eq. 5) has been selected. Therefore, we may now focus on the adsorption problem independently of electrostatics, to seek an appropriate functional form for Eq. 5.

BINDING STOICHIOMETRY

The form of the adsorption isotherm depends on the stoichiometry of binding of ions to the membrane phospholipids. In the case where one molecule of adsorbate (monomer) binds to one phospholipid (1:1 binding), the appropriate isotherm is of the Langmuir form. Consider the reversible interfacial reaction

$$\mathrm{M} + \mathrm{X} \rightleftharpoons \mathrm{MX} \tag{9}$$

where M is a monomer in solution, X is an unoccupied phospholipid binding site on the membrane, and MX is a monomer-occupied phospholipid binding site on the membrane. The mass-action equilibrium association constant for this reaction is

$$K_M \equiv \frac{(\mathrm{MX})}{[\mathrm{M}](\mathrm{X})}, \tag{10}$$

where [ ] denotes an aqueous volume concentration or activity, and ( ) denotes an area concentration or density on the membrane surface. The density of membrane phospholipids, (ℓ), is

$$(\ell) = (\mathrm{MX}) + (\mathrm{X}). \tag{11}$$

From Eqs. 10 and 11, we find that the fraction of sites bound, or the lattice coverage Θ_M, is

$$\Theta_M = \frac{(MX)}{(\ell)} = \frac{K_M[M]}{1 + K_M[M]}. \tag{12}$$

Eq. 12 is the Langmuir isotherm. In the event that two distinct molecular species M and M′ bind competitively to the lattice with 1:1 stoichiometries, the straightforward generalization of Eq. 12 is

$$\Theta_M + \Theta_{M'} = \frac{K_M[M] + K_{M'}[M']}{1 + K_M[M] + K_{M'}[M']}. \tag{13}$$

In the case where one molecule of adsorbate (dimer) binds to two phospholipids (1:2 binding), the reaction to be considered is

$$D + X_2 \rightleftharpoons DX_2 \tag{14}$$

where D is a dimer is solution; X_2 is an unoccupied "pair" binding site on the membrane, i.e., a pair of adjacent unoccupied phospholipid sites; and DX_2 is a dimer-occupied pair binding site on the membrane. We consider here only the equilibrium state of Eq. 14 and not the detailed manner in which the final state is achieved. The equilibrium association constant for this reaction is

$$K_D \equiv \frac{(DX_2)}{[D](X_2)}. \tag{15}$$

The lipid density is now

$$(\ell) = 2(DX_2) + (X). \tag{16}$$

By use of Eqs. 15 and 16, the fraction of lipids bound by dimers, or the lattice coverage Θ_D, is found to be

$$\Theta_D = \frac{2(DX_2)}{(\ell)} = \frac{K_D[D] \cdot 2(X_2)/(X)}{1 + K_D[D] \cdot 2(X_2)/(X)}. \tag{17}$$

Eq. 17 is of a Langmuir form only if $(X_2)/(X)$ remains constant as the lattice fills. In particular, if $(X_2)/(X) = 1/2$, one obtains the Langmuir isotherm for dimers:

$$\Theta_D = \frac{K_D[D]}{1 + K_D[D]} \quad \text{for} \; \frac{(X_2)}{(X)} = \frac{1}{2}. \tag{18}$$

It has been shown for the case of monovalent-cation binding to negatively charged phospholipid membranes that the data can be treated in terms of a 1:1 Langmuir isotherm (Eisenberg et al., 1979). For the case of divalent-cation binding to phospholipid membranes in which a binding stoichiometry of 1:2 has been assumed (McLaughlin et al., 1971; Ohki and Sauve, 1978; Nir et al., 1978; Ohki and Kurland, 1981), the data have been analyzed in terms of Eq. 18 or a simple generalization of it to include 1:1 monovalent-cation binding. Our claim in this paper is that, in general, the conditions of Eq. 18 are not physically valid, so that 1:2 binding cannot be described by a Langmuir isotherm, and the more complex case of simultaneous 1:1 and 1:2 binding is not obtained simply.

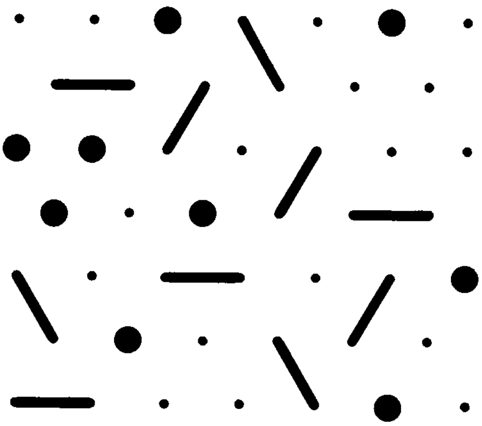

FIGURE 1 Monomers and dimers adsorbed on a $q = 6$ lattice. The lattice binding sites are indicated by dots, 1:1-bound monomers by filled circles, and 1:2-bound dimers by bars. In this picture, two empty sites are unavailable for dimer binding.

THE MEMBRANE LATTICE

If ions indeed bind specifically to one or more phospholipids, then the discrete nature of the membrane, as an array of binding sites, must be considered.[6] We refer to such a two-dimensional array as the membrane "lattice" which may, or may not, be ordered. In this paper, we treat only the case of identical lattice sites, i.e., single-component phospholipid membranes.

We assume each site to have, on the average, q nearest neighbors. A maximally packed array corresponds to $q = 6$, the hexagonal (or triangular) lattice as shown in Fig. 1, while the value $q = 4$ denotes a square-coordinated lattice, which is quite loosely packed. Thus the values $4 \leq q \leq 6$ appear to span the range of packing geometries physically appropriate for membranes. It will be shown later that our results are insensitive to the choice of q in this range.

Since a monomer can bind to a single empty lattice site (1:1 binding), while a dimer requires two neighboring empty sites (1:2 binding), a given lattice site either is empty or is

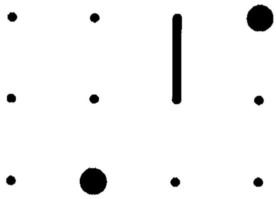

FIGURE 2 A $q = 4$ lattice with $N = 12$, $N_M = 2$, $N_D = 1$, $N_X = 8$, and $N_{X_2} = 7$.

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[6] The apparent inconsistency whereby membrane discreteness is ignored in the electrostatic treatment (Gouy-Chapman theory) yet retained in the binding formalism has been discussed (Aveyard and Haydon, 1973; McLaughlin, 1977). The necessity for maintaining lattice discreteness for binding and not for screening results from the much shorter range of the binding (relative to the Coulombic) interaction.

filled by a bound monomer or half a dimer. We assume that a filled site cannot bind any further molecules. (This competition between monomers and dimers for empty sites is easily relaxed. We treat the noncompetitive case later.) Fig. 1 illustrates the statistical complexity of the manner in which bound monomers and dimers affect the number of unoccupied pairs available for further dimer binding. It must be appreciated that, on the lattice, a given site X may participate in several different pair vacancies X_2. For example, in Fig. 2 there are seven vacant pairs capable of binding further dimers.

It is clear from Fig. 1 that as the lattice fills (with either monomers or dimers or both) the ratio $(X_2)/(X)$ does not remain constant. On the empty lattice $(X_2)/(X) = q/2$. The ratio of empty pairs to empty sites decreases as the lattice fills, approaching zero as the lattice approaches saturation.[7] The constancy of $(X_2)/(X)$ required for the validity of Eq. 18, and hence for use of the Langmuir isotherm, is not maintained.

THE MONOMER-DIMER PROBLEM

We now derive the monomer-dimer adsorption isotherm; that is, we obtain an expression for the equilibrium lattice coverage of monomers and dimers as a function of [M], [D], K_M, K_D, and q.

We assume the membrane lattice to be in equilibrium with the aqueous solution. Molecules from the solution impinging randomly on the lattice are either monomers or dimers. Thus there are four reactions occurring on the lattice: adsorption and desorption of monomers, and adsorption and desorption of dimers. These reactions are represented by Eqs. 9 and 14. We assume there are no interactions between molecules either in solution or on the lattice and therefore expect the reactions to obey laws of mass action.[8] The respective mass-action equilibrium constants K_M and K_D are given by Eqs. 10 and 15. For purposes of statistical treatment we also write Eqs. 10 and 15 in the form

$$K_M = \frac{\overline{N}_M/N}{[M]\overline{N}_X/N} \tag{19}$$

and

$$K_D = \frac{\overline{N}_D/N}{[D]\overline{N}_{X_2}/N}, \tag{20}$$

where N is the total number of sites on the lattice;
$\overline{N}_M$ is the average number of adsorbed monomers;
$\overline{N}_D$ is the average number of adsorbed dimers;

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[7] An exception to this statement is the case $q = 1$, where in the absence of bound monomers $(X_2)/(X) = \text{constant} = \frac{1}{2}$ for all degrees of dimer coverage. Even for $q = 1$, however, $(X_2)/(X)$ is no longer constant when monomers are on the lattice.

[8] One might think that electrostatic interactions between the lattice and the molecules in solution, which are explicitly taken into account in our treatment, would invalidate this assumption. However, it can be shown that if these interactions are treated in a mean field approximation, which is equivalent to the Gouy-Chapman theory, then laws of mass action are still valid provided bulk concentrations are replaced by concentrations at the membrane-water interface.

$\overline{N}_X$	is the average number of vacant sites on the lattice;
$\overline{N}_{X_2}$	is the average number of vacant nearest-neighbor pairs on the lattice;
[M], [D]	are, respectively, the number of monomers and dimers per unit volume in solution, expressed in either molecular or molar units;
K_M, K_D	are constants, independent of [M] and [D], but dependent on temperature. The units of these constants are, respectively, $[M]^{-1}$ and $[D]^{-1}$.

For purposes of interconversion between the above statistical parameters and the surface-density notation, we note that the membrane area = $N/(\ell)$ so that

$$(MX) = (\ell)\frac{\overline{N}_M}{N}, \quad (DX_2) = (\ell)\frac{\overline{N}_D}{N}, \quad (X) = (\ell)\frac{\overline{N}_X}{N}, \quad (X_2) = (\ell)\frac{\overline{N}_{X_2}}{N}. \tag{21}$$

We now want to calculate $\overline{N}_M/N$ and $\overline{N}_D/N$ for given values of [M], [D], K_M, K_D, and q. The four unknown quantities $\overline{N}_M/N$, $\overline{N}_D/N$, $\overline{N}_X/N$, and $\overline{N}_{X_2}/N$ are related by Eqs. 19 and 20. An obvious additional equation is

$$\frac{\overline{N}_M}{N} + \frac{2\overline{N}_D}{N} + \frac{\overline{N}_X}{N} = 1, \tag{22}$$

which simply states that every lattice site is either occupied by a monomer or by half a dimer or is vacant. To make the system determinate we need another equation which should relate $\overline{N}_M/N$, $\overline{N}_D/N$, and $\overline{N}_{X_2}/N$. This is the equation that involves counting the number of ways a specified number of monomers and dimers can be put on the lattice.

If there are no adsorbed dimers ($K_D = 0$ or $[D] = 0$ and thus $\overline{N}_D/N = 0$), Eqs. 19 and 22 yield

$$\frac{\overline{N}_M}{N} = \frac{K_M[M]}{1 + K_M[M]}, \tag{23}$$

i.e., a Langmuir isotherm for monomers (cf. Eq. 12). If there are no adsorbed monomers ($K_M = 0$ or $[M] = 0$ so that $\overline{N}_M/N = 0$), the adsorption problem for dimers is not soluble in closed analytic form. However, the counting of configurations of dimers on the lattice has been combined with sophisticated numerical extrapolation procedures (Gaunt, 1969) to yield numerically accurate results over the full range of parameters. We shall exhibit the solution of our problem (which we call "the monomer-dimer problem") in terms of the above solution of the dimer problem.[9] It is instructive to solve the problem two ways: first, by using the law of mass action (Eqs. 19 and 20) together with Eq. 22 and an appropriate combinatorial result; and second, directly from first principles of statistical mechanics. The latter method also enables us to verify that the law of mass action is indeed applicable to the reactions given by Eqs. 9 and 14.

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[9] We use the term "dimer problem" to refer to the problem of a lattice in equilibrium with a solution containing only dimers and no monomers. Unfortunately, Gaunt (1969) calls this situation "the monomer-dimer problem," using the word "monomer" to refer to an empty site on the lattice. He does not treat the case in which both monomeric and dimeric adsorbents are present.

The Dimer Isotherm

In the absence of adsorbed monomers, calculation of the dimer isotherm requires that we know $g_N(N_D)$, the number of ways of putting N_D indistinguishable dimers on a q-coordinated lattice of N sites. (The subscript q is deleted for brevity.) It is convenient for this purpose to define the function

$$\Xi_N(z) \equiv \sum_{N_D} g_N(N_D)\, z^{N_D}, \tag{24}$$

usually called the generating function for $g_N(N_D)$, where z is, at this point, a formal variable used for bookkeeping purposes whose physical significance will become apparent shortly. The maximum value of N_D in Eq. 24 is $qN/2$. Gaunt (1969) defines

$$\Xi(z) \equiv \lim_{N\to\infty} [\Xi_N(z)]^{1/N} \tag{25}$$

and gives many coefficients g_s in the power-series expansion

$$\Xi(z) = 1 + \sum_{s=1}^{\infty} g_s z^s. \tag{26}$$

We also define[10]

$$F(z) \equiv \ln \Xi(z). \tag{27}$$

Now, the average number of dimer vacancies $\overline{N}_{X_2}$ is related to the average number of adsorbed dimers $\overline{N}_D$ by the equation

$$\overline{N}_{X_2} = (\overline{N}_D + 1)\frac{g_N(\overline{N}_D + 1)}{g_N(\overline{N}_D)}. \tag{28}$$

The proof of Eq. 28 is simply to observe that if we have an arrangement of N_D dimers on the lattice, we can generate an arrangement of $N_D + 1$ dimers in N_{X_2} different ways, by putting a single dimer in any of the N_{X_2} dimer vacancies. If we do this for all the $g_N(N_D)$ arrangements of N_D dimers, we generate each arrangement of $N_D + 1$ dimers $N_D + 1$ times. (See Fig. 3.) Since there are $g_N(N_D + 1)$ different arrangements of $N_D + 1$ dimers, we have $N_{X_2} g_N(N_D) = (N_D + 1)\, g_N(N_D + 1)$, which yields Eq. 28. Note that Eq. 28 is true only when $\overline{N}_D$ is large (which is the only case of interest); otherwise, it is not correct to assume that almost all configurations characterized by a given value of N_D have approximately the same number of dimer vacancies N_{X_2}. Therefore, on the right side of Eq. 28 we may replace the factor $(\overline{N}_D + 1)$ by $\overline{N}_D$.

Eqs. 28 and 20 may be combined to yield

$$K_D[\mathrm{D}]\frac{g_N(\overline{N}_D + 1)}{g_N(\overline{N}_D)} = 1. \tag{29}$$

Eq. 29 may now be rewritten in a more useful form which explicitly yields $\overline{N}_D/N$ in terms of K_D, [D], and the (for practical purposes known) function $F(z)$. On very general grounds we

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[10] Gaunt defines $\Gamma(z) = (2/q) \ln \Xi(z)$, but we prefer to deal with $F(z)$.

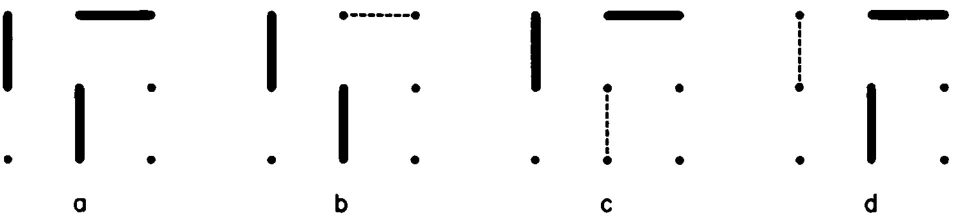

FIGURE 3 The $(N_D + 1)$-dimer configuration (*a*) can be generated in $N_D + 1$ ways by adding a single dimer (dashed bar) to the N_D-dimer configurations *b*, *c*, and *d*. Here $N_D = 2$ and $N_D + 1 = 3$.

know that if the lattice size N is large, the summand $g_N(N_D)\, z^{N_D}$ in Eq. 24 peaks sharply in the vicinity of a certain value $\overline{N}_D$. This value $\overline{N}_D$ can be determined in two ways: on one hand, using the peaking property, we can write

$$\overline{N}_D = \frac{\Sigma\, N_D g_N(N_D) z^{N_D}}{\Sigma\, g_N(N_D) z^{N_D}} = \frac{z\, \Xi_N'(z)}{\Xi_N(z)} = z \frac{d}{dz} \ln \Xi_N(z) = NzF'(z), \tag{30}$$

but we can also assert that the largest term in Eq. 24 occurs when the ratio of successive terms is unity (otherwise the terms are increasing or decreasing), i.e.,

$$\frac{z g_N(\overline{N}_D + 1)}{g_N(\overline{N}_D)} = 1. \tag{31}$$

Eq. 31 is of exactly the same form as Eq. 29, with $z = K_D$ [D]. But since Eqs. 31 and 30 are equivalent, we can rewrite Eq. 29 in the more useful form

$$\frac{\overline{N}_D}{N} = z\, F'(z) \tag{32a}$$

where

$$z = K_D[\mathrm{D}]. \tag{32b}$$

Eq. 32 is the solution of our problem when no monomers are involved ($K_M = 0$ or $[\mathrm{M}] = 0$).

The solution can also be obtained directly from the principles of statistical mechanics. This is done in Appendix A, where the law of mass action for dimers (Eq. 20) is verified and K_D expressed directly in terms of statistical-mechanical quantities.

The Monomer-Dimer Isotherm

When both monomers and dimers are present, the problem can again be solved either directly from the law of mass action (Eqs. 19 and 20) or from the principles of statistical mechanics.

In either case, we must introduce the quantity $g_N(N_M, N_D)$ = the number of ways of placing N_M indistinguishable monomers and N_D indistinguishable dimers on a q-coordinated lattice of N sites. (For simplicity we again suppress the q subscript.) What makes the problem soluble (in terms of the solution of the dimer problem) is the simple relation

$$g_N(N_M, N_D) = g_N(N_D) \binom{N - 2N_D}{N_M}, \tag{33}$$

where the first factor on the right is the number of ways of placing the dimers on the lattice in the absence of monomers and the second factor is the binomial coefficient $(N - 2N_D)!/N_M!(N - 2N_D - N_M)!$ which is the number of ways of placing the monomers in the remaining vacancies. Furthermore, the reasoning that led to Eq. 28 is easily extended to the case where both monomers and dimers are present, yielding

$$\overline{N}_{X_2} = (\overline{N}_D + 1)\frac{g_N(\overline{N}_M, \overline{N}_D + 1)}{g_N(\overline{N}_M, \overline{N}_D)} \tag{34}$$

and

$$\overline{N}_X = (\overline{N}_M + 1)\frac{g_N(\overline{N}_M + 1, \overline{N}_D)}{g_N(\overline{N}_M, \overline{N}_D)}. \tag{35}$$

Eqs. 35 and 33 yield

$$\overline{N}_X = N - 2\overline{N}_D - \overline{N}_M \tag{36}$$

which is identical to Eq. 22. Combining Eqs. 33 and 34 we obtain the less trivial equation

$$\overline{N}_{X_2} = \overline{N}_D \frac{g_N(\overline{N}_D + 1)}{g_N(\overline{N}_D)}\left(\frac{N - 2\overline{N}_D - \overline{N}_M}{N - 2\overline{N}_D}\right)^2, \tag{37}$$

where we have used $N_D \gg 1$ and $N_M \gg 1$ to simplify several factors. Combining Eq. 37 with the law of mass action for dimers (Eq. 20), we find

$$\frac{g_N(\overline{N}_D + 1)}{g_N(\overline{N}_D)}\left(\frac{N - 2\overline{N}_D - \overline{N}_M}{N - 2\overline{N}_D}\right)^2 K_D[D] = 1. \tag{38}$$

Similarly, combining Eq. 36 with the law of mass action for monomers (Eq. 19), we get

$$\frac{\overline{N}_M}{N - 2\overline{N}_D - \overline{N}_M} = K_M[M], \tag{39}$$

and thus

$$\frac{\overline{N}_M}{N} = \frac{K_M[M]}{1 + K_M[M]}\left(1 - \frac{2\overline{N}_D}{N}\right), \tag{40}$$

and

$$\frac{N - 2\overline{N}_D}{N - 2\overline{N}_D - \overline{N}_M} = 1 + K_M[M]. \tag{41}$$

From Eq. 40 it is seen that the monomers bind, via a Langmuir isotherm, to the reduced lattice comprised of sites unoccupied by dimers. From Eqs. 38 and 41 we find

$$\frac{g_N(\overline{N}_D + 1)}{g_N(\overline{N}_D)}\frac{K_D[D]}{(1 + K_M[M])^2} = 1. \tag{42}$$

We have already seen (Eqs. 30 and 31) that Eq. 42 is equivalent to

$$\frac{\overline{N}_D}{N} = z\,F'(z), \tag{43a}$$

where

$$z = \frac{K_D[D]}{(1 + K_M[M])^2}. \tag{43b}$$

Eqs. 43 and 40 solve the problem, giving $\overline{N}_D/N$ and $\overline{N}_M/N$ explicity in terms of [M], [D], K_M, and K_D.

The solution of the monomer-dimer problem by statistical mechanics is given in Appendix B, where the laws of mass action for monomer and dimer adsorption to the lattice (Eqs. 19 and 20) are verified and expressions for K_M and K_D obtained in terms of partition functions.

NUMERICAL EVALUATION

We now want to use Eq. 43 to evaluate $\overline{N}_D/N$ as a function of z for all z; that is, we want to evaluate the expression $z\,F'(z)$. Gaunt (1969) has calculated the first 15 coefficients g_s of Eq. 26 for the square lattice, and the first 10 g_s for the hexagonal lattice. These coefficients alternate in sign and increase rapidly in magnitude. Thus, when $\overline{N}_D/N$ is expressed as a power series in z, the series converges only when z is quite small; Gaunt estimates the radius of convergence as $|z| = 0.08895$ (square lattice) and $|z| = 0.05600$ (hexagonal lattice). These values of z correspond to values of $\overline{N}_D/N$ that are only about 20% of the saturation value $\overline{N}_D/N = 0.5$. However, Heilmann and Lieb (1972) have shown that there is no phase transition in the adsorbed dimer system in the entire physical region $0 \leq \overline{N}_D/N \leq 0.5$. Thus, the fact that the power series in z has a limited radius of convergence indicates only that we need a better method of calculation at high densities of adsorbed dimers (large z).

A much more useful series, which contains the same information as Eq. 43*a*, expresses z as a power series in $\overline{N}_D/N$. Gaunt gives 15 coefficients of such a series for the square lattice, and 10 coefficients for the hexagonal lattice.[11] This series contains only positive coefficients (see Gaunt, Table II) and converges over the entire physical range $0 \leq \overline{N}_D/N \leq 0.5$. When $\overline{N}_D/N \ll 1$, the first term of the series suffices and we have

$$z \approx \frac{2}{q}\frac{\overline{N}_D}{N} \qquad \left(\frac{\overline{N}_D}{N} \ll 1\right), \tag{44a}$$

whence

$$\frac{\overline{N}_D}{N} \approx \frac{q}{2} z \qquad (z \ll 1). \tag{44b}$$

This corresponds to the dilute-dimer regime and Henry's law of adsorption (McLaughlin et al., 1978).

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[11] Gaunt's series are in terms of the quantity $\rho = (2/q)\,\overline{N}_D/N$.

By analyzing the asymptotic behavior of the coefficients, Gaunt has determined that at high dimer coverage

$$z \approx \frac{A}{\left(1 - 2\dfrac{\overline{N}_D}{N}\right)^{\gamma}} \qquad \left(\frac{\overline{N}_D}{N} \to 0.5\right), \tag{45a}$$

whence

$$\frac{\overline{N}_D}{N} \approx \frac{1}{2}\left[1 - \left(\frac{A}{z}\right)^{1/\gamma}\right] \qquad (z \to \infty), \tag{45b}$$

where

$$\gamma = 7/4, \quad A = 0.3030 \qquad \text{(square lattice)}$$

$$\gamma = 2, \quad A = 0.149 \qquad \text{(hexagonal lattice)}.$$

TABLE I
EXACT SERIES SOLUTION OF EQ. 43A AND COMPARISON WITH THE BETHE APPROXIMATION (EQ. 46A)

Square ($q = 4$) lattice			Hexagonal ($q = 6$) lattice		
$\overline{N}_D/N$	z(series)	z(Bethe)	$\overline{N}_D/N$	z(series)	z(Bethe)
0	0	0	0	0	0
0.01	0.005180	0.005180	0.01	0.003459	0.003459
0.02	0.010742	0.010742	0.03	0.011206	0.011204
0.04	0.023156	0.023157	0.06	0.025331	0.025310
0.06	0.037575	0.037577	0.09	0.043360	0.043278
0.08	0.054413	0.054422	0.12	0.066712	0.066482
0.10	0.074195	0.074219	0.15	0.097473	0.096939
0.12	0.097587	0.097645	0.18	0.138811	0.137695
0.14	0.125452	0.125579	0.21	0.195705	0.193520
0.16	0.158915	0.159170	0.24	0.276329	0.272189
0.18	0.199466	0.199951	0.27	0.394826	0.387051
0.20	0.249111	0.250000	0.30	0.577329	0.562500
0.22	0.310597	0.312181	0.33	0.876264	0.846886
0.24	0.387766	0.390533	0.36	1.408720	1.346939
0.26	0.486115	0.490885	0.39	2.477977	2.336777
0.28	0.613728	0.621901	0.42	5.067826	4.703125
0.30	0.782881	0.796875	0.45	13.932546	12.750000
0.32	1.012941	1.037037	0.48	91.850988	84.000000
0.34	1.335914	1.377930	0.50	∞	∞
0.36	1.807776	1.882653			
0.38	2.533661	2.671875			
0.40	3.730072	4.000000			
0.42	5.902289	6.480469			
0.44	10.466788	11.916667			
0.46	22.802101	27.671875			
0.48	81.673705	114.000000			
0.50	∞	∞			

Thus, when z is expressed as a power series in $\overline{N}_D/N$, the high-order coefficients ($n > 15$ for the square lattice and $n > 10$ for the hexagonal lattice) may be replaced by the binomial coefficients obtained by expanding Eq. 45*a*. We have summed the resulting series, and the results are presented in Table I and Fig. 4, which are the solutions of Eq. 43*a* for the square and hexagonal lattices.

THE BETHE APPROXIMATION

For most practical purposes it is sufficient to replace the exact numerical solution of Eq. 43*a* with the Bethe approximation, which corresponds to a simple approximate scheme for counting dimer configurations on the lattice (Nagle, 1966). In the Bethe approximation the expression for z in terms of $\overline{N}_D/N$ is (Gaunt, 1969)

$$z = \frac{2}{q}\frac{\overline{N}_D}{N}\left(1 - \frac{2}{q}\frac{\overline{N}_D}{N}\right)\left(1 - 2\frac{\overline{N}_D}{N}\right)^{-2}, \tag{46a}$$

or equivalently,

$$\frac{\overline{N}_D}{N} = \frac{q}{4}\left(\frac{1 + 2qz - \sqrt{1 + 4(q-1)z}}{1 + q^2 z}\right). \tag{46b}$$

The accuracy of this approximation can be assessed from Table I and Fig. 4. It is clear that Eq. 46 suffices except (perhaps) very near saturation, where Eq. 45 can be used.

COMPARISON OF BETHE AND LANGMUIR DIMER ISOTHERMS

It is instructive to examine the dimer isotherm in the Bethe approximation (Eqs. 46*b* and 43*b*) further. In the absence of bound monomers ($K_M[M] = 0$), we plot in Fig. 5 the lattice coverage of dimers ($\Theta_D = 2\overline{N}_D/N$) as a function of $qz = qK_D[D]$ for $q = 4$ and 6 (which span the coordination numbers of physical interest) as well as for the case $q = 1$. We have chosen the abscissa to produce coincidence of all curves in the low-coverage limit according to Eqs.

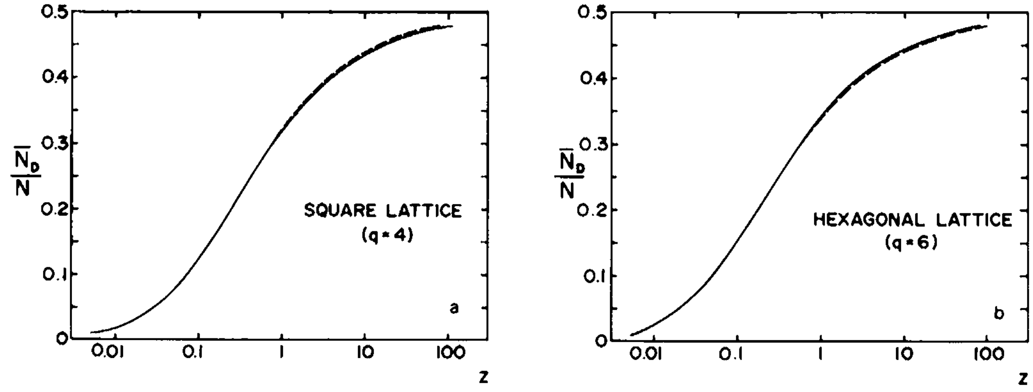

FIGURE 4 Plot of Table I; solution of Eq. 43*a* for (*a*) square lattice and (*b*) hexagonal lattice. ----, exact series solution. ——, Bethe approximation.

44*b* and 43*b*. It is apparent in Fig. 5 that the $q = 4$ and $q = 6$ curves are nearly identical. The choice of q in this range, therefore, has little effect on the shape of the isotherm.

The case $q = 1$ is noteworthy. When no bound monomers are present, Eqs. 46*b* and 43*b* give

$$\Theta_D = \frac{2\overline{N}_D}{N} = \frac{z}{1+z} = \frac{K_D[D]}{1 + K_D[D]} \qquad (q = 1, K_M[M] = 0). \tag{47}$$

Thus, by setting $q = 1$ we recover the Langmuir isotherm for dimers (cf. Eq. 18). It is apparent, therefore, that the approximation used by previous authors to treat the 1:2 binding of divalent cations to phospholipid membranes, in the absence of monovalent-cation binding, is tantamount to treating the membrane as a $q = 1$ lattice. On such a lattice each phospholipid has only one nearest neighbor; i.e., all phospholipids are "paired off" into isolated pair binding sites, even before any dimer adsorption occurs. We consider it unlikely that such a highly constrained model is applicable to phospholipid membranes.

The $q = 4$ and 6 isotherms in Fig. 5 clearly differ from the $q = 1$ (Langmuir) isotherm in the intermediate and high-coverage regimes. However, even in the low-coverage limit where the isotherms become coincident, an important distinction remains. When values of K_D are determined experimentally in this regime, the parameter actually obtained is qK_D. Therefore, the assumption $q = 1$ yields values of K_D that are a factor of 4–6 too high. This is because the assumption $q = 1$ underestimates the number of dimer binding sites on the empty lattice, which is $qN/2$, by a factor of 4–6.

When bound monomers are present, the dimer coverage for the $q = 1$ lattice is given by

$$\Theta_D = \frac{z}{1+z} = \frac{K_D[D]}{(1 + K_M[M])^2 + K_D[D]} \qquad (q = 1), \tag{48}$$

where Eqs. 43*b* and 46*b* have been used. Eq. 48 differs from the expression used by Nir et al. (1978, cf. their Eq. 16) to treat dimer binding in the presence of monomers, which is

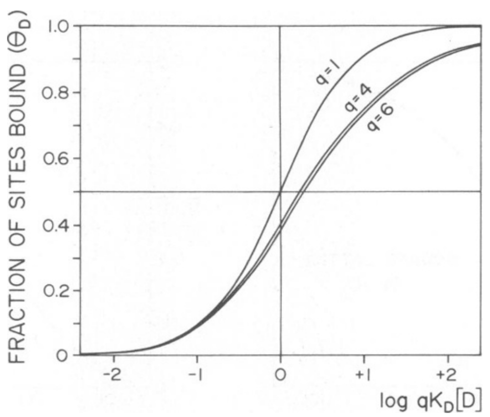

FIGURE 5 Dimer adsorption isotherms in the Bethe approximation with no adsorbed monomers. Dimer lattice coverage $\Theta_D = 2\overline{N}_D/N = 2(DX_2)/(\ell)$ is plotted vs. log $qK_D[D]$ where $K_M[M] = 0$. Isotherms are shown for the cases $q = 1$, 4, and 6. The $q = 1$ isotherm is identical to the Langmuir isotherm for dimers that has been used by previous authors.

equivalent to

$$\Theta_D = \frac{K_D[D]}{1 + K_M[M] + K_D[D]}. \tag{49}$$

Since the Bethe approximation (Eq. 48) is exact when $q = 1$ (Nagle, 1966), we see that in the presence of bound monomers Eq. 49 is not valid, even if one assumes a $q = 1$ lattice.

ADSORBED CHARGE DENSITY

It is now possible to write explicitly the adsorption-isotherm function f_A (cf. Eq. 5) to be used in Eq. 8. For generality, we include the possibility of both 1:1 and 1:2 binding of C^{++} to the membrane, but C^+ binding is restricted to a 1:1 stoichiometry. The monvalent cations are monomers. The 1:1-bound divalent cations may be treated simply as a second monomeric species according to Eq. 13. We use the notation K_{D1} to denote the divalent-cation 1:1 association constant (equivalent to $K_{M'}$ in Eq. 13). The 1:2-bound divalent cations are dimers whose association constant we now call K_{D2} (cf. Eq. 15) and whose coverage of the lattice $(2\overline{N}_D/N)$ we call Θ_{D2}. Because

$$\sigma_{\text{adsorbed}} = e(C_0{}^+X) + 2e(C_0{}^{++}X) + 2e(C_0{}^{++}X_2), \tag{50}$$

Eq. 5 becomes

$$f_A(C_0{}^+, C_0{}^{++}, K_M, K_{D1}, K_{D2}) = (\ell)' \frac{K_M C_0{}^+ + 2K_{D1}\, C_0{}^{++}}{1 + K_M\, C_0{}^+ + K_{D1}\, C_0{}^{++}} \{1 - \Theta_{D2}\} + (\ell)'\, \Theta_{D2}, \tag{51a}$$

where

$$\Theta_{D2} = 2z\, F'(z) \qquad \text{(exact solution)}, \tag{51b}$$

or

$$\Theta_{D2} = \frac{q}{2}\left\{\frac{1 + 2qz - \sqrt{1 + 4(q-1)z}}{1 + q^2 z}\right\} \qquad \text{(Bethe approximation)}, \tag{51c}$$

and

$$z = \frac{K_{D2}\, C_0{}^{++}}{(1 + K_M\, C_0{}^+ + K_{D1}\, C_0^{++})^2}. \tag{51d}$$

Here we have used Eqs. 21, 40, 43*a*, 46*b*, and 43*b* generalized to the case of two distinct monomeric species. The first term on the right of Eq. 51*a* is the sum of surface-charge densities due to 1:1-bound monovalent cations and 1:1-bound divalent cations, while the second term is that due to 1:2-bound divalent cations. Eq. 51 *a*–*d* (in conjunction with Eqs. 6 and 8) give the solution to the problem of simultaneous 1:1 binding of monovalent cations, and 1:1 and 1:2 binding of divalent cations, to single-component phospholipid membranes.

We also give the solution for the case in which monovalent- and divalent-cation binding are completely noncompetitive. This solution is relevant if, for example, monovalent cations intercalate in the head-group region of the membrane rather than binding chemically to specific phospholipid sites. In this case, assuming that n monovalent cations can associate with the membrane per phospholipid, where $n \geq 1$,

$$f_A(C_0^+, C_0^{++}, K_M, K_{D1}, K_{D2}) = n(\ell)' \frac{K_M C_0^+}{1 + K_M C_0^+} + (\ell)' \frac{2K_{D1} C_0^{++}}{1 + K_{D1} C_0^{++}} \{1 - \Theta_{D2}\} + (\ell)'\Theta_{D2}, \quad (52a)$$

where Θ_{D2} is evaluated as in Eq. 51*b* or 51*c*, and

$$z = \frac{K_{D2} C_0^{++}}{(1 + K_{D1} C_0^{++})^2}. \quad (52b)$$

When K_{D2} and $\Theta_{D2} = 0$, Eqs. 51 and 52 reduce to the competitive and noncompetitive 1:1 Stern isotherms, respectively, for the case of monovalent and divalent cations binding solely with 1:1 stoichiometries.[12]

The analogue of Eq. 51 from the treatment by Nir et al. (1978) is

$$f_A(C_0^+, C_0^{++}, K_M, K_{D2}) = (\ell)' \frac{K_M C_0^+ + K_{D2} C_0^{++}}{1 + K_M C_0^+ + K_{D2} C_0^{++}}. \quad (53)$$

THEORETICAL CURVES

In Fig. 6 we show some plots of Ψ_0 vs. $\log C^{++}$ obtained from Eq. 8 for a PS membrane in 0.1 M monovalent salt. We illustrate the case of no monovalent-cation binding ($K_M = 0$) as well as the case $K_M = 0.8$ liters/mol (M^{-1}), a value that has been reported for the binding of Na^+ to PS membranes (Nir et al., 1978).[13] In Fig. 6 we examine only 1:2 divalent-cation binding to the membrane and hence set $K_{D1} = 0$. The isotherms shown are those treated here in the Bethe approximation (Eqs. 51 and 52) and those of the approximate treatment previously used by Nir et al. (Eq. 53). For illustrative purposes we choose $qK_{D2} = 100\ M^{-1}$, a 1:2 binding constant which, as we shall show, is relevant for Ca^{++} binding to PS membranes; we also set $q = 5$, a value intermediate between the extremes 4 and 6. In Fig. 6, curves A and D are shown for reference purposes: A is the Grahame curve, representing pure screening behavior with no ion binding to the membrane ($K_M = K_{D2} = 0$); D is the monovalent-cation Stern curve, representing monovalent- but no divalent-cation binding to the membrane ($K_M = 0.8\ M^{-1}$, $K_{D2} = 0$). Curves B and C are calculated, respectively, from the present treatment and from that of Nir et al. with $K_M = 0$ and $qK_{D2} = K_{D2}^N = 100\ M^{-1}$.[14] The coincidence of curves B and C in the dilute C^{++} limit is ensured by this choice of the relative K_Ds (cf. Fig. 5 and Eq. 47). Curves E and F are the generalizations of B and C where monovalent-cation binding is included. Curve G is obtained from the noncompetitive version of the present treatment with $K_M = 0.8\ M^{-1}$, $qK_{D2}^* = 100\ M^{-1}$, and $n = 1$ (Eq. 52).[14] Curves E, F, and G reduce to curve D,

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[12] Actually, we have permitted n:1 adsorption of monovalent cations in the noncompetitive case (Eq. 52).

[13] Other recently reported values for K_M (Na·PS) are 0.6 M^{-1} (Eisenberg et al., 1979), 0.4 – 1.2 M^{-1} (Kurland et al., 1979), and 0.6 M^{-1} (Ohki and Kurland, 1981).

[14] We employ the notation K_{D2}^N to denote the value of K_{D2} used in the Nir et al. isotherm. We also employ the notation K_{D2}^* and K_{D1}^* to denote, respectively, the values of K_{D2} and K_{D1} used in the noncompetitive isotherms.

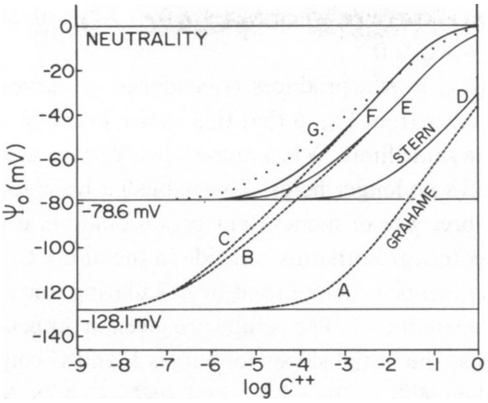

FIGURE 6 Theoretical plots of membrane surface potential vs. log C^{++} for a PS membrane in 0.1 M monovalent salt. Parameters are $T = 22°C$, $A' = 1/272.7\ e\ M^{-1/2}\ Å^{-2}$, $\langle \ell \rangle = 1/70\ Å^{-2}$, divalent salt is 2:1 (e.g. $CaCl_2$), $K_{D1} = 0$. (A, B, C): No monovalent-cation binding ($K_M = 0$); initial surface potential = −128.1 mV (from Gouy equation). Curve A: $K_{D2} = 0$, Grahame equation. Curve B: $qK_{D2} = 100\ M^{-1}$, present treatment, Bethe approximation ($q = 5$). Curve C: $K_{D2}^N = 100\ M^{-1}$, Nir approximation (same as Bethe, $q = 1$). (D, E, F, G): Monovalent-cation binding ($K_M = 0.8\ M^{-1}$); initial surface potential = −78.6 mV (from Stern equation). Curve D: $K_{D2} = 0$, Stern equation for 1:1 monovalent-cation binding. Curve E: $qK_{D2} = 100\ M^{-1}$, present treatment, competitive, Bethe approximation ($q = 5$). Curve F: $K_{D2}^N = 100\ M^{-1}$, Nir approximation. Curve G: $qK_{D2}^* = 100\ M^{-1}$, present treatment, noncompetitive ($n = 1$), Bethe approximation ($q = 5$).

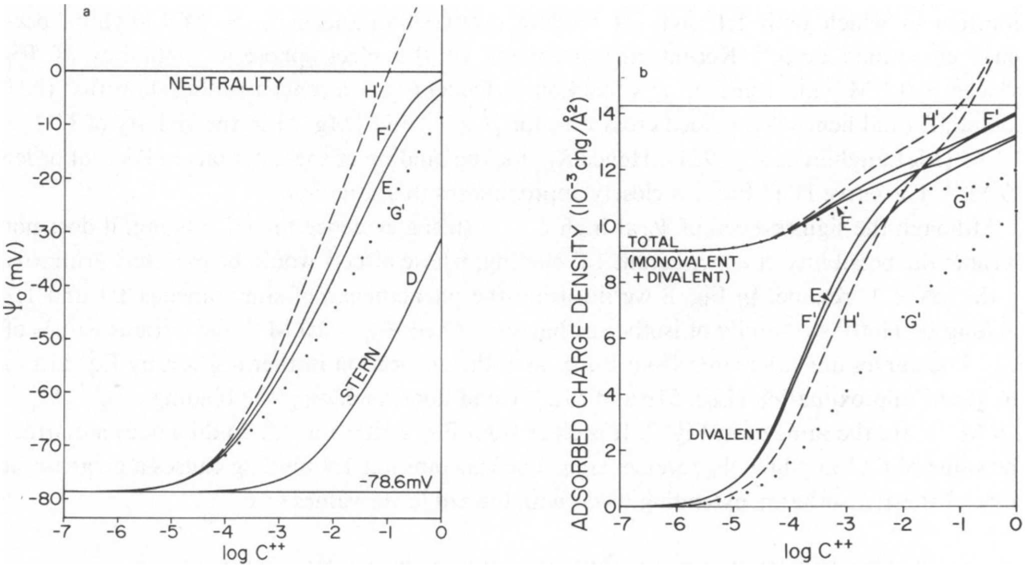

FIGURE 7 Theoretical plots of (*a*) membrane surface potential and (*b*) adsorbed charge density vs. log C^{++} for a PS membrane in 0.1 M monovalent salt. Conditions are the same as in Fig. 6, with $K_M = 0.8\ M^{-1}$. Divalent-cation binding constants are chosen to produce coincidence of Ψ_0 curves in the dilute C^{++} limit as described in the text. Curves D and E are the same as in Fig. 6. Primes denote curves whose K_D values have been changed from those in Fig. 6. In Fig. 7 *b* the horizontal line at 14.29×10^{-3} charge/Å^2 denotes an adsorbed charge density of 1 charge/70 Å^2, i.e., membrane neutrality. Curve D: $K_{D2} = 0$, Stern equation for 1:1 monovalent-cation binding. Curve E: $qK_{D2} = 100\ M^{-1}$, present treatment, competitive, Bethe approximation ($q = 5$). Curve F′: $K_{D2}^N = 36.3\ M^{-1}$, treatment by Nir et al. Curve G′: $qK_{D2}^* = 4.78\ M^{-1}$, present treatment, noncompetitive ($n = 1$), Bethe approximation ($q = 5$). Curve H′: $K_{D1} = 9.66\ M^{-1}$, 1:1 Stern treatment, competitive.

and curves B and C reduce to curve A, when $K_{D2} = K_{D2}^{N} = K_{D2}^{*} = 0$; all curves reduce to curve A when $K_{D2} = K_{D2}^{N} = K_{D2}^{*} = K_{M} = 0$.

Although our choice $qK_{D2} = K_{D2}^{N}$ produces coincidence of curves B and C in the dilute divalent-cation limit, it is clear from Fig. 6 that this choice does not also produce coincidence of curves E, F, and G in the same limit. When monovalent cations are bound to the lattice, the above correspondence of K_Ds no longer holds. To establish a basis for comparison among the various treatments in the presence of monovalent-cation binding, it is necessary to scale the various K_Ds so that the theoretical isotherms coincide in the dilute C^{++} limit.

The requisite K_D scaling can be accomplished by calculating, for each treatment, the slope of Ψ_0 vs. C^{++} in the limit of small C^{++}. The results are given in Appendix C. With reference to Fig. 6, Eq. C4 *a–c* show that the initial slopes for curves F and G coincide with that for curve E ($qK_{D2} = 100\ M^{-1}$) when $K_{D2}^{N} = 36.3\ M^{-1}$ and $qK_{D2}^{*} = 4.78\ M^{-1}$, respectively. These isotherms are plotted in Fig. 7 *a* (the primes denote rescaled curves), where we also include the case of 1:1 divalent-cation binding (curve H′) for which Eq. C4*a* yields $K_{D1} = 9.66\ M^{-1}$. In Fig. 7 *b* we plot adsorbed charge density as a function of log C^{++} for the same cases, indicating both the divalent and the total (monovalent + divalent) adsorbed charge.

COMPARISON WITH EXPERIMENTAL DATA: HIGH C^{++} REGIME

A salient feature of curve H′ in Fig. 7 *a* is the fact that, at sufficiently high C^{++}, Ψ_0 becomes positive. This feature is characteristic of either noncompetitive, or 1:1 competitive, binding of divalent cations to the PS membrane. For the competitive case the value of C^{++} at which Ψ_0 crosses zero can be shown to equal $1/K_{D1}$ under quite general conditions, including the situation in which both 1:1 and 1:2 binding occur simultaneously (S. McLaughlin, personal communication).[15] Recent measurements of the electrophoretic mobilities of PS vesicles in 0.1 M NaCl solution, as a function of divalent-cation concentration, show that the ζ potentials (and hence Ψ_0) indeed cross zero for [Ca^{++}] and [Mg^{++}] in the vicinity of $C^{++} = 0.1$ M (McLaughlin et al., 1981). Hence K_{D1} for the binding of these cations to PS is of order $10\ M^{-1}$. The curve H′ in Fig. 7 *a* closely approximates this situation.

Although the sign reversal of Ψ_0 at high C^{++} is strong evidence for 1:1 binding, it does not exclude the possibility of concomitant 1:2 binding, whose effects would be manifest primarily in the low C^{++} regime. In Fig. 8 we illustrate the phenomenon of simultaneous 1:1 and 1:2 binding by plotting a family of isotherms having a fixed $K_{D1} = 10\ M^{-1}$ and various values of K_{D2}. The curves are calculated from Eq. 8 with the adsorption isotherm given by Eq. 51*a* in the Bethe approximation (Eqs. 51*c* and 51*d*). Conditions, including the binding of C^{+} ($K_M = 0.8\ M^{-1}$), are the same as in Fig. 7. It is clear from Fig. 8 that the 1:2 binding does not affect the value of C^{++} at which Ψ_0 reverses sign. The concomitant 1:2 binding causes a decrease in slope of the 1:1 isotherm, extending its domain toward lower values of C^{++}.

COMPARISON WITH EXPERIMENTAL DATA: LOW C^{++} REGIME

In the dilute to moderate range of C^{++} concentrations, it is evident in Fig. 7 *a* that experiments which measure Ψ_0 (or shifts of Ψ_0) vs. log C^{++} are not likely to distinguish among

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[15] This fact is also seen from Eqs. 51*a*, 8, and 4 when $f_G \to 0$.

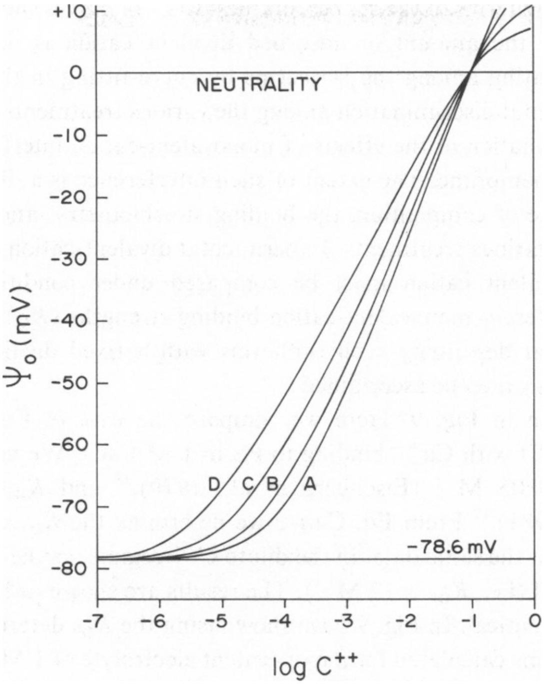
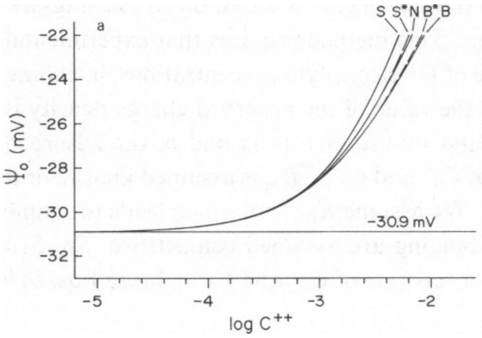


FIGURE 8 Theoretical plots of membrane surface potential vs. log C^{++} for a PS membrane in 0.1 M monovalent salt with 1:1 monovalent-cation binding ($K_M = 0.8\ M^{-1}$), 1:1 divalent-cation binding ($K_{D1} = 10\ M^{-1}$), and simultaneous 1:2 divalent-cation binding. Conditions are the same as in Fig. 6. All curves are calculated from the present treatment in the Bethe approximation ($q = 5$), competitive case. Curve A ($K_{D2} = 0$), curve B ($K_{D2} = 20\ M^{-1}$), curve C ($K_{D2} = 100\ M^{-1}$), and curve D ($K_{D2} = 400\ M^{-1}$).



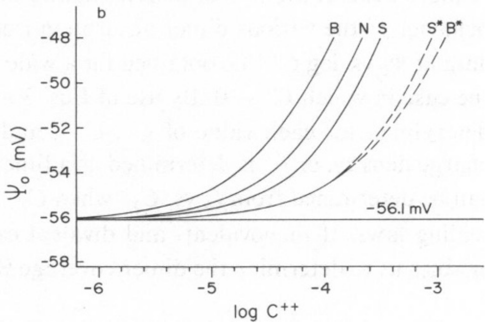


FIGURE 9(a) Theoretical plots of Ψ_0 vs. log C^{++} for a PS membrane in 1M monovalent salt, for which $K_M = 0.6\ M^{-1}$ (i.e., NaCl). Parameters are $T = 22°C$, $A' = 1/272.7\ e\ M^{-1/2}\ Å^{-2}$, $(\ell) = 1/70\ Å^{-2}$, divalent salt is 2:1 (i.e., $CaCl_2$). Curve S: $K_{D1} = 12.0\ M^{-1}$, 1:1 competitive Stern treatment (same as present treatment with $K_{D2} = 0$). Curve S*: $K_{D1}^{*} = 2.64\ M^{-1}$, 1:1 noncompetitive Stern treatment ($n = 1$); same as present treatment with $K_{D2}^{*} = 0$. Curve N: $K_{D2}^{N} = 48.2\ M^{-1}$, treatment by Nir et al. Curve B*: $K_{D2}^{*} = 1.06\ M^{-1}$, present treatment, noncompetitive ($n = 1$), Bethe approximation ($q = 5$), $K_{D1}^{*} = 0$. Curve B: $K_{D2} = 29.1\ M^{-1}$, present treatment, competitive, Bethe approximation ($q = 5$), $K_{D1} = 0$. Values of the association constants for curves S*, N, B*, and B were determined by use of Eq. C4a–c to produce coincidence with curve S in the dilute C^{++} limit.

FIGURE 9(b) Same as Fig. 9 a except that $K_M = 0.05\ M^{-1}$ (i.e., CsCl). All divalent-cation:membrane association constants are the same as those in Fig. 9 a.

the various treatments in terms of the shapes, or "best fits," of curves such as E, F′, G′, and H′. Experiments in which the amount of adsorbed divalent cation is detected are similarly incapable of differentiating among the isotherms by curve-fitting in the dilute to moderate C^{++} regime. Experimental discrimination among the various treatments in this regime can be accomplished by examination of the effects of monovalent-cation interference with divalent-cation binding to the membrane. The extent of such interference is a direct manifestation of the presence or absence of competition, the binding stoichiometry, and the lattice statistics which distinguish the various treatments. Experimental divalent-cation isotherms (Ψ_0 vs. log C^{++}) for a given divalent cation must be compared under conditions of different C^+ concentrations and different monovalent-cation binding strengths (K_M). The success of each theoretical treatment at describing such isotherms with a fixed divalent-cation:membrane association constant may then be ascertained.

An example is given in Fig. 9. Here we compare the case of Ca^{++} binding to a PS membrane in 1 M NaCl with Ca^{++} binding to PS in 1 M CsCl. We use $K_M(\text{Na}\cdot\text{PS}) = 0.6$ M^{-1}, $K_M(\text{Cs}\cdot\text{PS}) = 0.05$ M^{-1} (Eisenberg et al., 1979),[16] and $K_{D1}(\text{Ca}\cdot\text{PS}) = 12$ M^{-1} (McLaughlin et al., 1981).[17] From Eq. C4*a–c* we determine the K_D values required by the various treatments to fit the same data, in the dilute C^{++} regime, as the competitive 1:1 Stern treatment in 1 M NaCl (i.e., $K_{D1} = 12$ M^{-1}). The results are shown in Fig. 9 *a*, where indeed all curves are nearly identical. In Fig. 9 *b* we show, using the K_Ds determined in Fig. 9 *a*, the C^{++} adsorption isotherms calculated for a monovalent electrolyte of 1 M CsCl. The isotherms predicted by the various treatments differ enough to permit experimental discrimination. However, the difficulty of differentiating between the 1:1 and 1:2 noncompetitive isotherms should be noted.[18]

SCALING LAWS

A more general method of discriminating among the treatments is based on the scaling-law behavior of the various dimer adsorption isotherms. This method requires that experimental data of Ψ_0 vs. log C^{++} be obtained for a wide range of C^+ electrolyte concentrations, including the case in which $C^+ = 0$. By use of Eqs. 3 and 8, the value of the adsorbed charge density is determined for each value of C^+, C^{++}, and Ψ_0; and by use of Eq. 6*a* and *b*, the adsorbed charge density, or f_A, is determined as a function of $C_0{}^+$ and $C_0{}^{++}$. K_M is assumed known, or it can be determined from f_A vs. $C_0{}^+$ when $C_0{}^{++} = 0$. We assume $K_{D1} = 0$, which leads to simple scaling laws. If monovalent- and divalent-cation binding are assumed competitive, Eq. 51*a* enables us to determine the dimer coverage Θ_{D2} as a function of $C_0{}^+$ and $C_0{}^{++}$. From Eqs. 51*b*

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[16] We choose this example because the differences illustrated here are enhanced in highly concentrated C^+ solutions having widely different values of K_M.

[17] The value $K_{D1} = 12$ M^{-1} was determined by McLaughlin et al. (1981), for Ca^{++} binding to PS in 0.1 M NaCl, by use of the 1:1 competitive Stern treatment. We use this value here for illustrative purposes only. To our knowledge, data on Ca^{++} binding to PS membranes in various 1-M monovalent electrolytes are not yet available.

[18] It has been pointed out (S. McLaughlin et al., 1981 and personal communication) that the question of 1:1 vs. 1:2 binding can be tested by incorporation of relatively inert lipids, such as cholesterol or glycerolmonooleate, into the membrane, thus decreasing the PS density. An increase in the mean PS-PS distance should alter K_{D2} much more drastically than K_{D1}.

and 51*d* it follows that

$$\Theta_{D2}(C_0{}^{+}, C_0{}^{++}) = \Theta_{D2}\left(0, \frac{C_0{}^{++}}{(1 + K_M C_0{}^{+})^2}\right). \tag{54a}$$

The corresponding scaling law for the treatment by Nir et al. (cf. Eq. 53) is

$$\Theta_{D2}^{N}(C_0{}^{+}, C_0{}^{++}) = \Theta_{D2}^{N}\left(0, \frac{C_0{}^{++}}{1 + K_M C_0{}^{+}}\right). \tag{54b}$$

If the monovalent- and divalent-cation binding are assumed to be noncompetitive, then the dimer coverage is determined from Eq. 52*a*, and Eqs. 51*b* and 52*b* yield the scaling law

$$\Theta_{D2}^{*}(C_0{}^{+}, C_0{}^{++}) = \Theta_{D2}^{*}(0, C_0^{++}). \tag{54c}$$

Scaling laws also exist when both $K_{D1} \neq 0$ and $K_{D2} \neq 0$. However, experimental testing of these laws requires prior determination of K_{D1} in addition to K_M.

In conclusion, we have presented a theory to treat the simultaneous adsorption of monovalent cations and divalent cations by single-component charged membranes. This treatment may be used to investigate whether monovalent- and divalent-cation binding are competitive or noncompetitive, and whether the divalent cations obey binding stoichiometries of 1:1, 1:2, or both concurrently.

APPENDIX A

Statistical-Mechanical Derivation of the Dimer Isotherm (Eq. 32)

The statistical mechanics of the adsorbed dimers can be approached in either of two ways. We can regard the adsorbed dimers as an open system, with the dimers in solution acting as a particle reservoir, in which case the appropriate ensemble for the description of the adsorbed dimers is the "grand canonical ensemble," or we can treat the entire system (lattice + solution) as a closed system, described by the "canonical ensemble." We choose to do the latter; furthermore, we assume that the dimers in solution do not interact with each other (only in this case do we expect the law of mass action to hold), and that the total number of dimers (which we call $\mathcal{N}_D$) is large compared with the number of sites N on the lattice. We also treat the dimers as distinguishable, although it will be seen that this assumption does not affect the answer. The probability that there are N_D dimers on the lattice and $\mathcal{N}_D - N_D$ dimers in solution is proportional to $Z(N_D)$, where

$$Z(N_D) = \binom{\mathcal{N}_D}{N_D} g_N(N_D) N_D! c^{N_D} d^{\mathcal{N}_D - N_D}. \tag{A1}$$

The binomial coefficient

$$\binom{\mathcal{N}_D}{N_D} \equiv \frac{\mathcal{N}_D!}{N_D!(\mathcal{N}_D - N_D)!} \tag{A2}$$

counts the number of ways of choosing the N_D adsorbed dimers out of a total population of $\mathcal{N}_D$ dimers; $g_N(N_D)$ counts the number of ways of choosing the sites that are to be occupied by the dimers, and $N_D!$ counts the number of ways of assigning N_D dimers to N_D sites. Finally, *c* is the partition function for a single adsorbed dimer (including a factor of 2 for the two ways in which a dimer can be placed on an adsorption site) and d is the partition function for a single dimer in solution. If we had treated the dimers as identical particles, the binomial coefficient and $N_D!$ would be omitted, but a factor $1/(\mathcal{N}_D - N_D)!$ would now be necessary to compensate for the fact that $d^{\mathcal{N}_D - N_D}$ has overcounted the phase space for the solution; thus we would still obtain Eq. A1 except for the factor $\mathcal{N}_D!$ which does not depend on N_D and cancels out when the probabilities are normalized.[19]

The average number of adsorbed dimers is

$$\overline{N}_D = \frac{\Sigma\, N_D Z(N_D)}{\Sigma\, Z(N_D)}. \tag{A3}$$

Since $N_D \ll \mathcal{N}_D$, we have

$$\frac{\mathcal{N}_D!}{(\mathcal{N}_D - N_D)!} = \mathcal{N}_D(\mathcal{N}_D - 1)\cdot\cdot\cdot\cdot\cdot(\mathcal{N}_D - N_D + 1) \approx \mathcal{N}_D^{N_D} \tag{A4}$$

and thus Eq. A3 becomes

$$\overline{N}_D = \frac{\Sigma\, N_D g_N(N_D)(\mathcal{N}_D c/d)^{N_D}}{\Sigma\, g_N(N_D)(\mathcal{N}_D c/d)^{N_D}}. \tag{A5}$$

Eq. A5 is identical with Eq. 30 if we let

$$z = \frac{\mathcal{N}_D c}{d}. \tag{A6}$$

Comparing Eqs. 32*b* and A6 we see that the law of mass action and statistical mechanics yield identical solutions of the dimer problem if we make the identification

$$K_D[D] = \frac{\mathcal{N}_D c}{d}. \tag{A7}$$

The translational part of the partition function d includes a factor V (the volume of the solution); thus we can write

$$\frac{\mathcal{N}_D c}{d} = \left(\frac{\mathcal{N}_D}{V}\right)\left(\frac{Vc}{d}\right) = [D]\left(\frac{Vc}{d}\right) \tag{A8}$$

where [D] is in molecular units. Eq. A7 thus becomes

$$K_D = \frac{Vc}{d}. \tag{A9}$$

Eq. A9 makes it clear that K_D is independent of [D] but may depend on the temperature.

From Eq. A6 we can prove directly that the law of mass action (Eq. 20) describes the dimer

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[19] It also does not matter whether the two ends of a dimer are regarded as distinguishable or indistinguishable, since only the ratio c/d appears in the normalized probability distribution. If the two ends are indistinguishable, c is diminished by a factor of 2; however, d includes as a factor the rotational partition function for a dimer in solution, which is also diminished by a factor of 2 if the two ends are indistinguishable.

adsorption reaction (Eq. 14). From Eq. A5, using the peaking property of the summand, we obtain

$$\frac{g_N(\overline{N}_{\mathrm{D}} + 1)(\mathcal{N}_{\mathrm{D}}c/d)}{g_N(\overline{N}_{\mathrm{D}})} = 1. \tag{A10}$$

Combining Eq. A10 with Eq. 28 (which is based only on counting), and using Eq. A8, we obtain

$$\frac{\overline{N}_{\mathrm{D}}/N}{[\mathrm{D}]\overline{N}_{\mathrm{X}_2}/N} = \frac{Vc}{d}, \tag{A11}$$

which is the law of the mass action (Eq. 20) with $K_{\mathrm{D}} = Vc/d$.

APPENDIX B

Statistical-Mechanical Derivation of the Monomer-Dimer Isotherm (Eqs. 40 and 43)

We consider the closed system consisting of $\mathcal{N}_{\mathrm{M}}$ monomers and $\mathcal{N}_{\mathrm{D}}$ dimers (where $\mathcal{N}_{\mathrm{M}} \gg N$ and $\mathcal{N}_{\mathrm{D}} \gg N$). A monomer (or dimer) may be on the lattice or in the solution; there are no monomer-monomer, dimer-dimer, or monomer-dimer interactions in the solution. The probability that there are N_{M} monomers and N_{D} dimers on the lattice, and $\mathcal{N}_{\mathrm{M}} - N_{\mathrm{M}}$ monomers and $\mathcal{N}_{\mathrm{D}} - N_{\mathrm{D}}$ dimers in solution, is proportional to $\mathrm{Z}(N_{\mathrm{M}}, N_{\mathrm{D}})$ where

$$\mathrm{Z}(N_{\mathrm{M}}, N_{\mathrm{D}}) = \binom{\mathcal{N}_{\mathrm{M}}}{N_{\mathrm{M}}}\binom{\mathcal{N}_{\mathrm{D}}}{N_{\mathrm{D}}} g_N(N_{\mathrm{M}}, N_{\mathrm{D}}) N_{\mathrm{M}}! N_{\mathrm{D}}! a^{N_{\mathrm{M}}} b^{\mathcal{N}_{\mathrm{M}} - N_{\mathrm{M}}} c^{N_{\mathrm{D}}} d^{\mathcal{N}_{\mathrm{D}} - N_{\mathrm{D}}}. \tag{B1}$$

The explanation of the factors in Eq. B1 is identical with that of the corresponding factors in Eq. A1; $g_N(N_{\mathrm{M}}, N_{\mathrm{D}})$ is explained in Eq. 33, a is the partition function for a single monomer on the lattice, and b is the partition function for a single monomer in solution. The average number of monomers on the lattice is

$$\overline{N}_{\mathrm{M}} = \frac{\sum\limits_{N_{\mathrm{M}}} \sum\limits_{N_{\mathrm{D}}} N_{\mathrm{M}}\, \mathrm{Z}(N_{\mathrm{M}}, N_{\mathrm{D}})}{\sum\limits_{N_{\mathrm{M}}} \sum\limits_{N_{\mathrm{D}}} \mathrm{Z}(N_{\mathrm{M}}, N_{\mathrm{D}})}, \tag{B2}$$

and similarly

$$\overline{N}_{\mathrm{D}} = \frac{\sum\limits_{N_{\mathrm{M}}} \sum\limits_{N_{\mathrm{D}}} N_{\mathrm{D}}\, \mathrm{Z}(N_{\mathrm{M}}, N_{\mathrm{D}})}{\sum\limits_{N_{\mathrm{M}}} \sum\limits_{N_{\mathrm{D}}} \mathrm{Z}(N_{\mathrm{M}}, N_{\mathrm{D}})}. \tag{B3}$$

If we define

$$A(N_{\mathrm{M}}, N_{\mathrm{D}}) \equiv \frac{\mathcal{N}_{\mathrm{M}}}{(\mathcal{N}_{\mathrm{M}} - N_{\mathrm{M}})!} \frac{\mathcal{N}_{\mathrm{D}}}{(\mathcal{N}_{\mathrm{D}} - N_{\mathrm{D}})!} g_N(N_{\mathrm{D}}) \binom{N - 2N_{\mathrm{D}}}{N_{\mathrm{M}}} \tag{B4}$$

and

$$W\left(\frac{a}{b},\frac{c}{d}\right) \equiv \sum_{N_M}\sum_{N_D} A(N_M, N_D)\left(\frac{a}{b}\right)^{N_M}\left(\frac{c}{d}\right)^{N_D}, \tag{B5}$$

then Eqs. B2 and B3 become

$$\overline{N}_M = \left(\frac{a}{b}\right)\frac{\partial \ln W}{\partial(a/b)} \tag{B6}$$

and

$$\overline{N}_D = \left(\frac{c}{d}\right)\frac{\partial \ln W}{\partial(c/d)}. \tag{B7}$$

Since $\mathcal{N}_M \gg N$ and $\mathcal{N}_D \gg N$ we can write

$$\frac{\mathcal{N}_M!}{(\mathcal{N}_M - N_M)!} \approx \mathcal{N}_M^{N_M} \quad \text{and} \quad \frac{\mathcal{N}_D!}{(\mathcal{N}_D - N_D)!} \approx \mathcal{N}_D^{N_D}, \tag{B8}$$

and thus

$$\begin{aligned} W &= \sum_{N_D}\sum_{N_M} g_N(N_D)\binom{N-2N_D}{N_M}\left(\frac{\mathcal{N}_M a}{b}\right)^{N_M}\left(\frac{\mathcal{N}_D c}{d}\right)^{N_D} \\ &= \sum_{N_D} g_N(N_D)\left(1+\frac{\mathcal{N}_M a}{b}\right)^{N-2N_D}\left(\frac{\mathcal{N}_D c}{d}\right)^{N_D} \\ &= \left(1+\frac{\mathcal{N}_M a}{b}\right)^{N}\Xi_N(z), \end{aligned}$$

where

$$z = \frac{\mathcal{N}_D c/d}{(1+\mathcal{N}_M a/b)^2}. \tag{B9}$$

The binomial theorem was used to perform the sum on N_M. Using

$$\ln W = N\ln(1+\mathcal{N}_M a/b) + NF(z) \tag{B10}$$

and Eqs. B6 and B7, we find

$$\frac{\overline{N}_M}{N} = \frac{\mathcal{N}_M a/b}{1+\mathcal{N}_M a/b}(1-2zF'(z)) \tag{B11}$$

and

$$\frac{\overline{N}_D}{N} = zF'(z), \tag{B12}$$

where

$$z = \frac{\mathcal{N}_D c/d}{(1+\mathcal{N}_M a/b)^2}. \tag{B13}$$

Using Eq. B12 we can rewrite Eq. B11 as

$$\frac{\overline{N}_{\mathrm{M}}}{N} = \frac{\mathcal{N}_{\mathrm{M}} a/b}{1 + \mathcal{N}_{\mathrm{M}} a/b}\left(1 - \frac{2\overline{N}_{\mathrm{D}}}{N}\right). \tag{B14}$$

Eqs. B14 and B12 are identical with Eqs. 40 and 43, which were derived from the law of mass action, if we make the identifications

$$K_{\mathrm{M}} = \frac{Va}{b} \quad \text{and} \quad K_{\mathrm{D}} = \frac{Vc}{d}. \tag{B15}$$

Using the expressions given by Eqs. 35 and 34 for $\overline{N}_{\mathrm{X}}$ and $\overline{N}_{\mathrm{X}_2}$, it is readily shown that the law of mass action (cf. Eqs. 19 and 20) is true, with the above identifications of K_{M} and K_{D}.

APPENDIX C

Slopes of Ψ_0 vs. C^{++} in the Limit $C^{++} \longrightarrow 0$

We assume that

$$\lim_{C^{++}\to 0} \Psi_0 = \Psi_{00} + \alpha C^{++}, \tag{C1}$$

where Ψ_{00} is the value of Ψ_0 when $C^{++} = 0$ and α is the slope that we wish to evaluate in terms of the adsorption isotherms discussed in the text. The Grahame function $f_{\mathrm{G}}(C^+, C^{++}, \Psi_0)$ and the adsorption-isotherm function $f_{\mathrm{G}}(C^+, C^{++}, \Psi_0, K_{\mathrm{M}}, K_{\mathrm{D1}}, K_{\mathrm{D2}})$ are expanded to first order in C^{++} about the values $f_{\mathrm{G}}(C^+, 0, \Psi_{00})$ and $f_{\mathrm{A}}(C^+, 0, \Psi_{00}, K_{\mathrm{M}}, K_{\mathrm{D1}}, K_{\mathrm{D2}})$, respectively. From Eq. 8 it is obvious that

$$f_{\mathrm{G}}(C^+, C^{++}, \Psi_0) - f_{\mathrm{G}}(C^+, 0, \Psi_{00}) = f_{\mathrm{A}}(C^+, C^{++}, \Psi_0, K_{\mathrm{M}}, K_{\mathrm{D1}}, K_{\mathrm{D2}}) - f_{\mathrm{A}}(C^+, 0, \Psi_{00}, K_{\mathrm{M}}, K_{\mathrm{D1}}, K_{\mathrm{D2}}). \tag{C2}$$

The left and right sides of Eq. C2 are the linear terms in the first-order expansions of f_G and f_{A}, respectively, and they contain α as a parameter. The resultant expression can be solved for α, yielding

$$\alpha = \frac{kT}{e}\left\{(\ell)' \exp\frac{-2e\Psi_{00}}{kT}\, h(C^+, \Psi_{00}, K_{\mathrm{M}}, K_{\mathrm{D1}}, K_{\mathrm{D2}}) - \frac{\dfrac{\Psi_{00}}{|\Psi_{00}|}\dfrac{A'}{2}\left(\lambda \exp\dfrac{2e\Psi_{00}}{\lambda kT} + \exp\dfrac{-2e\Psi_{00}}{kT} - (1+\lambda)\right)}{\left\{C^+\left(\exp\dfrac{e\Psi_{00}}{kT} + \exp\dfrac{-e\Psi_{00}}{kT} - 2\right)\right\}^{1/2}}\right\}$$
$$\times \left\{\frac{\dfrac{\Psi_{00}}{|\Psi_{00}|}\dfrac{A'}{2}C^+\left(\exp\dfrac{e\Psi_{00}}{kT} - \exp\dfrac{-e\Psi_{00}}{kT}\right)}{\left\{C^+\left(\exp\dfrac{e\Psi_{00}}{kT} + \exp\dfrac{-e\Psi_{00}}{kT} - 2\right)\right\}^{1/2}} + \frac{(\ell)' K_{\mathrm{M}} C^+ \exp\dfrac{-e\Psi_{00}}{kT}}{\left(1 + K_{\mathrm{M}} C^+ \exp\dfrac{-e\Psi_{00}}{kT}\right)^2}\right\}^{-1}, \tag{C3}$$

where, for the present treatment,

$$h(C^+, \Psi_{00}, K_{\mathrm{M}}, K_{\mathrm{D1}}, K_{\mathrm{D2}}) = \frac{K_{\mathrm{D1}}(2 + K_{\mathrm{M}} C_{00}{}^+)}{(1 + K_{\mathrm{M}} C_{00}{}^+)^2} + \frac{qK_{\mathrm{D2}}}{(1 + K_{\mathrm{M}} C_{00}{}^+)^3} \tag{C4a}$$

with

$$C_{00}{}^{+} = C^{+} \exp \frac{-e\Psi_{00}}{kT}.$$

In Eq. C3, λ is a parameter that depends on the valence symmetry of the C^{++} electrolyte. For a 2:1 electrolyte (e.g., $MgCl_2$), $\lambda = 2$; for a 2:2 electrolyte (e.g., $MgSO_4$), $\lambda = 1$ (cf. Eqs. 3*a* and 3*b*). For the treatment by Nir et al. (1978) the expression for α is the same as Eq. C3, but instead of Eq. C4*a* one has

$$h(C^{+}, \Psi_{00}, K_M, K_{D2}^{N}) = \frac{K_{D2}^{N}}{(1 + K_M C_{00}{}^{+})^2}. \tag{C4b}$$

For the noncompetitive isotherm,

$$h(C^{+}, \Psi_{00}, K_M, K_{D1}^{*}, K_{D2}^{*}) = 2K_{D1}^{*} + qK_{D2}^{*}. \tag{C4c}$$

Under any conditions, α can be evaluated from Eqs. C3 and C4. However, if one is only interested in finding relative values of the various K_Ds which produce identical values of α under fixed C^+, Ψ_{00}, and K_M conditions, it is sufficient to equate Eq. C4*a*–*c* to one another and to solve for the relative K_Ds. This is the procedure adopted for Figs. 7 *a* and 9 *a*.

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