Surface charging by large multivalent molecules

Extending the standard Gouy-Chapman treatment

Stefan Stankowski
Biocenter of the University, Klingelbergstrasse 70, CH-4056 Basel, Switzerland

ABSTRACT Traditionally, Gouy-Chapman theory has been used to calculate the distribution of ions in the diffuse layer next to a charged surface. In recent years, the same theory has found application to adsorption (incorporation, partitioning) of charged peptides, hormones, or drugs at the membrane-water interface. Empirically it has been found that an effective charge, smaller than the physical charge, must often be used in the Gouy-Chapman formula. In addition, the large size of these molecules can be expected to influence their adsorption isotherms. To improve evaluation techniques for such experiments, comparatively simple extensions of the standard Gouy-Chapman formalism have been studied which are based on a discrete charge virial expansion. The model allows for the mobility of charged groups at the interface. It accounts for finite size of the adsorbed macromolecules and for discrete charge effects arising from pair interactions in the interface plane. In contrast to previous discrete charge treatments this model nearly coincides with the Gouy-Chapman formalism in the case where the adsorbing molecules are univalent. Large discrepancies are found for multivalent molecules. This could explain the reduced effective charges needed in the standard Gouy-Chapman treatment. The reduction factor can be predicted. The model is mainly limited to low surface coverage, typical for the adsorption studies in question.

INTRODUCTION

Biochemical reactions occurring at the surfaces of cell or organelle membranes are likely to be influenced by surface charges due to the ionizable groups of lipids and proteins. Apart from governing specific interactions, surface charge generally plays an important role in modulating the distribution of counterions and co-ions in the vicinity of the membrane (see Cevc, 1990, for a recent review). Usually, these charge effects can be taken into account in a fully satisfactory way by use of the Gouy-Chapman theory (Aveyard and Haydon, 1973). Despite its simplicity and the relatively crude approximations made (in particular, considering the surface charge to be homogeneously smeared over the water-membrane interface), this formalism has proved to be remarkably successful. Excellent reviews are available to document this fact (McLaughlin, 1989). Problems appear, however, in using the Gouy-Chapman approach, if the charged groups on the surface are not univalent (e.g., phosphatidylglycerol or phosphatidyl-serine lipid head groups) but multivalent (such as the trivalent phosphatidyl-inositol, PIP2) (Langner et al., 1990). The simple formalism then overestimates strongly the charge effect on the co-ions. Complex statistical thermodynamics calculations have been invoked to explain these findings (Langner et al., 1990 and references to Kjellander and Marcelja therein). In the work covered by the above-mentioned reviews the typical situation is that of a membrane carrying a fixed surface charge (e.g., a given mole fraction of charged lipid). The interest in these cases is to determine the surface potential or the ion distribution close to the surface. Though some corrections can be made to account for ion absorption (Gouy-Chapman-Stern model, cf. e.g., Aveyard and Haydon 1973) the predominant contribution always comes from the diffuse double layer of ions near the surface. Surface charges considered are relatively high, typically corresponding to between 10% and 100% charged lipid in the membrane.

In recent years, it has become very popular to apply the Gouy-Chapman formalism to a different kind of experiment, namely the binding or incorporation of charged drugs, hormones, peptides, or proteins at a lipid bilayer or biomembrane (e.g., McLaughlin and Harary, 1976; Lee, 1978; Altenbach and Seelig, 1985; Schwyzer, 1986; Seelig and McDonald, 1989; Seelig et al., 1988; Sauterau et al., 1989; Schwarz and Beschiaschvili, 1989; Beschiaschvili and Seelig, 1990*a*, *b*). If these molecules adsorb to a surface containing lipids of opposite charge, their affinity is increased. If they associate with a neutral or zwitterionic bilayer they build up a surface charge by themselves and thereby discourage association, leading to a flattening of the isotherm at increasing free concentration. In principle, such effects can readily be evaluated using the Gouy-Chapman approach. However, if the membrane-associating molecules are multivalent, isotherms can often be fitted only by inserting a formal "effective charge" into the equations. In several cases, this effective charge was found to be much smaller than the actual physical charge (Chung et al., 1985; Seelig and

McDonald, 1989; Beschiaschvili and Seelig, 1990*a*). The most dramatic case is the hexavalent peptide melittin where the effective charge needed for fitting was only ~2 (Schwarz and Beschiaschvili, 1989; Kuchinka and Seelig, 1989; Beschiaschvili and Seelig, 1990*b*; Stankowski and Schwarz, 1990). Very recently, a similar situation has been found for a hexavalent signal peptide (Frey and Tamm, 1990). Of course, there could be some physical mechanism operating to produce this apparent charge reduction, e.g., close association of counterions with the charged groups or their localization at some distance from the interface. On the other hand, one must also examine the possibility of a deficiency of the Gouy-Chapman model, at least for multivalent adsorbates, in analogy to the situation found with the phosphatidylinositol, PIP2, where co-ion repulsion was also smaller than predicted by the simple model (Langner et al., 1990).

Considering association of charged molecules with a membrane, the situation is quite different than the one sketched previously: the surface charge is not fixed, but changes continuously as more and more material absorbs. The parameter of interest is not the surface potential but the deviation from ideality, or the activity coefficient of the absorbing species. Furthermore, in this kind of experiment, interest focuses on the interface itself, not on the diffuse ionic double layer. The adsorbing or incorporating molecules are often quite large, so that effects due to their finite size are expected to play an important role. On the other hand, average surface charges often remain small, at least if adsorption (incorporation) is to an uncharged membrane, so that the surface charge is exclusively due to the adsorbed species.

There exists a vast body of literature considering various extensions of the Gouy-Chapman theory. Much of this work is related to electrode/electrolyte interfaces, but could in principle be applied or rewritten for insulator/electrolyte interfaces which would be more appropriate in the case of biological membranes. Unfortunately, most of these theories contain a certain amount of mathematical sophistication and/or a number of parameters which are unknown in the case of protein or peptide adsorption to membranes. As a result, these theories are currently ignored by most workers studying peptide adsorption to lipid membranes. Instead, the simple Gouy-Chapman theory is generally applied along with the use of "effective" values of the most elementary parameters such as the charge number of the adsorbing molecule (Schwarz and Beschiaschvili, 1989; Beschiaschvili and Seelig, 1990*a*, *b*; Kuchinka and Seelig, 1989; Frey and Tamm, 1990).

As a first step in an attempt to remedy this unsatisfactory situation, I try to give the simplest extensions of the Gouy-Chapman theory which account for finite size of the membrane-absorbed particles and explain the appearance of "effective charges" when treating multivalent adsorbed ions with the standard methods. To keep the formalism as simple as possible, I shall take profit from the following two experimental features: firstly, surface coverage is often low, especially if adsorption to uncharged membranes is considered. This fact allows one to use simple linear approximations. Secondly, the absorbing macro-ions are large and their shape in the adsorbed conformation is usually unknown. Spherical shapes would rather seem unprobable as compared with flattened conformations accomodating to the surface. The latter are in fact easier to treat than spheres. Peptides are not expected to loose their hydration shells upon adsorption. The significance of introducing inner and outer Helmholtz planes (Grahame, 1947) as for ionic adsorption on electrode surfaces is thus doubtful. I shall avoid the appearance of undefined parameters by representing the highly complex lipid membrane, water interface by a formal interface between a high dielectric (water) containing electrolyte and a low dielectric (membrane). From experimental considerations, it has been concluded that the mobility of charged groups at the surface of lipid membranes is high (Winiski et al., 1986). This fact is emphasized in the present treatment and contrasts with the majority of existing discrete-charge models which consider adsorption to a lattice.

In summary, the present approach is not intended to introduce completely new theoretical concepts. Instead it is thought to help for a better understanding of the standard treatment and to provide simple corrections for the experimental situations typical for macromolecular adsorption to lipid bilayers or biomembranes. The main concept is to use a virial approach (thereby considering explicitly pair interactions at the adsorption interface), while retaining the mean-field treatment characteristic for the standard Gouy-Chapman theory for the electrolyte phase. This formalism, which may be considered the next higher order correction to the classical approach, will be shown to nearly coincide with the latter in the case of small univalent adsorbates, but to yield large differences for large size and/or multivalent adsorbates. The formalism can either be taken as it stands or else be used to predict finite-size corrections and effective parameters in the standard treatment.

THE MODEL

Smeared charges: Gouy-Chapman approach

For comparison with the formalism to be developed below, we first state those results of the Gouy-Chapman

treatment which are of interest in our context: the absorbed charge is considered to be smeared uniformly over the interface, giving rise to an average surface charge, σ (in Coulomb per square meter, say). The natural variable to measure the concentration of adsorbed (or incorporated) molecules at the interface is r = moles of adsorbed material per moles of total lipid. For the purpose of our discussion it is more convenient to introduce

$$x = \text{moles of adsorbed material/moles of accessible lipid} = r\beta, \quad (1)$$

where β is an accessibility factor. β is equal to unity if the molecule of interest has access to both sides of the bilayers, as with lipid dispersions. If adsorption is from aqueous solution to the outer leaflet of lipid vesicles, β will be between 0.5 and $\sim$0.65, depending on vesicle size, the larger figure referring to very small unilamellar vesicles where nearly two-thirds of the lipids stay in the outer shell; in multilamellar dispersions, β may in fact be much smaller than 0.5 if only the outermost shell is accessible. We shall give our formulae in terms of x, conversion to r being dependent on the relevant β value for the particular experimental system. If the adsorbing molecule inserts deeply into the bilayer thereby increasing the area of the interface, x should be replaced by the corrected parameter $\hat{x}$:

$$\hat{x} = x/(1 + xA_{ins}/A_{lip}) \quad (2)$$

with A_{ins} and A_{lip} being the molecular areas of the inserted molecule and the lipid, respectively.

The surface charge σ adsorbed on a neutral lipid bilayer can be expressed as

$$\sigma = (ze/A_{lip})x \quad (3)$$

with z the charge number of the adsorbed species and e the elementary charge.

The Poisson-Boltzmann equation can be used to describe the ionic distribution near the interface as a function of the surface potential ψ induced by the smeared charge. Specializing to a 1:1 electrolyte bathing the membrane (see McLaughlin, 1977, for more general formulae), this differential equation can be solved to yield (Schwarz and Beschiaschvili, 1989):

$$\psi = 2kT/e\ (\sinh^{-1} zbx) \quad (4)$$

with $b = e^2/(2A_{lip}\kappa\epsilon\epsilon_o kT)$

$\kappa^2 = 2e^2 IN_A/(\epsilon\epsilon_o kT)$, κ is the inverse Debye length.

(ϵ_o = permittivity of vacuum, ϵ = dielectric constant of water, kT = thermal energy, I = ionic strength, N_A = Avogadro's number, e = electronic charge). For a 0.1-M electrolyte solution at 25°C, the value of b is 6.2 (using 70 Å^2 for the area of a lipid). Up to $zx = 0.1$ the $\sinh^{-1}$ can be simply replaced by its argument to $\sim$5% precision. Eq. 4 then simplifies to

$$\psi = \frac{zex}{A_{lip}\kappa\epsilon\epsilon_o} = \frac{\sigma}{\kappa\epsilon\epsilon_o}. \quad (5)$$

Let us assume that ideal incorporation takes place in the absence of electrostatic interactions, governed by a partition coefficient K:

$$x = Kc,$$

where c is the (bulk) aqueous concentration of the incorporating species. At low surface coverage the same equation is also applicable for adsorption or surface binding, with K the binding constant. At higher coverage, Langmuir or other isotherms can be used to account for the saturation of sites. Charge accumulation at the interface would reduce the adsorption due to repulsive interactions, which can be written in terms of an activity coefficient, α, giving

$$\alpha x = Kc. \quad (6)$$

With the Gouy-Chapman formalism, α is obtained as (Schwarz and Beschiaschvili, 1989)

$$\ln \alpha = 2z \sinh^{-1} zbx = ze\psi/kT. \quad (7)$$

Eq. 6 can thus be rewritten as

$$x = K c \exp(-ze\psi)/kT$$

so that $1/\alpha$ can be interpreted as a Boltzmann factor reducing the bulk aqueous concentration at the interface. Again, in Eq. 7, the $\sinh^{-1}$ can be replaced by its argument if the surface coverage is low:

$$\ln \alpha = 2z^2 bx. \quad (7a)$$

We note the activity coefficient goes with the square of the charge number. Thus, for an effective charge reduction by a factor of 3 ($z_{eff} = z/3$) as evaluated in the case of melittin, the corresponding reduction in the interaction energy is by a factor of 9.

So far, adsorption has been considered to proceed at an interface composed of uncharged or zwitterionic lipid head groups. If some of the lipid is charged, it contributes to the overall surface charge. It is the main virtue of the Gouy-Chapman approach that it allows immediately to incorporate this effect: zx in Eqs. 3, 4, 7 is simply replaced by $zx + z_{lip}x_{lip}$, with z_{lip} the valency of the lipid head group charge and x_{lip} the mole fraction of charged over total lipid.

Alternative derivation

Mathias et al. (1988) have proposed a very instructive alternative derivation of the Gouy-Chapman formula. Since the dielectric constant of a lipid membrane ($\epsilon_m \approx 2$) is low with respect to that of the aqueous phase ($\epsilon \approx 78$), the following screened Coulomb potential can be assumed for a point-like charge ze sitting right at the interface (Mathias et al., 1988; McLaughlin, 1989; Nelson and McQuarrie, 1975; Brown, 1974):

$$\psi = \frac{2ze}{4\pi\epsilon\epsilon_0}\frac{\exp(-\kappa r)}{r}. \quad (8)$$

The factor 2 accounts for the image charge effect arising at the interface. (More rigorously, this factor would be $1 + (\epsilon - \epsilon_m)/(\epsilon + \epsilon_m) = 1.95$ using the values of dielectric constants given above. The reader may refer, e.g., to Vorotyntsev and Ivanov (1989) for a more general treatment where the adsorption plane is distinct from the interface plane.)

I consider the point charge to be at the center of a circular membrane patch of radius $a \gg \kappa^{-1}$. Averaging the potential, Eq. 8, over this patch, one obtains

$$\langle\psi\rangle = \frac{1}{\pi a^2}\int_0^a 2\pi r\psi(r)dr = \frac{\sigma}{\kappa\epsilon\epsilon_0}. \quad (9)$$

The average surface charge has been defined as $\sigma = ze/\pi a^2$, and the exponential screening of the potential allows one to integrate to infinity if a is large with respect to the Debye length. This reproduces the Gouy-Chapman formula, Eq. 5. Of course, the same result will be obtained for several point charges ze positioned at distances from each other which are large with respect to the Debye length.

A similar result has been obtained in more general terms (i.e., including media of other dielectric constants) by Vorotyntsev and Ivanov (1988). They note that the equivalence of statistical averaging and smearing out of charges is related to the adopted model of Debye-Hückel-type electrolyte screening.

To conform with the formalism sketched under point 1, the activity coefficient of the adsorbate is calculated by setting the interaction energy of an ion of charge ze with the remaining membrane phase equal to $ze\langle\psi\rangle$. This represents a typical mean-field approach. According to Eq. 7, the Gouy-Chapman potential can be written

$$\psi_{GC} = kT \ln \alpha / ze. \quad (10)$$

Mobile discrete charges of finite size—virial approach

As the next higher order approximation I propose to consider pair interactions of charged molecules explicitly in the membrane phase, but to retain a mean-field approximation for the electrolyte screening effects.

The Gouy-Chapman theory considers the distribution of charge in the aqueous phase close to and up to a homogeneously charged surface. In the following I shall consider the membrane interface with the absorbed molecules as a separate phase, in equilibrium with the aqueous phase. The equilibrium condition is given by Eq. 7.

In practical work on adsorption of charged molecules to lipid membranes, typical electrolyte concentrations range between 10 and 100 mM ionic strength. Screening by counterions then makes the potential of discrete charges exposed to water essentially "short-range," decaying to low values at distances of about two Debye lengths (2×9.6 Å at 0.1 M ionic strength, 25°C). Consequently, the adsorbed molecules will not repel each other when surface coverage is low enough that the average distance between two charges is large with respect to the Debye length, κ^{-1}. As the concentration of absorbed molecules, x, increases, pair interactions will come into play; at still higher concentrations triplet interactions come in, etc. The situation is fully analogous to modeling a real gas by a virial expansion. From general thermodynamics, the activity coefficient of the adsorbed species is given by (Tsien, 1978)

$$\ln \alpha = \Sigma_k[(k+1)/k]B_{k+1}x^k \quad (11)$$

expanded in powers of x. (Others like Hill [1960] define the virial expansion as a power expansion of α instead of $\ln \alpha$. Both definitions are equivalent as far as the second virial coefficient is concerned.) The B_{k+1} are the virial coefficients. Since I measure surface concentration per lipid and not per square meter, the B as defined here are obtained from the ordinary coefficients by dividing by the molecular lipid area. For low surface density only B_2 is relevant. It can be calculated from the following integral over the two-dimensional surface phase (Tsien, 1978)

$$B_2 = \frac{\pi}{A_{lip}}\int_0^\infty [1 - \exp(-U/kT)]rdr. \quad (12)$$

Here, r is the distance between the two interacting charges in the plane and $U(r)$ is the pair interaction energy.

Treating the aqueous phase as a continuum, I use the screened Coulomb potential, Eq. 8, to describe the potential produced by an absorbed molecule. This procedure is in line with previous discrete charge treatments of membrane surfaces (Nelson and McQuarrie, 1975; Brown, 1974). The present approach differs from typical models of that kind in so far as the charges are taken to be mobile in the surface plane and are not restricted to a

lattice or otherwise a priori restricted in their position. Of course there is a sort of inconsistency to consider direct pair correlations of discrete charges at the interface while treating the aqueous phase in a mean field continuum model. Such an assumption would appear to be justified as an approximation only as long as attention is limited to events in the interface plane, as will be the case in the remainder of this article.

Using the potential of Eq. 8, the pair interaction energy U, is given, in units of kT:

$$U/kT = ze\psi/kT$$

$$= z^2 w \exp(-\kappa r)/r \quad (13)$$

$$w = 2e^2/(4\pi\epsilon\epsilon_0 kT). \quad (14)$$

Inserting this into Eq. 12, the second virial coefficient, B_2, can be computed numerically. The integral converges well, and there is no need of sophisticated algorithms. Once B_2 is known, the activity coefficient can be obtained for any (sufficiently low) surface coverage x by setting

$$\alpha = \exp(2B_2 x). \quad (15)$$

The condition of low surface coverage means that $2B_2x \ll 1$ (e.g., $2B_2x < 0.1$) to justify truncation of the virial expansion after the second, linear term. I shall come back to this condition in the Discussion.

Finite size

Finite size of the adsorbing ions is quite naturally incorporated in the virial approach. For the sake of simplicity, I shall limit myself to shapes which are circularly symmetric in the interface plane (extension to more general shapes being straightforward). Two extreme cases are considered: (*a*) the z-valent charge remains point-like but is surrounded by a "hard" disk-shaped belt of radius R; (*b*) the z-valent charge is smeared homogeneously over the surface of a hemisphere of radius R, sitting with its center on the interface. The image charges then complete this charge distribution approximately to a full sphere of charge 2ze.

For case *a*, the potential of Eq. 8 remains valid in the range of $r > 2R$, the distance of closest approach of two large ions. For $r < 2R$, U becomes infinite. The integral, Eq. 12 then yields

$$B_2 = 2\pi R^2/A_{lip} + \frac{\pi}{A_{lip}} \int_{2R}^{\infty} [1 - \exp(-U/kT)] r dr. \quad (16)$$

The first term is the excluded-area contribution which gives the entropic effect due to steric exclusion (Stankowski, 1983, 1984). Since an integrand smaller than unity has been replaced by unity in part of the integration range, the resulting virial coefficient is larger than the one for a point charge.

For case *b*, the hemispherical charge distribution, completed to a sphere by the image charges, the potential is taken in analogy to Debye Hückel theory of large ions (Moore, 1972):

$$\psi(r) = \frac{2ze}{4\pi\epsilon\epsilon_0} \frac{\exp(\kappa R)}{(1 + \kappa R)} \frac{\exp(-\kappa r)}{r}. \quad (17)$$

This potential is valid in the part of the space containing the electrolyte solution and (by virtue of the continuity conditions) including the interface plane itself. To calculate the pair interaction energy, this expression has to be integrated over the surface of a hemisphere, the center of which is in the interface plane at a distance r from the hemisphere producing the potential (see Tsien, 1978, for an argument of why integration is only over the distribution of real charges whereas the potential includes image charges). The result is

$$U(r)/kT = \int_0^{\infty} ze\psi(s) \sin\Theta d\Theta$$

with $s = (r^2 + R^2 - 2rR\cos\Theta)^{1/2}$ and $\psi(s)$ given by Eq. 17 with r replaced by s. The final result is

$$U(r)/kT = \frac{ze}{kT} \frac{\sinh\kappa R}{\kappa R} \psi(r). \quad (18)$$

Again $\psi(r)$ is given by Eq. 17. Inserting this into Eq. 16 yields the second virial coefficient for case *b*. The distance of closest approach, $2R$, and the excluded-area term are the same in the two cases considered.

Adsorption to charged bilayers

There is no problem to calculate B_2 for repulsion between point charges, but for attractive interactions the interaction energy diverges as the distance of closest approach goes to zero. This can only be avoided by introducing the finite size of the charged groups. The choice of the size is very critical because it determines where the attractive potential trough is cut off. (In contrast, for repulsive interactions, finite size merely steepens the potential in the hard core region, see Fig. 1.) In the following, I shall give numerical values for a range of trial radii of ionic groups, starting at ~ 2 Å.

Combining positive and negative charges at the interface, the virial approach has to be extended in analogy to the well-known case of a binary mixture of real gases (Hill, 1960). The activity coefficient of the absorbing (incorporating) species then comes out as

$$\ln\alpha = 2B_2(\text{rep})\, x + 2B_2(\text{att})\, x_{lip} + \ldots \quad (19)$$

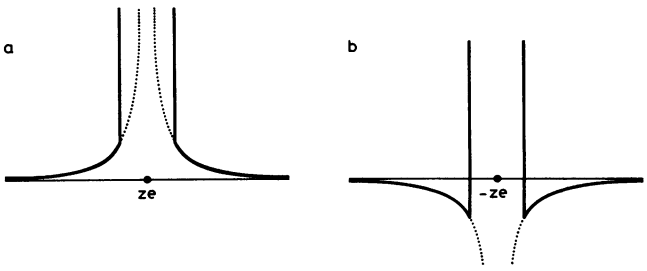

FIGURE 1 Hard core effect on repulsive (*a*) and attractive (*b*) electrostatic potentials.

B_2(rep), the repulsive pair interaction of adsorbed molecules, is the same as above, that is Eq. 16 together with Eqs. 8 or 17. B_2(att), the attractive pair interaction between adsorbing species and lipid head groups, is given by the same Eq. 16, but the z^2 appearing in the pair interaction energy $U(r)$, Eqs. 8 and 17, is replaced by $z \cdot z_{lip}$. Obviously, B_2(att) is negative. (In textbook notation, B_2[att] would be twice as large due to a symmetry factor; the factor 2 in front of it in Eq. 19 is to compensate for this in our notation.) $2R$ is the distance of closest approach, equal to the sum of the individual radii for the adsorbate and for the lipid head group. (For case *b* note that the result, Eq. 18, implies the assumption of equal radii of the interacting molecules; the more general result is readily obtainable, but for the sort of estimate of interest here it is fully sufficient to use Eq. 18 as it stands, with R taken as the arithmetic average of the radii involved.)

It should be remembered that in any case this approach is limited to low surface coverage of all ionic species in the interface, so that each term in Eq. 19 remains smaller than unity.

Simplified finite-size model

In general, evaluation of the virial integral, Eq. 16, has to be done numerically. The situation simplifies considerably, however, if the absorbing particles are very large: the pair interaction energy $U(r)$ has to be evaluated in the range of r between $2R$ and infinity. Being a homogeneously decreasing function of r, $U(r)$ will clearly remain $\ll kT$ in the complete integration range, if R is large enough. The exponential $\exp(-U/kT)$ can then be expanded and the integral solved analytically. Assuming "case *b*," this yields

$$2B_2 = 4\pi R^2/A_{lip} + 2z^2 b \exp(-2\kappa R), \qquad (20)$$

where b is the same constant as appearing in the Gouy-Chapman formalism, Eq. 4. Multiplying Eq. 20 by x one obtains ln α which is thus composed of an excluded-area and an electrostatic term. The latter has the form of a mean-field potential, $\langle\psi\rangle$, multiplied by ze/kT. In fact, $\langle\psi\rangle$ is identical to the ordinary Gouy-Chapman potential, apart from the exponential term containing the size R.

Because the parameter b is proportional to the Debye length, κ^{-1}, the elecrostatic term dominates the excluded-area term at sufficiently low ionic strength, and $\exp(-2\kappa R)$ approaches unity in that same limit. Thus the virial approach goes over to the Gouy-Chapman theory under the following limiting conditions: (*a*) $U \ll kT$ everywhere in the physically available part of the interface (no "discrete-charge effects") and (*b*) low enough ionic strength so that $\kappa R \ll 1$ (no "finite-size effects").

Of course, $U \ll kT$ at $r = 2R$ is not at all satisfied for ordinary particle radii of a few Angstroms, even for $z = 1$. This is the reason why the virial expansion yields different results than the mean-field treatment: in the statistical averaging, strong repulsion at close distance is weighed more correctly than in the smeared-charge treatment. The effect should be the more pronounced the larger the interaction energy at the distance of closest approach, i.e., the larger z. The corresponding numerical values are discussed in Results.

From an intuitive point of view, one may thus state that the smearing out of charges is equivalent to eliminating the peaks in the interaction energies arising in discrete charge distributions at close distances between charged groups. Vorotyntsev and Ivanov (1989) have noted in a similar way (though in a different context involving an intermediate dielectric layer) exaggerated particle–particle repulsion due to the "smearing out of the charge within the Bjerrum region around the ion." I propose the following limiting procedure to make the argument explicit: starting with a given distribution in the interface plane of discrete charges ze (surface coverage x), smearing out of these charges is simulated by breaking them up into smaller entities of valency $z' = z/n$ (surface coverage nx). In the limit of n tending to infinity, the distribution represents a homogeneously smeared surface charge. Clearly $U(r)$ will be smaller than kT at any fixed value of $r = 2R$ if only n is made large enough. The virial integral then goes over to Eq. 20 (where the finite-size contributions are negligible if R is small with respect to the Debye length). As before, the activity coefficient is found as $kT \ln \alpha = z'e\langle\psi\rangle$, with $\langle\psi\rangle$ equal to the Gouy-Chapman potential.

In the above discussion, finite-size effects were neglected to allow direct comparison with the standard Gouy-Chapman formalism. Obviously, however, even with finite-size contributions included, the electrostatic term in Eq. 20 is still of a mean-field type. This equation can therefore be considered as a generalization of the

ordinary GC formalism to include finite-size effects. It is obtained from the virial expansion approach either directly (for large R) or, more generally, by applying the limiting procedure of splitting up the charges. The latter corresponds to smearing out the charge while retaining the steric structure of the adsorbed particles. Calculations can be done with particles of "case a" as well as of "case b" type. The resulting formulae are:

$$\ln \alpha = 4\pi R^2 x/A_{lip} + 2z^2 bx\phi\,, \tag{21}$$

where the factor ϕ is

$$\phi_{(a)} = \exp(-2\kappa R) \quad \text{for case } a \tag{22a}$$

$$\phi_{(b)} = \frac{\sinh\kappa R}{\kappa R}\frac{\exp(-\kappa R)}{(1+\kappa R)} \quad \text{for case } b. \tag{22b}$$

Obviously, both cases give the same result if κR is much smaller than unity.

The model represented by Eqs. 21 and 22 is clearly less general than the virial approach, because it only incorporates finite-size corrections, but not the "discrete-charge effects" in the sense of the above discussion. This simplified model is nevertheless useful to estimate the size contributions alone in the framework of ordinary smeared-charge treatments.

RESULTS

Adsorption (incorporation) of charged molecules to an uncharged lipid bilayer is considered in the first instance. Low surface coverage x is assumed throughout. The logarithm of the activity coefficient of the adsorbing (incorporating) material is then found to be a linear function of x both in the standard Gouy-Chapman (GC) treatment and in the virial approach (VE):

$$(\ln \alpha)/x = 2B_2 \quad \text{for VE} \tag{23}$$

$$(\ln \alpha)/x = 2z^2 b \quad \text{for GC}. \tag{24}$$

Thus, the two models can simply be compared by looking at the coefficient $C = \ln \alpha/x$, which still depends on the valency of the adsorbate and on the ionic strength of the electrolyte solution. In analogy to current evaluation practice, one may also write

$$2B_2 = 2z_{eff}^2 b \tag{25}$$

and compare the physical valency z with the "effective charge number" z_{eff} needed in the GC formalism to match the virial expansion results.

Numerical values of $C = \ln \alpha/x$ for the two models are compiled in Table 1 for various ionic strengths and $z = 1$ ($\epsilon = 78$ and $A_{lip} = 70$ Å^2 is assumed throughout). They agree very well at low ionic strength, but deviate as the electrolyte concentration increases.

TABLE 1 Coefficients C = ln α /x defining the activity coefficients of membrane-adsorbed ions, calculated from virial expansion (VE) and from Gouy-Chapman theory (GC): ionic strength dependence

Ionic strength	VE (point)	VE (2 Å)*	GC (point)‡	GC (2 Å)§
1mM	106	106	123	119
10mM	29	29	39	35
20mM	19	19	27.5	23.7
50mM	10.7	10.9	17.4	13.9
100mM	7.0	7.1	12.3	9.0 (8.8)
500mM	2.4	2.6	5.5	3.2 (2.9)
1 M	1.5	1.8	3.9	2.0 (1.8)

*Hemispherical charge distribution of radius R = 2 Å (Eqs. 16, 18); case a would give nearly the same results.
‡Given is $2z^2b$, cf. Eq. 7a.
§Simplified finite-size correction, Eqs. 21, 22b. Case a results are given in brackets where they deviate.

Using the simplified finite-size correction of the previous section (last column of Table 1), it becomes evident that the discrepancy is mainly due to finite-size effects. These effects obviously become important already in the range of ionic strengths between 10 and 100 mM, which is the one of primary interest in experimental work. Upon comparing z_{eff} and z in this range of salt concentration, the discrepancies amount to 15–25% without finite-size correction and to ~10% with finite-size correction in the GC treatment, along the lines of the simplified model given above.

The discrepancies between the coefficients C in the GC and VE models become much more important for larger values of z, as shown in Table 2 (ionic strengths: 0.01 and 0.1 M). $2B_2$ values are considerably smaller than the corresponding Gouy-Chapman counterparts. In fact, they increase rather more linearly with z in contrast to the z^2 dependence of the Gouy-Chapman parameter. (In

TABLE 2 Coefficients C = ln α /x at 0.01 M and 0.1 M ionic strength: dependence on valency z

	I = 0.01 M			I = 0.1 M		
z	VE (point)	GC (point)	GC (2 Å)	VE (point)	GC (point)	GC (2 Å)
1	29	39	35	7.0	12.3	9.0
2	82	156	137	17	49	34
3	140	350	308	27	111	76
4	197	622	546	36	197	134
5	252	973	853	45	308	209
6	304	1400	1228	52	444	301

Calculations as for Table 1 (VE [2 Å] is within 2% of the values for VE [point] throughout). GC (2 Å) represents simplified finite-size correction.

fact, using zbx instead of z^2bx in the Gouy-Chapman formula nearly exactly reproduces the second virial coefficients for $z > 1$.) We have also calculated coefficients at 10, 20, and 50 mM ionic strength and find the following approximate empirical relations valid for $z \geq 2$:

$$2B_2 = 2zb \quad \text{at 100mM ionic strength} \tag{26a}$$

$$2B_2 = 1.6z^{1.05}b \quad \text{at 50mM ionic strength} \tag{26b}$$

$$2B_2 = 1.86z^{1.1}b \quad \text{at 20mM ionic strength} \tag{26c}$$

$$2B_2 = 2z^{1.15}b \quad \text{at 10mM ionic strength} \tag{26d}$$

In a purely operational way, these equations can be used to define an "effective" charge number, z_{eff}, setting the above equations equal to $2\,z_{eff}^2\,b$. For example, at 100 mM ionic strength $z_{eff} = z^{0.5}$, or at 20 mM, $2z_{eff}^2 = 1.86z^{1.1}$, i.e. $z_{eff} = 0.96z^{0.55}$.

Fig. 2 summarizes the main conclusions by showing water-membrane partition isotherms for a molecule with $z = 3$, assumed to be hemispherical with a radius of 2 Å, at 0.1 M inert 1:1 electrolyte. Curve c flattens much less at high concentrations than the Gouy-Chapman curve a, as predicted by the virial approach. It can be reproduced in the standard model by introducing an "effective charge number" smaller than 3, namely $z_{eff} = 1.5$. Curve b corresponds to the simplified model which corrects only for finite-size effects. The dashed curve is calculated with the virial model and represents finite-size effects for a very large particle, of radius equal to the Debye length (9.6 Å). The simplified model then gives a very good approximation to the virial expansion, in fact so close to it that it had to be omitted from the figure for clarity. (The effective charge number corresponding to the dashed curve is 1.7.)

In principle, the model can be applied also to the case of adsorption to charged lipid bilayers, provided that the charge density is low enough. Some size estimate (distance of closest approach or average radius of charged groups) has to be made. As more and more data accumulate, it should become feasible to define a sort of calibration parameter by comparison. In any event, as shown in Table 3, the attractive interaction energies evaluated with reasonable assumptions about size are found in the same range of magnitude as the repulsive energies. However, for the dependence on z, there is apparently a stronger increase of attraction than of repulsion. Of course, if the adsorbing molecule is very large, much of the attractive effect may be compensated by the excluded area.

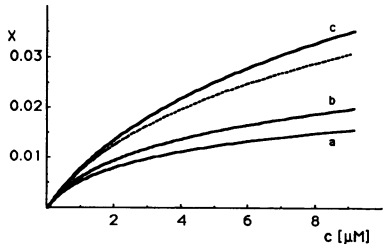

FIGURE 2 Water-membrane partitioning of a molecule with charge number $z = 3$ and radius 2 Å in 0.1 M inert 1:1 electrolyte. Partition coefficient $K = 10^4\ M^{-1}$. x = membrane-associated molecule per lipid, c = aqueous concentration. (*a*) Standard Gouy-Chapman model; (*b*) smeared-charge model accounting for finite size; (*c*) second virial coefficient model. (*Full lines*) particle radius 2 Å, (*dashed line*) very large particle of radius 9.6 Å (i.e., equal to the Debye length); curve represents case *c*, case *b* would be very slightly below it.

DISCUSSION

Recently, Gouy-Chapman theory has been applied by various authors to describe the adsorption (incorporation) of relatively large molecules (hormones, peptides, proteins) at lipid bilayers. Two problems are obvious from these reports: first the finite size of these molecules is expected to cause excluded area effects and may also have some influence on the electrical potentials. In addition, fitting isotherms for multivalent molecules by the Gouy-Chapman formula often required that a reduced "effective charge" was used.

To analyze problems with the standard treatment in these cases, a virial expansion approach has been proposed. It may be considered as the next higher level of approximation in so far as pair interactions in the interface plane are taken into account, as compared with the mean-field character of the standard model. The approach is, however, limited to low surface coverage of ions, making it particularly appropriate for studying the adsorption of peptides or other large molecules to

TABLE 3 **Coefficients for attractive interaction, $2B_2$ (att), for different valencies z and different radii R as indicated**

z	$2B_2(2Å)$	$2B_2(3Å)$	$2B_2(4Å)$	$2B_2(6Å)$	$2B_2(9.6Å)$	GC (point)
1	−51/ −12.5	−40/ −8.3	−34/ −4.1	−24/ +1.7	−7/+14	−39/ −12.3
2	−274/−59	−128/−25	−94/−14	−64/ −3.9	−34/+11	−156/ −49
3	−2620/−325	−400/−60	−211/−30	−121/−10.7	−67/ +8	−350/−111

Values given refer to 0.01 M (first item) and 0.1 M (second item) ionic strength, separated by slashes. $z_{lip} = -1$.

uncharged (zwitterionic) lipid membranes. Nevertheless, a generalization has been given which includes the presence of charged lipid in the membrane in not too high amounts.

In the virial approach, the charged molecules are considered to be mobile in the interface plane and not in any way a priori restricted in their position (this is the main difference with respect to the majority of existing discrete-charge treatments). The resulting functional form of the activity coefficient of the adsorbate is the same as in the Gouy-Chapman model (at low surface coverage x), $\ln \alpha$ being a linear function of x. It should be pointed out that a completely different functional form has been postulated in one of the few reports in the literature where a discrete-charge model has been applied to peptide absorption data (Schoch and Sargent, 1980). In fact, in that treatment the charges were assumed to adsorb always at the points where the electrostatic potential is minimal, and this assumption was combined with a Langmuir isotherm. Using this isotherm, however, implicitly assumes random and unrestricted adsorption, which seems to conflict with the first assumption.

Because the virial expansion as given here yields the same functional dependence on surface coverage as the standard Gouy-Chapman treatment, it predicts that the latter should be able to correctly describe experimental isotherms, if the coefficients are suitably adjusted. For instance, it may be necessary to introduce an "effective charge number," z_{eff}, instead of the physical valency, z. According to the numerical results listed in Tables 1 and 2, z_{eff} is predicted to be smaller than z, the effect being more pronounced at large z. This is exactly the situation found in experimental work on peptide absorption to lipid membranes. The "effective" reduction of the physical valency to be expected when applying the standard treatment is given by Eq. 26.

One of the best documented cases is that of the bee venom peptide melittin (Schwarz and Beschiaschvili, 1989; Kuchinka and Seelig, 1989; Stankowski and Schwarz, 1990). It has been argued that out of its six charged groups the two arginines probably do not contribute very much to the surface charge at a bilayer-water interface due to a close association of counterions or a localization far away from the interface plane (Stankowski and Schwarz, 1990). With Eq. 26, the remaining valency of $z = 4$ then reduces to an "effective charge" of 2 at 0.1 M ionic strength and 2.2 at 0.01 M. This would explain the experimental values, reported to lie between 1.85 (Schwarz and Beschiaschvili, 1989) and 2.2 (Kuchinka and Seelig, 1989). Similar arguments apply to the hexavalent signal peptide from cytochrome c oxidase subunit IV for which Frey and Tamm (1990) report a comparable charge reduction. For a $z = 2$ analogue of somatostatin, Beschiaschvili and Seelig (1990*a*) find $z_{eff} = 1.3$ at 0.1 M NaCl, in line with the prediction of $z_{eff} = 1.4$ from Eq. 26.

The present model can be generalized in principle to describe the absorption to charged lipid bilayers. However, application of the corresponding formalism is limited by the condition that the surface density of every charged species must be low. The results compiled in Table 3 are nevertheless interesting in that they show that the contributions due to attractive interactions are of similar magnitude as in the standard GC theory.

Although the virial model is meant to describe the charge distribution at the interface plane and not in the ionic double layer, it is of interest to note the qualitative agreement with recent experimental results obtained using the multivalent lipid, inositol-trisphosphate (Langner et al., 1990). In that study, co-ion repulsion was found to be much weaker than that predicted by the simple Gouy-Chapman model, in accordance with an effective charge reduction very much like the one obtained with the present model. In contrast, counterion attraction was stronger and more closely matched the Gouy-Chapman value. I would predict the same behavior if an estimated average radius of 2.5 Å is used for the charged groups involved. This is about the same size as that used by the authors to rationalize their results in the framework of a rather sophisticated thermodynamic model of the hypernetted chain type.

Previous discrete charge theories had rather predicted co-ion interactions to agree with the standard Gouy-Chapman results and counterion attraction to be much stronger (Nelson and McQuarrie, 1975; Winiski et al., 1986). The main problems in the treatment have been discussed by Winiski et al. (1986) on the basis of numerical calculations. Finite size and mobility of the charges were considered as major factors affecting the results, in agreement with conclusions from the present model.

In fact, accounting for finite-size effects is the second major advantage of the virial approach, apart from rationalizing "effective charge numbers." Two extreme cases have been considered in detail: a point-like charge surrounded by a circular belt of radius R ("case *a*") and a charge distribution smeared homogeneously over the surface of a hemisphere of radius R ("case *b*"). Sizes of the order encountered with simple ions do not markedly affect the second virial coefficient, as shown in Table 1. For very large molecules, the excluded-area contribution can become dominant. The importance of this term has been stressed previously (Levine et al., 1962; Stankowski, 1983, 1984).

A simplified model has been presented which combines the steric properties of molecules with a smeared-charge approach. This simplified model provides a very

convenient correction to the standard GC treatment, improving the results especially at not too high valencies (cf. Tables 1 and 2). Improvement is due to cutting off the potential in those parts of the plane which remain inaccessible due to steric constraints. The quality of this simplified model can be judged by comparison with the more general virial expansion formalism.

Limitations

The main advantage of the formalism appears to be its simplicity, especially when compared with sophisticated thermodynamical theories of the hypernetted chain or mean-spherical-approximation type (Kjellander and Marcelja, 1986; Attard et al., 1988). However, this can clearly not go without serious limitations.

First of all, the formalism as developed here is only valid at low surface coverage ($2B_2x < 1$). This corresponds approximately to the regime where the Gouy-Chapman theory can be linearized. At higher surface coverage it is necessary to take account of higher order virial coefficients which are more difficult to calculate. I evaluated the third virial coefficient numerically at 0.1 M ionic strength for univalent point charges and found $B_3 = 0.3\ B_2^2$. This is less than in the case of uncharged hard disks in a plane, where $B_3 = 0.782\ B_2^2$. This figure allows one to estimate the importance of higher order terms, by using Eq. 11. It turns out that the contribution of these terms may have less importance than another point of concern which becomes evident when considering the Gouy-Chapman formula, Eq. 7. For $zbx > 1$, the $\sinh^{-1}$ is *smaller* than zbx (in contrast to the higher order virial coefficient which would tend to increase the linear term). Obviously this is due to rearrangement of the ions in the diffuse double layer: the total ion concentration, $c_+ + c_-$, remains constant near the interface as long as the $\sinh^{-1}$ can be linearized (i.e., the increase in counterion concentration is counterbalanced by the decrease of co-ion concentration). Beyond that regime, counterion concentration increase exceeds the decrease of co-ion concentration, leading to more efficient screening of the membrane-bound charges. Possible corrections would involve appropriate modification of the Debye parameter κ. At the present stage, the simplest procedure is probably to use the Gouy-Chapman formalism with an effective charge number, z_{eff}, taken from a relation like Eq. 26. It is encouraging to note that Vorotyntsev and Ivanov (1989) have found linearization of the pair interaction function to remain meaningful up to much higher charge densities.

Another limitation of the virial coefficient approach as used here is the distinction between charges at the interface, modeled as individual entities, and in the aqueous phase, modeled as a continuum, respectively. This makes the model less appropriate to describe the potential or interactions in the ionic double layer occupying the aqueous phase close to the interface. The main scope was in fact to treat interactions among adsorbing molecules at the interface.

Received for publication 11 July 1990 and in final form 14 January 1991.

REFERENCES

Altenbach, D., and J. Seelig. 1985. Binding of the lipophilic cation tetraphenylphosphonium to phosphatidylcholine membranes. *Biochim. Biophys. Acta.* 818:410–415.

Attard, P., D. J. Mitchell, and B. W. Ninham. 1988. Beyond Poisson-Boltzmann: images and correlations in the electric double layer. II. Symmetric electrolyte. *J. Chem. Phys.* 89:4358–4367.

Aveyard, R., and D. A. Haydon. 1973. An introduction to the principles of surface chemistry. Cambridge University Press, Cambridge 40–52.

Beschiaschvili, G., and J. Seelig. 1990*a*. Peptide binding to lipid bilayers. Binding isotherms and zeta-potential of a cyclic somatostatin analog. *Biochemistry.* 29:10995–11000.

Beschiaschvili, G., and J. Seelig. 1990*b*. Melittin binding to mixed phosphatidylglycerol/phosphatidylcholine membranes. *Biochemistry.* 29:52–58.

Brown, R. H. 1974. Membrane surface charge: discrete and uniform modeling. *Prog. Biophys. Mol. Biol.* 28:343–370.

Cevc, G. 1990. Membrane electrostatics. *Biochim. Biophys. Acta.* 1031:311–382.

Chung, L., G. Kaloyanides, R. McDaniel, A. McLaughlin, and S. McLaughlin. 1985. Interaction of gentamicin and spermine with bilayer membranes containing negatively charged phospholipids. *Biochemistry.* 24:442–452.

Frey, S., and L. Tamm. 1990. Membrane insertion and lateral diffusion of fluorescence labelled cytochrome *c* oxidase subunit IV signal peptide in charged and uncharged phospholipid bilayers. *Biochem. J.* 272:713–719.

Grahame, D. C. 1947. The electric double layer and the theory of electrocapillarity. *Chem. Rev.* 41:441–501.

Hill, T. 1960. Introduction to Statistical Thermodynamics. Addison-Wesley, Reading, MA. 261–275.

Kjellander, R., and S. Marcelja. 1986. Double-layer interaction in the primitive model and the corresponding Poisson-Boltzmann description. *J. Phys. Chem.* 90:1230–1232.

Kuchinka, E., and J. Seelig. 1989. Interaction of melittin with phosphatidylcholine membranes. Binding isotherm and lipid head-group conformation. *Biochemistry.* 28:4216–4221.

Langner, M., D. Cafiso, S., Marcelja, and S. McLaughlin. 1990. Electrostatics of phosphoinositide membranes. Theoretical and experimental results. *Biophys. J.* 57:335–349.

Lee, A. G. 1978. Effects of charged drugs on the phase transition temperatures of phospholipid bilayers. *Biochim. Biophys. Acta.* 514:95–104.

Levine, S., G. M. Bell, and D. Calvert. 1962. The discreteness-of-charge effect in electric double layer theory. *Can. J. Chem.* 40:518–538.

Mathias, R. T., S. McLaughlin, G. Baldo, and K. Manivannan. 1988. The electrostatic potential due to a single fixed charge at a membrane-solution interface. *Biophys. J.* 53:128*a*. (Abstr.)

McLaughlin, S. 1977. Electrostatic potentials at membrane-solution interfaces. *Curr. Top. Membr. Transp.* 9:71–144.

McLaughlin, S. 1989. The electrostatic properties of membranes. *Annu. Rev. Biophys. Biophys. Chem.* 18:113–136.

McLaughlin, S., and H. Harary. 1976. The hydrophobic absorption of charged molecules to bilayer membranes: a test of the applicability of the Stern equation. *Biochemistry.*. 15:1941–1948.

Moore, W. J. 1972. Physical Chemistry. 5th ed. Longman, London. 453.

Nelson, A. P., and D. A. McQuarrie. 1975. The effect of discrete charges on the electrical properties of a membrane. *J. Theor. Biol.* 55:13–27.

Sautereau, A. M., M. Betermier, A. Altibelli, and J. F. Tocanne. 1989. Adsorption of the cationic antitumoral drug Celiptium to phosphatidylglycerol in membrane model systems. Effect on membrane electrical properties. *Biochim. Biophys. Acta.* 978:276–282.

Schoch, P., and D. F. Sargent. 1980. Quantitative analysis of the binding of melittin to planar lipid bilayers allowing for the discrete-charge effect. *Biochim. Biophys. Acta.* 602:234–247.

Schwarz, G., and G. Beschiaschvili. 1989. Thermodynamic and kinetic studies on the association of melittin with a phospholipid bilayer. *Biochim. Biophys. Acta.* 979:82–90.

Schwyzer, R. 1986. Molecular mechanism of opioid receptor selection. *Biochemistry.* 25:6335–6342.

Seelig, A., and P. McDonald. 1989. Binding of a neuropeptide, substance P, to neutral and negatively charged lipids. *Biochemistry.* 28:2490–2496.

Seelig, A., P. R. Allegrini, and J. Seelig. 1988. Partitioning of local anesthetics into membranes: surface charge effects monitored by the phospholipid head group. *Biochim. Biophys. Acta.* 939:267–276.

Stankowski, S. 1983. Large-ligand adsorption to membranes. II. Disk-like ligands and shape-dependence at low saturation. *Biochim. Biophys. Acta.* 735:353–360.

Stankowski, S. 1984. Large-ligand adsorption to membranes. III. Cooperativity and general ligand shapes. *Biochim. Biophys. Acta.* 777:167–182.

Stankowski, S., and G. Schwarz. 1990. Electrostatics of a peptide at a membrane/water interface. The pH dependence of melittin association with lipid vesicles. *Biochim. Biophys. Acta.* 1025:164–172.

Tsien, R. Y. 1978. A virial expansion for discrete charges buried in a membrane. *Biophys. J.* 24:561–567.

Vorotyntsev, M. A., and S. N. Ivanov. 1988. Potential drop at the insulator/electrolyte solution interface. *Elektrokhimiya.* 24:805–807.

Vorotyntsev, M. A., and S. N. Ivanov. 1989. Ionic adsorption isotherms at homogeneous insulator/electrolyte solution interfaces. *Elektrokhimiya.* 25:554–557.

Winiski, A. P., A. C. McLaughlin, V. McDaniel, M. Eisenberg, and S. McLaughlin. 1986. An experimental test of the discreteness-of-charge effect in positive and negative lipid bilayers. *Biochemistry.* 25:8206–8214.