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Polyhydroxy-mediated interactions between liposomes and bacterial biofilms

Malcolm N. Jones *, Michael Kaszuba

School of Biological Sciences, Division of Biochemistry and Molecular Biology, University of Manchester, Stopford Building, Oxford Road, Manchester, M13 9PT, UK

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Abstract

A theoretical model has been developed for the interaction of the surface polymers of the bacterial glycocalyx with liposomes incorporating lipids with polyhydroxy headgroups such as phosphatidylinositol (PI). The theory is based on a lattice model and equations are derived for the potential energy of interaction between the surfaces of a bacterium and a liposome as a function of their separation. It is shown that a relatively small energy of interaction, less than that of a single hydrogen bond, between the polyhydroxyl headgroup of the liposomal lipid and bacterium surface polymer residues could give rise to a potential energy of interaction in excess of the classical double layer repulsive force and attractive dispersion force interactions. The most important prediction of the theory is that the potential energy of interaction goes through a minimum as a function of the polyhydroxy lipid (PI) concentration in the liposomal surface, thus predicting an optimal liposomal composition for adsorption of liposome to bacterium. This result is in concordance with the adsorption of dipalmitoylphosphatidylcholine-PI liposomes to a range of biofilms of oral and skin-associated bacteria on solid supports, where optimum levels of PI for adsorption have been found. The theory demonstrates that subtle changes in the composition of liposomal and bacterial surfaces involving relatively small interaction energies can markedly influence the nature of their interactions.

Key words: Lattice theory; Bacterial glycocalyx; Phosphatidylinositol; Liposome; Interaction energy

1. Introduction

In a recent study on the targeting of liposomes to adsorbed films of oral bacteria it was found that liposomes incorporating low levels of phosphatidylinositol (PI) would target to the oral bacteria *Streptococcus mutans* (Table 3 in Ref. [1]). In these experiments targeting was assessed by the inhibition of an enzyme-linked immunosorbent assay (ELISA) using an antibody to antigen B in the cell wall of the bacterium. The adsorbed liposomes are thought to sterically hinder the antigen–antibody interaction and inhibit the ELISA. Subsequent experiments have shown that PI when incorporated in dipalmitoylphosphatidylcholine (DPPC) liposomes at mole percentages up to 20 also facilitates adsorption to strains of *Streptococcus sanguis, Staphylococcus epidermidis* and *Proteus vulgaris* [2] and that for each bacterium there is an optimum level of PI for adsorption. An example of such an optimum is shown in Fig. 1 for DPPC/PI liposomes (weight average diameter ~ 100 nm) prepared by the extrusion technique (VETs) [3] targeted to *Staphylococcus epidermidis* biofilms. Adsorption in the experiments was measured radiochemically and calculated from the projected area of the liposomes and the geometric area of the biofilm [4]. Fig. 2 shows the results of similar experiments with DPPC-dipalmitoylphosphatidylserine (DPPS) VETs. Although DPPS has a single negative charge at physiological pH, as has PI, the extent of liposome adsorption to the bacterial biofilm is very small.

These results demonstrate that the polyhydroxy nature of the inositol headgroup of PI significantly mediates the interaction with the bacteria. The use of phosphatidylinositol phosphates as targeting molecules to hydroxyapatite in the oral cavity has been previously observed but in this context the interaction was medi-

* Corresponding author.

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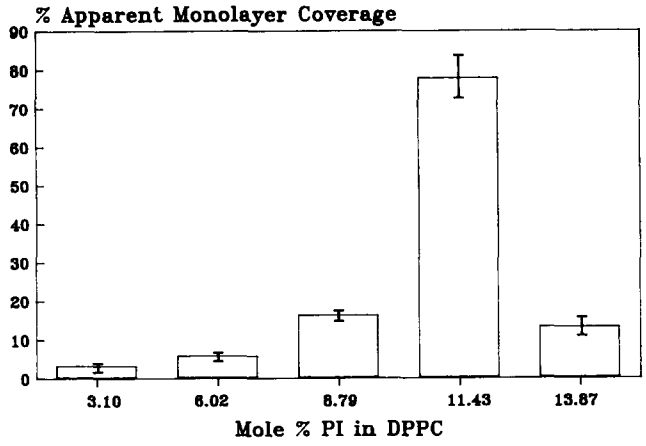

Fig. 1. The adsorption of dipalmitolyphosphatidylcholine (DPPC)-phosphatidylinositol (PI) liposomes to *Staphylococcus epidermidis* biofilms as a function of mol% PI in the liposomes. The liposomes were produced by extrusion and had a diameter (d) of approximately 100 nm. The biofilm was adsorbed on microtitre plate wells. Adsorption is expressed as monolayer coverage calculated from the projected area of the liposomes $\pi(d/2)^2$ and the geometric area of the biofilm. Since this calculation does not take into account the surface roughness of the biofilm the adsorption is described as apparent monolayer coverage. The solvent was phosphate-buffered saline (pH 7.4).

ated by phosphate group–metal ion interactions [5]. The purpose of the present study is to formulate a theoretical model for liposome–bacterium interactions to explain how optimum levels of PI for adsorption might come about by interactions between the PI head group and the surface polymers of bacteria (polyol phosphate polymers (teichoic acids) in the case of Gram negative bacteria [6]).

Several theoretical approaches to the interactions between surfaces with adsorbed polymer layers have been reviewed by De Gennes [7,8]. The interaction between polymer-coated surfaces has been treated theoretically for two particular cases. Firstly for a surface having anchored polymers whose separation exceeds their coil size (so-called 'mushroom' model) and secondly for surfaces with a high polymer density so that the polymer chains take up extended conformations (so-called 'brush' model). Both these models result in a repulsive force between the surfaces [8] which is important in the field of sterically stabilized liposomes [9]. These approaches do not however consider the problem of bridging between two surfaces by polymers which due to polymer interdiffusion and entanglements can lead to attractive interactions between particles [10].

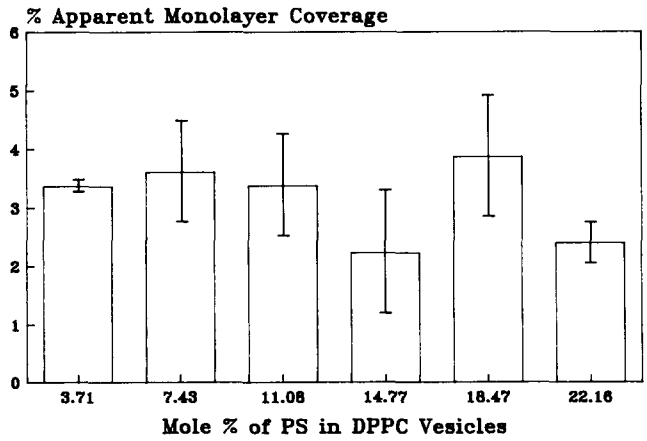

Fig. 2. The adsorption of DPPC-dipalmitoylphosphatidylserine (DPPS) liposomes to *Staphylococcus epidermidis* biofilms as a function of mol% DPPS in the liposomes. The conditions of the experiments, etc., were identical to those described in the legend to Fig. 1.

The problem of liposome–bacterium interactions is essentially a bridging problem and in the particular case of phosphatidylinositol-containing liposomes we take the view that attraction occurs between the inositol ring structure and most probably the monosaccharides in the bacterium polymeric glycocalyx. The origin of the attractive interaction is possibly hydrogen bonding although other types of interaction may be important. For example, it has recently been pointed out that monosaccharides can expose a significant area of hydrophobic surface in aqueous solution [11]. For glucose the ratio of the difference between the heat capacity of the solid state and the partial molar heat capacity of an infinitely dilute aqueous solution ($\Delta C°_p$), to the exposed non-polar surface area (ΔA_{np}), is -1.12 J mol^{-1} K^{-1} Å^{-2}. This figure is of the same sign and approximately the same value as that for the transfer of hydrocarbons in water at infinite dilution to the liquid state ($\Delta C°_p/\Delta A_{np} = -1.05 \pm 0.13$ J mol^{-1} K^{-1} Å^{-2}), processes which are dominated by hydrophobic interactions. The theoretical treatment below assumes only that there is an attractive interaction between the bacterial polymers and the PI headgroup. The model does not require the nature of this interaction to be precisely specified.

2. Theory

The theory is based on a three-dimensional lattice model resembling that of the Flory–Huggins theory of polymer solutions [12] for the bacterium glycocalyx and a two-dimensional lattice for the liposome surface. A similar approach was used in considering the role of hydrogen bonding in cellular cohesion [13]. We assume that the lattice site volume (V_s) is the volume of a monosaccharide residue and that the inositol headgroup on the liposome surface also approximates to this volume. Several studies have shown that the glucose residues of alkyl glycosides and other glycolipids adopt minimum energy conformations in which the glucose residues protrude out of the bilayer surface [14–16]. We assume that the inositol headgroup al-

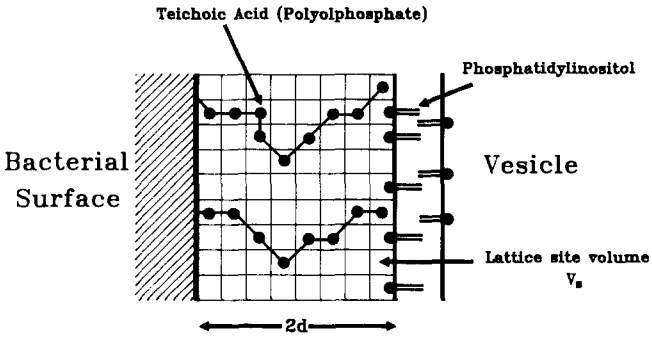

Fig. 3. Lattice model of bacterium–liposome bilayer interaction.

though not a sugar would resemble glucose in this respect. Fig. 3 shows a diagram of the lattice model in which we assume the bacterial and liposome walls are plane parallel in the region of interaction.

Considering the bacterium surface to have a polymer layer of thickness l and the total number of lattice sites per unit area to be N, then

$$N(\text{sites per unit area}) = \frac{l}{V_s} \tag{1}$$

If the number of sites occupied by surface polymer is n_s, then the fraction of sites occupied, x_s, will be given by

$$x_s = \frac{n_s}{N} = \frac{n_s V_s}{l} \tag{2}$$

If the lattice coordination number is Z, then the number of segment–segment interactions (n_{ss}), assuming random mixing between vacant and occupied sites, may be written

$$n_{ss} = x_s[x_s N(Z-2) + 1] \tag{3}$$

The 2 arises because any chain segment, apart from the terminal one, will have sites on either side of it occupied. If N is large, such that $N(Z-2) \gg 1$, then from Eqs. (3), (2) and (1).

$$n_{ss} = n_s^2 \frac{V_s^2}{l^2} N(Z-2) = n_s^2 \frac{V_s}{l}(Z-2) \tag{4}$$

Considering now the two-dimensional lattice representing the liposomal surface.

If the lattice has a thickness l' (the thickness of an inositol headgroup), the total number of lattice sites, N', will be given by

$$N' = \frac{l'}{V_s} \tag{5}$$

and if there are n'_s occupied sites per unit area, the fraction of sites occupied, x'_s, will be given by

$$x'_s = \frac{n'_s}{N'} = \frac{n'_s V_s}{l'} \tag{6}$$

The number of headgroup interactions (n_{hh}) in the surface lattice will then be

$$n_{hh} = x'_s[4N'x'_s] \tag{7}$$

where it has been assumed that each headgroup has four nearest neighbours. From Eqs. (7), (6) and (5) it follows that

$$n_{hh} = (x'_s)^2 4N' = (n'_s)^2 \frac{4V_s}{l'} \tag{8}$$

We consider now the situation when the bacterium and vesicle surfaces are brought into contact at a separation $2d$. The total number of sites per unit area between the surfaces, N_{2d}, will be given by

$$N_{2d} = \frac{2d}{V_s} \tag{9}$$

and the fraction of sites occupied x_s^i by

$$x_s^i = \left(\frac{n_s + n'_s}{2d}\right) V_s \tag{10}$$

If we assume that the lattice coordination number $Z = 6$, then n'_{ss} and n'_{hh} from Eq. (3), with $N(Z-2) \gg 1$ and Eq. (7) are given by exactly analogous expressions. If we assume that vacant and occupied sites randomly mix then the total number of site–site interactions, n_{ss}^t, irrespective of whether the site is occupied by a polymer segment or a headgroup may be written

$$n_{ss}^t = x_s^i[4N_{2d}x_s^i] \tag{11}$$

Substituting from Eqs. (10) and (9)

$$n_{ss}^t = \frac{(n_s + n'_s)^2}{d} V_s \tag{12}$$

The only site–site interactions which will contribute to an attractive interaction are those between a polymer segment and a head group. The number of these interacting sites will be given by n_{ss}^t minus the 'self' interactions on the bacterium and liposome surfaces given by Eqs. (4) and (8), respectively. Defining these site–site interactions as n_a, it follows

$$n_a = n_{ss}^t - n_{ss} - n_{hh} \tag{13}$$

$$n_a = 2V_s \frac{(n_s + n'_s)^2}{d} - 4V_s\left(\frac{n_s^2}{l} + \frac{(n'_s)^2}{l'}\right) \tag{14}$$

It is implicit in Eq. (14) that the self interactions are independent of the separation between the surfaces ($2d$). This assumption is reasonable for the liposomal surface but less so for the bacterium surface, particularly when $2d \ll l$. If there is an interaction energy E, between polymer segments and headgroups, then the number of such interactions, N_a, will be given by Eq. (14) times the Boltzmann factor $e^{-E/kT}$, thus

$$N_a = n_a e^{-E/kT} \tag{15}$$

The attractive energy of interaction per unit area, V_a', will be given by $N_a E$, hence

$$\therefore V_a' = \left[\frac{(n_s + n_s')^2}{d} - 2\left(\frac{n_s^2}{l} + \frac{n_s'^2}{l'}\right)\right] 2V_s E \, e^{-E/kT} \tag{16}$$

provided that $2d \ll l$. The dependence of self interactions between surface polymer segments on separation can be taken into account by putting $l = 2d$ in Eq. (16) which after manipulation gives the more general expression

$$V_a = \left[\frac{2n_s n_s'}{d} + n_s'^2\left(\frac{l' - 2d}{dl'}\right)\right] 2V_s E \, e^{-E/kT} \tag{17}$$

The other forces acting between the bacteria and liposomes will be those of the classical Derjaguin–Landau–Verwey–Overbeek (DLVO) theory [17,18]. These are the double layer repulsion and dispersion force attraction. The double layer repulsion for two surfaces of differing surface potentials has been considered by a number of authors [19,20,21]. We have used the following equation to calculate the repulsive energy of interaction (V_R) of parallel plates at a separation $2d$ [20].

$$V_R = \frac{2nkT}{\kappa}\left[2\bar{y} \ln\left(\frac{B + \bar{y} \coth(\kappa d/2)}{1 + \bar{y}}\right) - \ln(\bar{y}^2 + \cosh \kappa d + B \sinh \kappa d) + \kappa d\right] \tag{18}$$

where $\bar{y} = (y_1 + y_2)/2$; $y_1 = z \cdot e \cdot \psi_1/kT$; $y_2 = z \cdot e \cdot \psi_2/kT$

$$B = \left[1 + \bar{y}^2 \cdot \operatorname{csch}^2 \cdot (\kappa d/2)\right]^{1/2}$$

The surface potentials of the bacterium and vesicles are ψ_1 and ψ_2 and κ is the reciprocal Debye length given by $\kappa = 2e^2 n z^2/\epsilon_0 \epsilon_r kT$, where n is the electrolyte counter-ion (of charge z) concentration in ions per m^3, e the electronic charge and ϵ_0 and ϵ_r the permittivity of vacuum and the medium, respectively. A flat plate model has been used consistent with our model (Fig. 3). An analytical expression for the energy of interaction of two-spheres of differing radii is not in fact available because the appropriate integration of Eq. (18) cannot be done [20].

The dispersion force interaction (V_D) between two thick plates (thickness $\gg$ separation ($2d$)) is given by [22]

$$V_D = -\frac{A}{48\pi d^2} \tag{19}$$

where A is the London–Hamaker constant.

The total energy of interaction per unit area of surface (V_T) is thus given by

$$V_T = V_R - V_a - V_D \tag{20}$$

in writing Eq. (20) we have neglected contributions from the undulation force [23] and the hydration force which is only of importance at very small separations [24].

3. Parameterisation

The parameters required to apply Eq. (17) are n_s, n_s', l', V_s and E. From an analysis of the glucose content of the glycocalyx (extracellular slime) of a bacterium such as *Staphylococcus epidermidis* the number of glucose residues associated with the teichoic acid per unit area of the cell surface can be estimated to be of the order of 10^{18} m^{-2} [25,26]. We have taken this as the approximate value of n_s. The surface density of PI headgroups in the liposome surface (n_s') was calculated from the PI content (mol%) assuming that each phospholipid molecule occupies an area of $50 \cdot 10^{-20}$ m^2 [27]. For example for a liposome containing 5 mol% PI the number of PI headgroups per unit area of surface will be $0.05/50 \cdot 10^{-20} = 10^{17}$ m^{-2}. The lattice site volume (V_s) was taken to be that of a hexose unit ($180 \cdot 10^{-30}$ m^3 [13]) and the thickness of the headgroup layer on the surface of the liposome (l') as $V_s^{1/3}$, i.e., $5.6 \cdot 10^{-10}$ m. The energy E, was varied over a wide range from 0.1 to 25 kJ mol^{-1}. If interaction between a monosaccharide residue (M) and an inositol headgroup (I) occurred by hydrogen bonding, since both residues would be expected to be hydrogen bonded in an aqueous environment, then there would probably be no change in the overall number of hydrogen bonds, i.e.,

$$M \cdot H_2O + I \cdot H_2O \rightleftharpoons M \cdot I + H_2O \cdot H_2O \tag{21}$$

The value of the energy difference (E) would depend on the relative strength of the hydrogen bonds between proton donors and acceptors [28] which will be considerably less than the energy of formation of a single hydrogen bond ($\sim$ 25 kJ mol^{-1}).

The parameters required for the calculation of the energy of double layer repulsion (V_R) are the surface potentials of the bacterium and liposome. The zeta potential is usually taken to approximate to the surface potential. Zeta potentials determined by microelectrophoresis have been reported for a number of bacteria [29–32] including strains of the oral bacteria *Streptococcus sanguis*, *mutans* [31] and *salivarius* [30] in low ionic strength buffers. We have made measurements of zeta potentials at higher ionic strength appropriate to the conditions used in the targeting of liposomes to several strains of bacteria. Microelectrophoretic measurements were made using isotonic phosphate buffered salt (PBS) solutions at pH 7.4 with composition, [NaCl] = 160 mM, [KCl] = 3 mM, [Na_2HPO_4] = 8 mM and [KH_2PO_4] = 1 mM, so that the total counterion concentration was 180 mM (giving $n = 1.08 \cdot 10^{26}$

counterions m^{-3} in the expression for κ above). In this medium the zeta potentials of *Staphylococcus epidermidis*, *Streptococcus sanguis* and *Proteus vulgaris* were in the range −32 to −24 mV.

The surface potentials of the liposomes were calculated from the PI surface density (σ), assuming that each PI headgroup carried a single negative charge, from Eq. (22) (see Ref. [18])

$$\psi = \frac{2kT}{e}\sinh^{-1}\left(\frac{\sigma}{(8kTN\epsilon_0\epsilon_r)^{1/2}}\right) \quad (22)$$

where $\sigma = n_s' \cdot e$.

The London–Hamaker constant (A) in Eq. (19) for cells range from $0.2 \cdot 10^{-20}$ J to $3 \cdot 10^{-25}$ J [33]. We have used values in the range 10^{-20}–10^{-24} J.

V_R, V_a and V_{D_o} were calculated for values of $2d$ up to 50 nm (500 Å). Electron microscopic studies on *Staphylococcus epidermidis* and *Streptococcus salivarius* have shown that the thickness of the glycocalyx range up to approximately 18 nm depending on the particular strain [34–36]. The significant range of interaction between a bacterium and a liposome might be expected to be in a range of $2d$ up to approximately 18 nm.

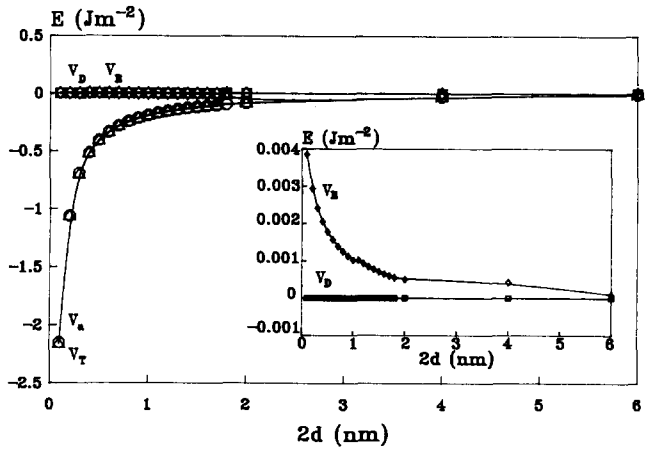

Fig. 4. Contributions to the total potential energy of interaction (V_T) between a bacterial and a liposomal surface as a function of separation ($2d$) at 298 K. In the calculation of the double layer repulsive energy (V_R) the surface potentials of the bacterium and liposome were −32 mV and −24 mV, respectively. The latter corresponds to a DPPC-PI liposome containing 7.5 mol% PI. The London–Hamaker constant used to calculate V_D was 10^{-24} J. The interaction energy (E) used in Eq. (17) for V_a was 10 kJ mol^{-1} and n_s was taken as 10^{18} m^{-2}. The other parameters were as described in the text. The inset shows V_R and V_D vs. $2d$ with the scale on the ordinate enlarged.

4. Results and discussion

We first consider the magnitude of the attractive energy of interaction (V_a) given by Eq. 17 in comparison with the electrical double layer repulsion (V_R) (Eq. 18) and attractive dispersion interactions (V_D) (Eq. 19). Fig. 4 shows the magnitudes of these contributions as a function of separation ($2d$), in aqueous solution at physiological ionic strength (total counterion concentration 180 mM at 25°C). In calculating V_R the surface potential of the bacterium was taken as −32 mV (the high end of the measured range) and the surface potential of the liposomes, calculated from Eq. (22), for a bilayer composition of 7.5 mol% PI in DPPC, was −24 mV. A London–Hamaker constant of 10^{-24} J was used in calculating V_D and an interaction energy (E) of 10 kJ mol^{-1} was used in calculating V_a. The other parameters were as described above.

It is seen that for these conditions, V_a is the dominating contribution to the net energy of interaction. An increase in the PI content of the liposomes will increase their surface potential and hence V_R with little change in V_D. Thus while classical DLVO theory might account for some decrease in interaction of liposomes on increasing their PI content this effect on V_R is

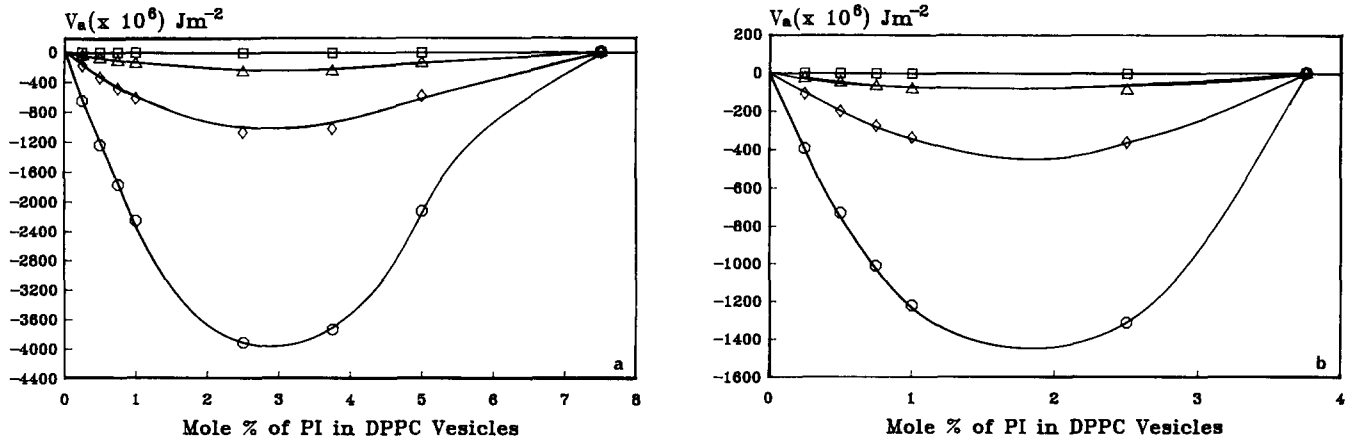

Fig. 5. The dependence of the potential energy of interaction (V_a) on the mol% PI for interaction of DPPC-PI liposomes with a bacterium surface ($n_s = 10^{18}$ m^{-2}) for various values of the interaction energy E: □, 1 kJ mol^{-1}; △, 5 kJ mol^{-1}; ◇, 7.5 kJ mol^{-1}; ○, 10 kJ mol^{-1}; for separations of 10 nm (a) and 16 nm (b).

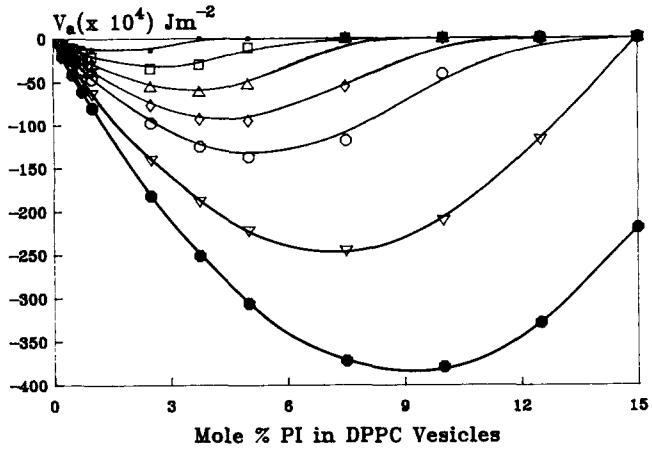

Fig. 6. The dependence of the potential energy of interaction (V_a) on mol% PI for interaction of DPPC-PI liposomes with a bacterium surface for various values of n_s. The bacterium–liposome separation was 16 nm and the interaction energy E was 10 kJ mol^{-1}. The values of n_s were as follows: ●, $1 \cdot 10^{18}$ m^{-2}; □, $1.5 \cdot 10^{18}$ m^{-2}; △, $2 \cdot 10^{18}$ m^{-2}; ◇, $2.5 \cdot 10^{18}$ m^{-2}; ○, $3 \cdot 10^{18}$ m^{-2}; ▽, $4 \cdot 10^{18}$ m^{-2}; ●, $5 \cdot 10^{18}$ m^{-2}.

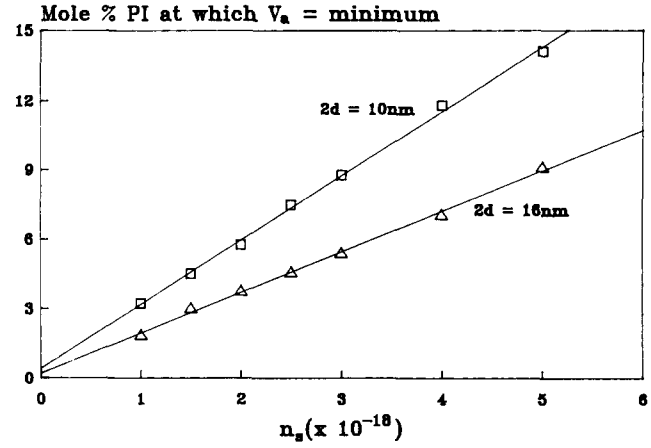

Fig. 7. The dependence of the mol% PI at the minimum in the V_a vs mol% PI plots (Fig. 6) as a function of n_s at two bacterium-liposome separations 10 nm and 16 nm.

negligible in comparison with V_a and it could not give rise to the optimum adsorption effects as depicted in Fig. 1. In classical terms if liposomes of high surface charge and surface potential do not adsorb due to double layer repulsion then liposomes of very low PI content should adsorb. As Fig. 1 shows this does not occur. We will henceforth assume that the observed effects arise predominantly from the interactions as given by Eq. (17).

The variation of V_a with PI content of the liposomes for various values of E and separations ($2d$) of 10 and 16 nm are shown in Fig. 5a and b, respectively. As Fig. 5 shows, plots of V_a pass through minima as a function of liposome PI content. The depth of the minima depend on the value of E and also the separation ($2d$) between the bacterium and liposome surfaces. Qualitatively the curves show that interaction is weaker for liposomes with low and high PI content and optimum PI levels for adsorption should occur as experimentally observed (Fig. 1). Quantitatively adsorption will depend on the magnitude of V_a/kT. This is not easy to estimate in that the contact area between liposome and bacterium is not precisely known nor is the separation ($2d$). At a separation of 16 nm (approx. 2 nm into the glycolalyx of a bacterium such as *Staphylococcus epidermidis*) for an interaction energy (E) of 10 kJ mol^{-1} the minimum value of V_a is ~ -0.0014 J m^{-2}. Assuming a bacterium contact area with a 100 nm diameter liposome of between $1/50^{th}$ and $1/100^{th}$ the surface area of the liposome leads to a range of V_a/kT between 214 and 107. Clearly this would give rise to very strong adsorption. Should E be smaller and/or the equilibrium separation be larger then V_a/kT at the optimum PI level for adsorption would be reduced.

For a given interaction energy (E), the position of the minima in the V_a plots, with respect to the liposome composition, is dependent on the surface concentration of sugar residues (n_s). Fig. 6 shows the relationship between V_a and mol% PI in DPPC liposomes for a range of values of n_s. As n_s increases the minimum values of V_a increase negatively and the position of the minima move to higher mol% PI. The dependence of V_a, at the minima, on n_s is a linear function of n_s at a constant separation ($2d$) (Fig. 7). Assuming a bacterium-liposome separation of 16 nm the experimental optima mol% PI levels for adsorption may be used to estimate values of n_s for a particular bacterium as shown in Table 1. These estimates suggest that rela-

Table 1
Targeting of PI-containing liposomes to bacteria

Bacterium-vesicle system [a]	d_m (nm)	Optimum mol% PI for adsorption	n_s (number of interaction sites m^{-2}) [b]
Streptococcus mutans strain NCTC 10449 (DPPC/PI) VETs	109 ± 5	8.8	$4.9 \cdot 10^{18}$
Streptococcus mutans strain D282 (DPPC/PI) VETs	105 ± 9	8.8	$4.9 \cdot 10^{18}$
Streptococcus sanguis strain CR2b [c] (DPPC/PI) REVs	191 ± 56	17.1	$9.6 \cdot 10^{18}$
Staphylococcus epidermidis strain NCTC 11047 (DPPC/PI) VETs	79.8 ± 6.7	11.4	$6.4 \cdot 10^{18}$

[a] Data from Ref. (4).
[b] Calculated assuming a bacterium-liposome separation of 16 nm.
[c] Since 1990 this strain has been renamed as *Streptococcus gordonii*.

tively small changes in the surface density of sugar residues on a bacterial strain can markedly change their adsorption characteristics for PI-containing liposomes.

5. Conclusions

The simple lattice theory described predicts semi-quantitatively the existence of minima in the potential energies of interaction between a bacterium and PI-containing liposomes as a function of their PI content. The existence of such potential energy minima predict optimum levels of PI for adsorption as found experimentally. The minima arise from a balance between the 'cross-interactions' between the residues (sugars) in the teichoic acid chains in the bacterium glycocalyx and the inositol headgroups of PI and the 'self-interactions' between the teichoic acid chains and the PI headgroups in the surfaces of the bacteria and liposomes, respectively. The 'cross' and 'self' interactions are represented by the first and second terms in the derived equations (Eqs. (16) and (17)). A relatively small energy of interaction (E), much less than the formation of a single hydrogen bond, can give rise to an attractive potential energy of interaction resulting in an optimum liposome composition for adsorption. The resulting attractive energy is significantly greater than the energies of the classical double layer repulsion and attractive dispersion interactions for bacteria and liposomes under physiological conditions. The concordance between the theoretical predictions and the experimental observations supports the proposed model for bacterium–liposome interaction.

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