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Fluidity-dependence of membrane adhesiveness can be explained by thermotropic shifts in surface potential

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It is demonstrated that the transition of both dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) from the gel to liquid-crystalline phase is paralleled by a pronounced increase in the negative surface potential of liposomes composed of either lipid. The Derjaguin-Landau-Verwey-Overbeek (DLVO) theory is applied to show that this phenomenon can serve as a simple explanation of diverse adhesive properties of solid and fluid lipid bilayers.

There is increasing evidence that most of the cell functions are mediated by cell-to-cell contacts, cell adhesion to extracellular matrix components and artificial substrata [1–10]. It is important therefore to examine the mechanisms underlying cell-to-substratum adhesion. Margolis and coworkers [11–13] demonstrated that different types of cell that adhere to and spread on glass surface coated with solid lipids fail to do so when the lipid is in fluid state. Moreover, the adhesiveness of DMPC and/or DPPC liposomes to Chinese hamster fibroblasts markedly decreases at the solid-to-fluid phase transition of the lipid [14]. Similarly, solid liposomes associate with several cell types much stronger than fluid liposomes [15]. Furthermore, lipid hydrocarbon chain melting is accompanied by a progressive increase in the equilibrium distance between phospholipid lamellae [16–18]. It is noticeable also that cell membrane fluidization by altering their lipid composition renders them nonadhesive [19–24] whereas membrane rigidification exerts the opposite effect [19–21,25]. These findings indicate that the adhesiveness between two membranes, cellular or artificial, is suppressed by enhanced fluidity of at least one of them. The physicochemical bases for striking difference in adhesiveness of solid and fluid lipid bilayers still remain obscure.

Experimental data and theoretical calculations are presented here to explain high adhesiveness of solid lipid layers and restricted adhesiveness of fluid lipid films.

Methods of preparation of multilamellar liposomes and measurement of their electrophoretic mobility are described elsewhere [26].

Curves of Fig. 1 exhibit abrupt changes in electrophoretic mobility of DMPC liposomes at the temperature of the main gel to liquid-crystalline phase transition of DMPC, $T_m = 23.6 \pm 0.3$°C [27–29]. Under certain conditions, the lipid phase transition results in the reverse of the sign of liposome mobility. As shown in Fig. 2, the transition of DMPC from the gel to liquid-crystalline phase in the presence of $CaCl_2$ in bathing solutions is followed by drastic increase in negative (or decrease in positive) surface potential of lipo-

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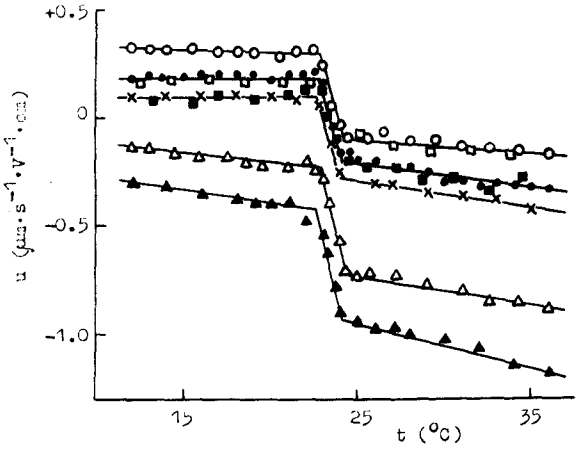

Fig. 1. Temperature dependence of the electrophoretic mobility of DMPC multilamellar liposomes in aqueous solutions containing 5 mM Tris-HCl (pH 7.2) and 10 mM NaCl (○), KCl (●), KNO_3 (×), NaSCN (△), KSCN (▲), or 100 mM NaCl (□) or KCl (■).

somes (at 20 ° C the value of $u = 1\ \mu m \cdot s^{-1} \cdot V^{-1} \cdot cm$ corresponds to the surface potential of 14 mV). Similar shifts in surface potential are detected for DPPC liposomes at 41.5 ± 0.3 ° C, corresponding to the main gel to liquid-crystalline phase transition temperature of this lipid [27–29] (Fig. 3). In this case, surface potential alterations take place also at the 'pretransition' temperature, T_p = 34.8 ± 0.5 ° C [27–29]. Besides, in the case of DPPC liposomes sharp reduction and subsequent restoration of positive surface potential is observed at about 50 ° C (Fig. 3). The latter phenomenon confirms our earlier finding that DPPC planar bilayers undergo thermotropic transformations in the range of 48 to 51 ° C [30]. The enhancement of T_m of DMPC and DPPC with increasing $CaCl_2$ concentration (Figs. 2,3) is evidently brought about by the formation of intermolecular Ca^{2+} 'bridges' and stabilization of the bilayer structure. Interestingly, the increase in Ca^{2+} concentration smooths over the DPPC pretransition and the latter is completely vanished at 500 mM $CaCl_2$. Temperature-dependence of the mobility beyond the temperature ranges of phase transitions can be ascribed to the changes in the medium viscosity.

Fig. 1 demonstrates that at a given temperature the magnitude of liposome surface potential depends on the ionic species present. In the presence of certain ions surface potential varies with their

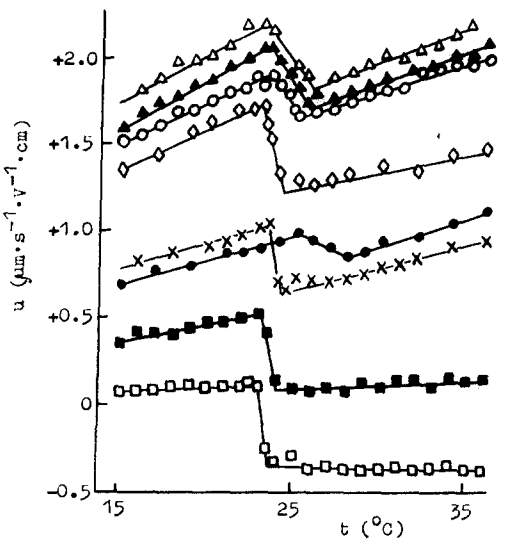

Fig. 2. Temperature dependence of the electrophoretic mobility of DMPC multilamellar liposomes in aqueous solutions containing 2 mM Tris-HCl (pH 7.2) and $CaCl_2$ in the following concentrations (mM): 0.1 (□), 0.3 (■), 1 (×), 4 (◇), 20 (△), 50 (▲), 100 (○), and 500 (●).

concentrations (Figs. 1–3). These facts lead to the conclusion that the surface potential of liposomes consisting of zwitterionic, net-neutral lipids, presumably arises from the selective adsorption of ions to their surface. Initial rise and further di-

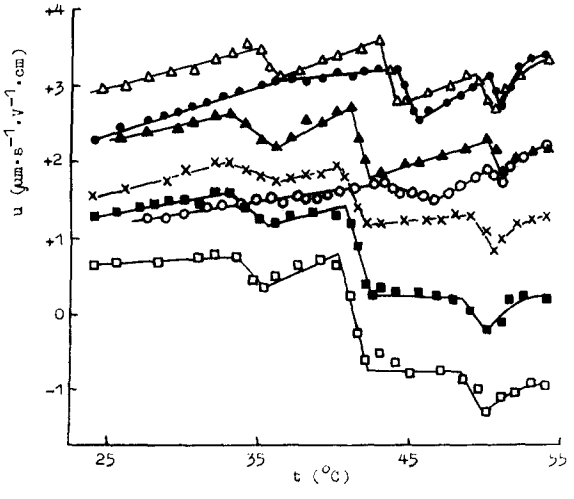

Fig. 3. Temperature dependence of the electrophoretic mobility of DPPC multilamellar liposomes in aqueous solutions containing 2 mM Tris-HCl (pH 7.2) and $CaCl_2$ in the following concentrations (mM): 0.1 (□), 0.3 (■), 1 (×), 3(▲), 30 (△), 100 (●), and 500 (○).

minution of the positive surface potential of liposomes as $CaCl_2$ concentration increases (Figs. 2, 3) is readily explained by the binding of Ca^{2+} ions to liposomes and origination of positive surface potential, and the screening of surface potential at higher $CaCl_2$ concentrations, when the adsorption approaches saturation. It is reasonable therefore to suggest that thermotropic shifts in the liposome surface potential are induced by desorption of cations or additional adsorption of anions at the lipid transition from the gel to liquid crystalline state. In fact, data of Figs. 2 and 3 allow the determination of the following values for Ca^{2+} binding constant to liposome membranes: $K = 392$ M^{-1} at $t < T_m$ and 256 M^{-1} at $t > T_m$ for DMPC and $K = 440$ M^{-1} at $t < T_m$ and 190 M^{-1} at $t > T_m$ for DPPC.

The question arises as to whether enhanced adhesiveness of solid lipid layers and low adhesiveness of fluid lipid films can be interpreted by thermal alterations of lipid bilayer surface potential detected in this work. To ascertain this question we invoke the DLVO theory [31,32], which regards the total energy of interaction between charged particles, V_T, as the sum of electrostatic (V_R) and van der Waals (V_A) interaction energies. At distances between a cell and a flat surface, $d < 10$ nm, the cell membrane at the contact region can be considered as a flat plate. Therefore we use equations describing from interactions between two plates [32,33]

$$V_A = -\frac{H}{12\pi}[d^{-2} + (d+2\delta)^{-2} - 2(d+\delta)^{-2}]$$

$$V_R = \frac{\epsilon\epsilon_0\varkappa}{2}\left[\frac{2\psi_1\psi_2}{\mathrm{sh}\varkappa d} + (\psi_1^2 + \psi_2^2)(\mathrm{cth}\varkappa d - 1)\right]$$

where H is the Hamaker constant of interacting plates, δ is the thickness of the plates, d is the distance between the plates, ϵ is the dielectric constant of the medium, ϵ_0 is the permittivity of free space, $\varkappa$ is the Debye-Hückel parameter, ψ_1 and ψ_2 are electrostatic surface potentials of separated plates.

Depicted in Fig. 4 are curves of the dependence of $V_T = V_R + V_A$ on d. Considering the interaction of cell membrane with a lipid bilayer leaflet a value of $\delta = 10$ nm is reasonable to choose. For the Hamaker constant we use values of $5 \cdot 10^{-22}$ J (Fig. 4A), 10^{-21} J (Fig. 4B), and $2 \cdot 10^{-21}$ J (Fig. 4C). Values of Hamaker constant varying in this range have been established for sheep polymor-

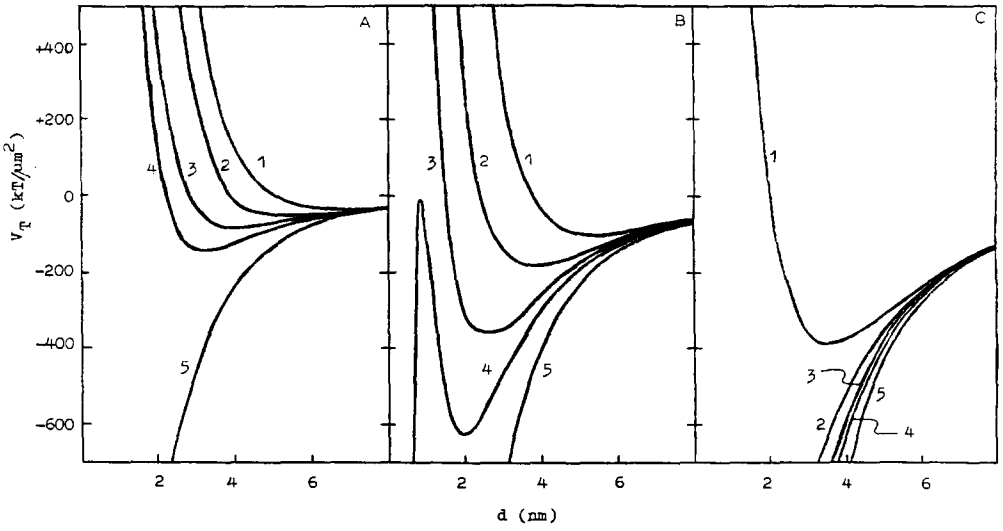

Fig. 4. Theoretical curves of the dependence of the interaction energy of a cell with the substratum, $V_T = V_R + V_A$, on the distance between them, d, in the 100 mM NaCl solution at 37 °C ($\varkappa = 1.05$ nm^{-1}, $\epsilon = 74$, $\epsilon_0 = 8.85 \cdot 10^{-12}$ F/m, $\delta = 10$ nm, $\psi_2 = -15$ mV). The curves 1, 2, 3, 4, and 5 correspond to $\psi_1 = -4$, -2, -1, -0.5, and $+1$ mV, respectively. The subscripts 1 and 2 correspond to the substratum and the cell, respectively. Hamaker contants of $H = 5 \cdot 10^{-22}$ J (A), $H = 1 \cdot 10^{-21}$ J (B), and $H = 2 \cdot 10^{-21}$ J (C) are used. Positive values of V_T correspond to the repulsion and vice versa. Parameters k and T are the Boltzmann constant and the absolute temperature, respectively.

phonuclear leucocytes [34], arachidic acid sols [35], and phospholipid bilayers [17,18]. Evidently, secondary minima deeper than 100 $kT/\mu m^2$ are sufficient for holding a cell attached to the substratum. Hence, lipid layers possessing surface potential (ψ_1) more negative than -2 mV are predicted to be nonadhesive for a cell (the surface potential of the cell is taken as -15 mV in 100 mM NaCl) provided that $H = 5 \cdot 10^{-22}$ J (Fig. 4A). The lowering of ψ_1 to -1 and then to -0.5 mV will provide the possibility for adhesion at the secondary minimum. When $H = 10^{-21}$ J, a decrease in ψ_1 from -4 to -0.5 mV is expected to result in closer and stronger secondary minimum adhesion (Fig. 4B). The enhancement of H up to $2 \cdot 10^{-21}$ J will cause the cell to stick tightly to the substratum (primary minimum adhesion) at ψ_1 values more positive than -2 mV (Fig. 4C). A stable secondary minimum adhesion is predicted in this case for $\psi_1 = -4$ mV. At $\psi_1 \geq +1$ mV the substratum is highly adhesive irrespective of the H value.

So it becomes clear that shifts in ψ_1 from $+2 \pm 1$ to -2 ± 1 mV, which occur at the lipid phase transition from the gel to liquid-crystalline state in the presence of 10–100 mM NaCl or KCl (Fig. 1) will strongly suppress the lipid layer-to-cell adhesiveness. Similar modulations are expected also in the presence of near-physiological concentrations (0.1–0.3 mM) of $CaCl_2$ (Figs. 2, 3). At higher $CaCl_2$ concentrations, when $\psi_1 > +5$ mV (Figs. 2, 3), the substratum is predicted to be highly adhesive (Fig. 4).

The influence of surface potential on interactions between both cellular and artificial membranes has been established theoretically and experimentally [17,18,36–44]. Maroudas [45], however, claimed that adhesive properties of lipid layers can hardly be explained from the electrostatic standpoint since optimum adhesion and spreading of BHK cells to polystyrene dishes was found at strongly negative surface charge densities (2–10 elementary charges per 1 nm^2) of the substratum [46]. The method implicated by Maroudas [46] to estimate charge density (binding of a cationic dye) was shown by Gingell and Vince [47] to be unreliable, so that the statement of Maroudas [45] against the role of electrostatic effects in cell-to-substratum adhesion is to be recognized as groundless.

Thus, as suggest our results, high adhesiveness of solid lipid bilayers, used as liposomes or flat substrata, and impaired adhesiveness of fluid lipid films [11–15] can be simply explained by an increase in the negative surface potential of the lipid layer at the solid-to-fluid phase transition, resulting in the enhancement of electrostatic repulsion between the lipid leaflet and negatively charged cell membrane.

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