BRIEF COMMUNICATION

FREE ENERGY POTENTIAL FOR AGGREGATION OF GIANT, NEUTRAL LIPID BILAYER VESICLES BY VAN DER WAALS ATTRACTION

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ABSTRACT Here, we report the first direct observation of Van der Waals' attraction between biomembrane capsules using measurements of the free energy reduction per unit area of membrane-membrane contact formation. In these studies, the membrane capsules were reconstituted neutral (egg phosphatidylcholine) lipid bilayers of giant ($>10^{-3}$ cm diam) vesicles. Micromanipulation methods were used to select and maneuver two vesicles into proximity for contact; after adhesion was allowed to occur, the extent of contact formation was regulated through the vesicle membrane tensions that were controlled by micropipette suction. The free energy reduction per unit area of contact formation was proportional to the membrane tension multiplied by a simple function of the pipette and vesicle dimensions. The free energy potential for Van der Waals attraction between the neutral bilayers in 120 mM NaCl solutions was 1.5×10^{-2} ergs/cm^2. Also, when human serum albumin was added to the medium in the range of 0–1 mg/ml, the free energy potential for bilayer-bilayer adhesion was not affected. Using published values for equilibrium spacing between lipid bilayers in multilamellar lipid-water dispersions and the theoretical equation for van der Waals attraction between continuous dielectric layers, we calculated the value for the Hamaker coefficient of the Van der Waals attraction to be 5.8×10^{-14} ergs.

INTRODUCTION

Although the influence of long range forces in cell-cell adhesion and aggregation processes has been discussed and analyzed theoretically for over a decade (Parsegian and Gingell, 1972; Nir and Andersen, 1977; Bongrand et al., 1982), how these electrodynamic forces contribute to the regulation of cell-cell contacts remains uncertain. Much of the uncertainty results from insufficient experimental quantitation of forces between biological membrane surfaces. Here, we report the first direct quantitation of Van der Waals attraction between biomembrane capsules using measurements of the free energy reduction per unit area of membrane contact formation for giant, neutral lecithin vesicles.

MATERIALS AND METHODS

Lipid vesicles were made from egg yolk lecithin, phosphatidylcholine (PC) that was dissolved in 10:1 chloroform-methanol. The solvent was evaporated in vacuo for 24 h, and then the lipid was rehydrated in a sucrose solution (200 mM). The resulting suspension was dialyzed against an osmotically equivalent sodium chloride solution to replace the external sugar with salt. Then a dilute suspension of these vesicles was injected into one side of a double microchamber on the microscope stage. Two vesicles were selected with a small suction micropipette and placed into a larger transfer pipette, which spanned the air gap between the two chambers on the microscope stage. The microscope stage was then positioned to leave both pipettes and the vesicles in the adjacent chamber. The second chamber contained a more concentrated sodium chloride buffer (110 mM NaCl) in order to slightly dehydrate the vesicles. The vesicles were slightly dehydrated to produce an initial state in which the membrane was tension free and the surface area was slightly larger than that for a sphere of equivalent volume. After the transfer, the lead vesicle in the transfer pipette was withdrawn by a small pipette and moved to a second small pipette that aspirated the vesicle with sufficient suction pressure to form a rigid spherical portion outside the pipette. Next, the second vesicle was withdrawn by the small micropipette and maneuvered close to the rigid vesicle surface as shown in Fig. 1. Finally, the second vesicle was allowed to adhere to the rigid vesicle surface in steps controlled by the aspiration pressure in the micropipette. The aspiration pressure was measured by a digital pressure transducer, which had a resolution of 10^{-6} atm. An example of an equilibrium configuration is shown in Fig. 1. Similarly, the vesicles were separated by stepwise increases in suction pressure in order to evaluate the reversibility of the adhesion process. Tension was induced in the initially flaccid vesicle membrane because of the adhesion to the rigid vesicle surface; this tension was measured directly by the suction pressure, ΔP, in the micropipette. The following relation approximates the tension T_m, in terms of ΔP:

$$T_m = \Delta P \cdot R_p/[2-(4 \cdot R_p/D_o)],$$

where R_p is the pipette inner radius and D_o is the outer cross sectional dimension of the vesicle. Results for a typical vesicle-vesicle adhesion and separation experiment are shown in Fig. 2, where the fractional coverage, x_c, of the rigid vesicle surface (i.e., the height of the adhesion zone normalized by the rigid vesicle diameter) is plotted against the reciprocal of the membrane tension, $1/T_m$, derived from the suction pressure

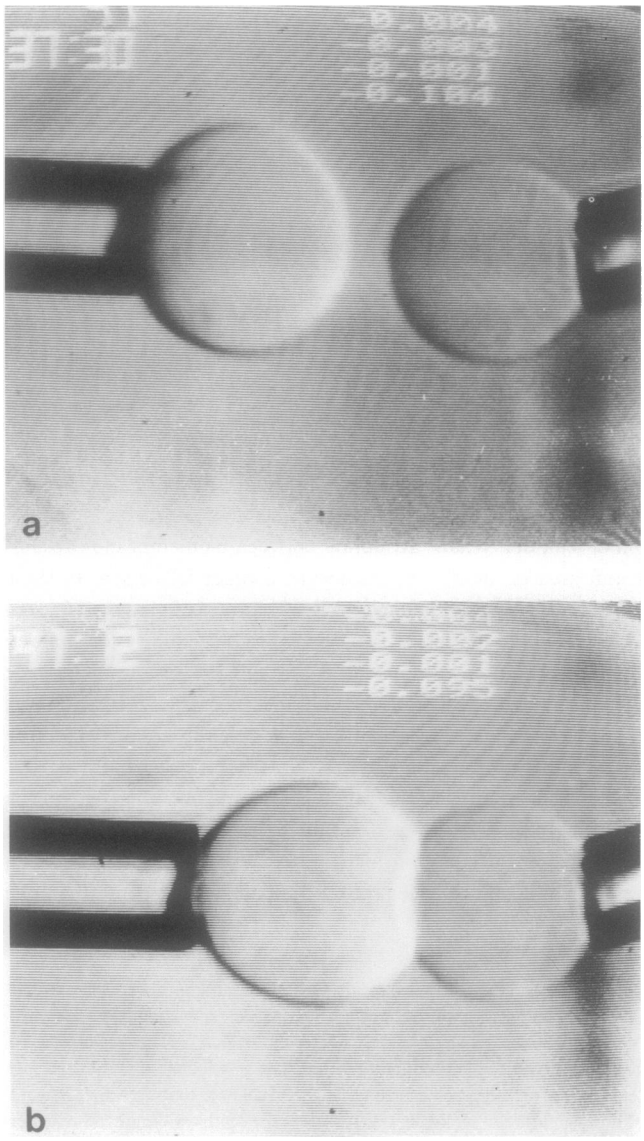

FIGURE 1 Video micrographs of controlled aggregation of two giant, neutral lecithin vesicles in 120 mM salt solution. (*a*) The vesicles with diameters on the order of 20 × 10^{-4} cm were first maneuvered into proximity for adhesion. The vesicle on the right was aspirated with sufficient suction pressure to form a rigid spherical test surface; the vesicle on the left was held with a low suction pressure that allowed formation of adhesive contact. (*b*) The left vesicle adhered spontaneously to the rigid vesicle surface and formed a stable equilibrium configuration as shown here, which was determined by the pipette suction pressure.

measurements. Note that an exact relation for tension in terms of suction pressure cannot be written analytically; the tension is derived from the analysis of vesicle geometry by numerical algorithm and from the observed pressure (see Evans and Metcalfe, 1984, for discussion). The maximum fractional coverage ranged from 0.3 to 0.5 in the tests.

RESULTS AND ANALYSIS

Because of the large size of the vesicles (>10^{-3} cm), the distances between surfaces are not discernible because of the limits of optical resolution. As such, the actions of the long-range forces are cumulated into an integral of force multiplied by displacement from a distance where the forces are negligible to the final position of stable contact. This integral is the work involved when the adhesive contact forms between locally flat surfaces, and is represented thermodynamically by a free energy reduction per unit area, γ. This free energy potential defines the chemical affinity for formation of membrane-membrane contact. In the experiment, mechanical equilibrium was established when small (virtual) decreases in free energy due to contact formation just balanced the work required to displace the pipette suction force. From this statement, it can be shown that the free energy potential for formation of a unit area of contact, γ, is proportional to the membrane tension, T_m, multiplied by a simple function of pipette and vesicle geometry, $f(geom)$, (Evans, 1980; Evans and Metcalfe, 1984), such that $\gamma = T_m \cdot f(geom)$. Hence, the tension induced in the vesicle membrane is a direct measure of the chemical affinity between the membrane surfaces. Because both pipettes were left at fixed positions throughout the adhesion process, the shape of the adherent vesicle was not an exact sphere, but was given by a surface of uniform total curvature. Consequently, the geometry was too complicated to permit a closed-form analytical solution for the geometric factor; but it can be obtained easily by numerical computation. An example in which the theoretical relation is correlated with the data for a particular vesicle-vesicle adhesion experiment is shown as the dashed curve in Fig. 2; the correlation is represented by a single value of the free energy potential per unit, area of contact formation of 1.5 × 10^{10-2} ergs/cm^2. It is clear by comparing the data for formation of contact (the triangles in Fig. 2) with that for separation of the contact (the stars in Fig. 2) that the adhesion process is

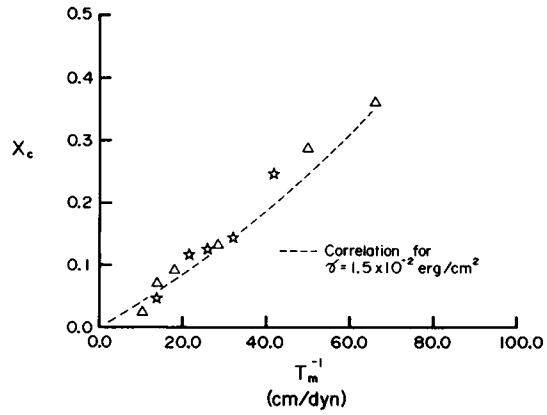

FIGURE 2 Data for a single vesicle-vesicle adhesion experiment. The fractional coverage X_c of the rigid vesicle is plotted vs. the reciprocal of the tension, T_m^{-1}, in the adherent vesicle membrane. The triangles are data for contact formation and the stars are data for contact separation. The dashed line is the correlation of the theoretical analysis with the observed data for a single value of the free energy reduction, γ, per unit area of contact formation.

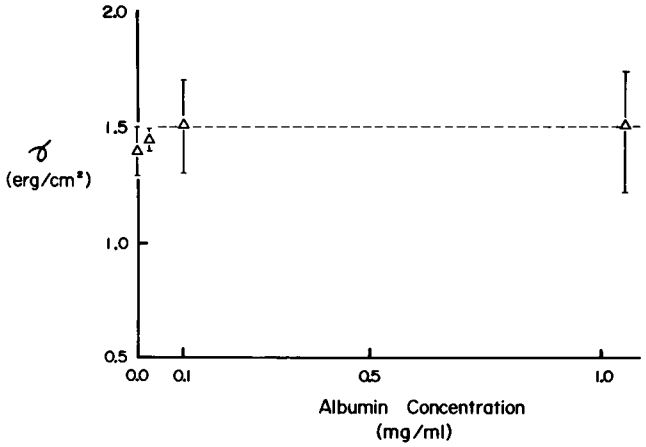

FIGURE 3 Values for the free energy reduction, γ, per unit area of contact formation between neutral lecithin membranes as a function of albumin concentration in solution.

reversible. The values found for the free energy potential for neutral vesicle aggregation in the salt buffer ranged from 1.4 to 1.5 $\times$ 10^{-2} ergs/cm^2 for 10 tests. In addition, incorporation of the negatively charged lipid, phosphatidylserine (PS), at a mole ratio of 1:10 PC was sufficient to prevent adhesion.

One significant difficulty in these experiments was that the neutral lecithin vesicles in the salt buffer were very fragile and ruptured easily. We found that the addition of a small amount of human serum albumin greatly reduced the vesicle fragility (probably because the glass pipettes became coated with albumin). We then tested the adhesion of neutral vesicles in the salt buffer with albumin in a concentration range of 0.01–1.0 mg/ml. The adhesion was unaffected by the presence of the albumin; the results for the free energy reduction per unit area of contact formation are plotted in Fig. 3 as a function of albumin concentration.

DISCUSSION

X-ray diffraction studies of the lamellar repeat spacing for lipid bilayers in multilamellar lipid-water dispersions have shown that the distance between bilayers is constant when the water content is in excess of ~40% by weight and that the mean water gap thickness is 27–28 $\times$ 10^{-8} cm for egg yolk lecithin (Parsegian et al., 1979). If we use the relation for the free energy of attraction per unit area between two semi-infinite layers of thickness, Z_1 (Hamaker, 1937; Parsegian et al., 1979), and bilayer separation distance, Z_w, with our measurements of the free energy reduction per unit area, γ, we can calculate the Hamaker coefficient, A_H, for Van der Waals attraction:

$$A_H = 12\pi \cdot Z_w^2 \cdot \gamma/\{1 - [2/(1 + t)^2] + [1/(1 + 2t)^2]\},$$

where $t = Z_1/Z_w$. If we use the data for bilayer thickness and separation distance obtained from x-ray diffraction studies, A_H is 5.8 $\times$ 10^{-14} ergs. Note that if the vesicular capsules were made of multilayers, only the outermost layer of each vesicle would contribute significantly to the Van der Waals attraction between vesicles. In this calculation, we have neglected the work associated with the repulsive hydration forces between the bilayers. This is a reasonable assumption based on the observations that these forces decay exponentially with a characteristic distance of 2 $\times$ 10^{-8} cm; also, calculations based on studies of dehydration of multilamellar lipid-water dispersions (Parsegian et al., 1979) show that the work per unit area of membrane surface required to overcome the repulsive hydration forces would only be ~1 $\times$ 10^{-3} ergs/cm^2 to reach a separation distance of 27 $\times$ 10^{-8} cm.

Our measurements of the free energy potential for Van der Waals attraction between neutral lecithin surfaces are in good agreement with theoretical predictions made many years ago (Parsegian and Gingell, 1972). Furthermore, these data agree well with the values deduced from x-ray diffraction studies of dehydration of multilamellar lipid-water dispersions (Parsegian et al., 1979). To derive values for the Hamaker coefficient from the dehydration studies, it is necessary to assume that the spatial decay of the strong (but short range) repulsive hydration force can be extrapolated to the final separation distance where the forces are very weak. The correlation between our measurements and the dehydration studies demonstrates that the form of the hydration force is maintained for large separations. Also, note that the Hamaker coefficient determined from free energies of thinning of "black" lipid films (Requena et al., 1977) is in good agreement with the value we have deduced from our free energy measurements. Finally, it is significant that low concentrations of the plasma protein, albumin, do not affect the free energy potential for Van der Waals attraction between the neutral lipid surfaces. Here, the indication is that there is little association of albumin with the lipid surface for the range of concentrations 0–1 mg/ml. Finally, even though the Van der Waals attraction appears to have little involvement in cell-cell adhesion because of the thick superficial layer of carbohydrates on cell surfaces (50–75 $\times$ 10^{-8} cm thick), the interaction may be important when lipid-encapsulated drugs adhere to cells and tissues, and thus may be important in the targeting of these particles.

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