From Macroscopic Adhesion Energy to Molecular Bonds: A Test of the Theory

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We present a statistical mechanical treatment relating the macroscopic adhesion energy of two surfaces, which can be obtained by micropipette aspiration studies, to the microscopic adhesion energy between individual bonds. The treatment deals with the case of weak reversible bonds, so that the equilibrium partition function has significance. This description is coherent with previous theories. Experiment and theory are compared to probe the nature of weak bonds in membranes, where local equilibria can be obtained. The case of a bead and a vesicle decorated by nucleosides was considered.

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Biological media provide a great variety of physicochemical phenomena through molecular recognition. Everything biological is accomplished with weak interactions and bonds. Strong interactions would stop the dynamical processes which are indispensable to life. The adhesion of surfaces exhibiting binding sites has been widely investigated theoretically [1–4]. Because of the diffusion process, the adhesion sites are attracted to the contact zone. A previous pioneering work [5] has indeed analyzed and confirmed the kinetics of the enrichment in the contact zone of two vesicles. In contrast, the experimental test of the theory was hindered by the lack of available weak binding sites with reversible adhesion. Recently designed lipid molecules and end-functionalized polymers now allow such experiments. The aim of this paper is to extend the equilibrium models and test them with these new molecular tools: the static adhesion energy was measured between a lipid vesicle and a polymer bead both functionalized with nucleosides that provide weak binding sites. The lipid vesicle can act as a reservoir of adhesion molecules which equilibrate with the contact zone while the adhesion groups on the bead are relatively immobile. Experiment and theory are compared to probe the nature of weak bonds in membranes, where local equilibria can be obtained. These are limiting physical behaviors, of general interest in biological and nonbiological applications.

The adhesion measurements are carried out by the micropipette aspiration technique developed by Evans [6]. Observations are made by differential interferometric contrast microscopy in order to see the vesicle (Fig. 1). To measure their adhesion, the osmotically controlled vesicle and the bead are aspirated in micropipettes and micromanipulated into contact. The (negative) pressure ΔP in the pipette controls the (positive) hydrostatic pressure in the vesicle and thus the mechanical tension τ_m in its membrane [5],

$$\tau_m = \frac{\Delta P}{2(\frac{1}{r_p} - \frac{1}{r_v})}, \tag{1}$$

where r_p and r_v are, respectively, the radius of the micropipette and of the vesicle. The adherent vesicle is held with low pressure and remains deformable. The adhesion free energy W_{adh} is obtained by determining the contact angle θ_c (Fig. 1) and the tension τ_m of the flaccid vesicle membrane,

$$W_{\mathrm{adh}} = \tau_m(1 - \cos\theta_c). \tag{2}$$

By combining (1) and (2), it is easy to relate ΔP to W_{adh},

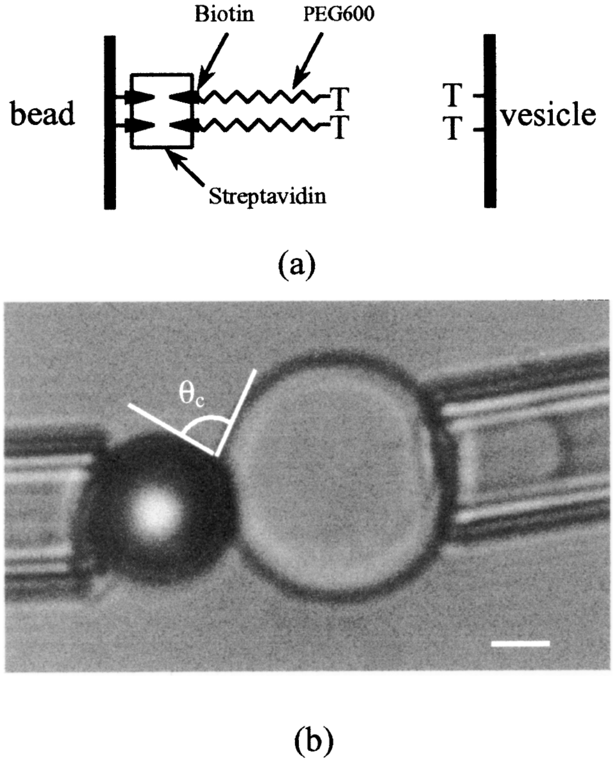

FIG. 1. (a) Functionalized bead with an end-grafted polyethylene glycol (PEG) bearing biotin at one end and one nucleoside at the other. (b) Micromanipulated vesicle in contact with a bead held by a micropipette. The bar represents 5 μm.

$$\Delta P = C \cdot W_{\mathrm{adh}}\,, \tag{3}$$

where C depends only on the geometry of the system,

$$C = \frac{2(\frac{1}{r_p} - \frac{1}{r_v})}{1 - \cos\theta_c}\,. \tag{4}$$

The measurement of θ_c was numerically deduced from geometrical parameters as indicated by Evans [7]. Following Eq. (3), the slope of ΔP as a function of C is W_{adh}.

Lipids carrying one nucleoside (adenosine, A, or thymidine, T) as a polar headgroup were synthesized [8]. Giant vesicles were formed by hydrating a lipid mixture stearoyl-oleoyl phosphocholine (SOPC)/nucleoside lipid (9/1) in 320 mOsm sucrose solution [9,10]. The vesicle suspension was added to an aqueous glucose solution chamber of a slightly higher osmolarity (360 mOsm) than that of the vesicle in order to deflate it and make it micromanipulable.

Superavidin coated polystyrene beads were purchased from Bangs Labs. The average area per attachment site for biotin was between 50 and 180 nm^2 [11]. In order to coat them with nucleosides, a polyethyleneglycol (PEG) spacer arm (containing 14 ethyleneoxide units) was dissymmetrically functionalized at one end with biotin, and at the other end with A or T (Fig. 1) [8]. The beads were incubated in water (50 μg/ml) with one of these heterobifunctionalized PEG arms (1 mg/ml) for one day. The contact angle was measured for several tension values of the flaccid vesicle membrane by decreasing the aspiration and then increasing it in order to check for the reversibility of the adhesion. This decreasing/increasing aspiration process was repeated several times on the same vesicle/bead couple, and it was indeed observed that this adhesion was reversible within the experimental error [12].

Four adhesion situations were investigated: bead A–vesicle A, bead T–vesicle T, bead T–vesicle A, and bead A–vesicle T. A control experiment was performed in which the vesicle was made of only SOPC and adhered to a T-coated bead. It gives the nonspecific contribution W_{nspe} to the adhesion.

Figure 2 shows the aspiration pressure as a function of C [cf. Eq. (4)] for these cases. According to Eq. (3), the slopes in Fig. 2 are equal to W_{adh}. The values of W_{adh} are independent of the vesicle size. The specific adhesion energies W_{spe} are given by $W_{\mathrm{spe}} = W_{\mathrm{adh}} - W_{\mathrm{nspe}}$ (cf. Table I).

Given the experimental error, the bead A-vesicle T and bead T-vesicle A systems have the same adhesion energy as expected. The specific adhesion energies follow the increasing order: $W_{\mathrm{spe}}(T/T) < W_{\mathrm{spe}}(A/A) < W_{\mathrm{spe}}(A/T)$. This is consistent with the data reported in the literature [13,14]. The short lifetime of the bonds is illustrated by the small fraction of occupied bead sites (5%) that can be estimated from the specific adhesion energy, the known bond energy, and the bead site density.

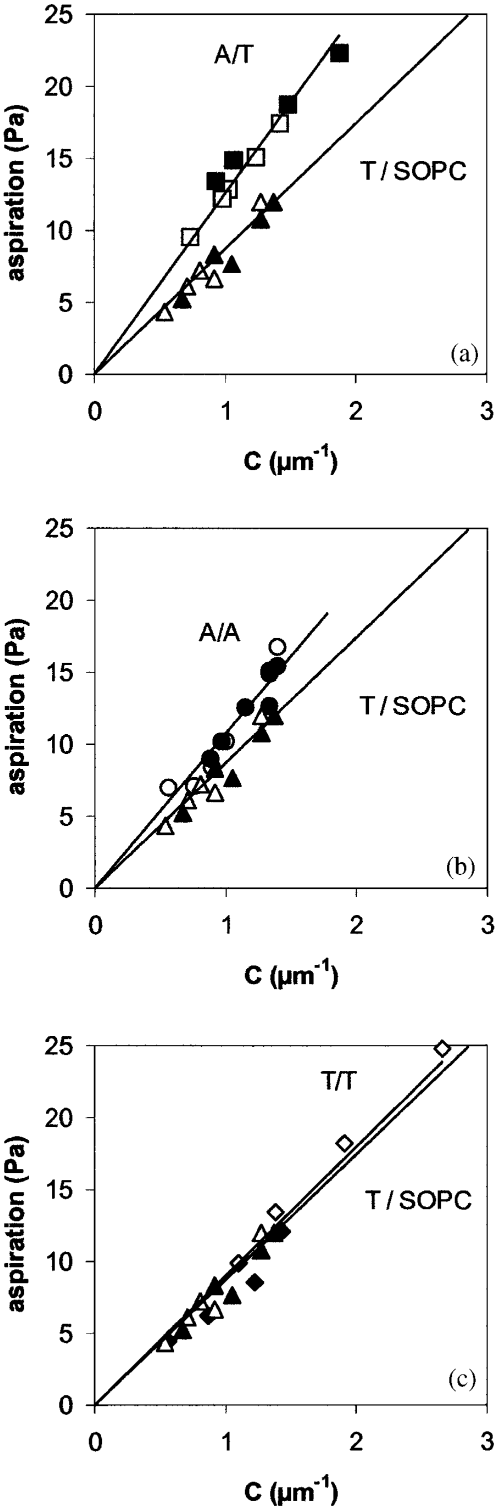

FIG. 2. Aspiration pressure as a function of C [cf. Eq. (4)] for a specific interaction and comparison with the nonspecific case T/SOPC (control). According to Eq. (3), the slopes are equal to W_{adh}. The solid lines are fits forced through zero and they roughly represent the adhesion energy. However, the values reported in Table I are averages from fits not forced through zero and in which the increasing pressure is treated separately from the decreasing one. Closed (open) symbols correspond to the increase (decrease) of the aspiration pressure. (a) bead A/vesicle T, (b) bead A/vesicle A, (c) bead T/vesicle T.

TABLE I. Adhesion energy values (W_{adh}) averaged as indicated in Fig. 2, from at least two separate experiments and six cycles. The error is $\pm 1 \times 10^{-6}$ J · m^{-2}. W_{spe} is equal to $W_{\mathrm{adh}} - W_{\mathrm{nspe}}$ where W_{nspe} is the T/PC adhesion energy.

	A/T	T/A	A/A	T/T	T/PC
W_{adh} (10^{-6} J/m^2)	12.0	12.9	9.9	9.3	8.6
W_{spe} (10^{-6} J/m^2)	3.4	4.3	1.3	0.7	

The expression of W_{spe} for stronger bonds has already been derived in the mid 1980s [1,2] but was never experimentally checked. It relates W_{spe} to the enrichment of the contact zone in adhesion sites,

$$W_{\mathrm{spe}} = (\rho_c - \rho_{\mathrm{nc}}) k_B T\,, \tag{5}$$

where ρ is the surface density of sites at the vesicle surface; c refers to the contact zone and nc to the rest of the vesicle.

Here we extend this approach through a microcanonical description that allows one to directly relate W_{spe} to the bond energy. We consider a bead with immobile adhesion sites with a density $1/A_0$. This bead interacts with a vesicle of area A_V displaying N mobile sites. We assume that each adhesion site has a field of attraction with an area α. A vesicle site can be bound to a given bead site if it is located within an area α around the bead site. Such a vesicle site can have two states, bound or unbound. α is assumed to be the same for thymidine and adenosine. Finally, we call A_f the part of the bead surface located in the contact zone A_c in which a site of the vesicle can be bound: $A_f = A_{\mathrm{c}}\alpha/A_0$. In our case, A_f is very small as compared to A_V. In this system, the partition function Z is obtained by summing over all the configurations in which n sites are in A_f, and among them, m are bound [15],

$$Z = K \sum_{n=0}^{N} \sum_{m=0}^{n} \frac{N!}{n!\,(N-n)!}\,\frac{n!}{m!\,(n-m)!} \times (A_v - A_f)^{N-n} \left(\frac{1}{2}\right)^n A_f^n e^{m e_b}, \tag{6}$$

where e_b is the binding energy and can represent in the more general sense an exchange energy; K is independent of A_{c}. Because the sites in A_f can be bound or not bound while the sites out of A_f have only one state, the factor $1/2^n$ is necessary to avoid counting twice the ones in A_f. By summing over m and then over n, one obtains

$$Z = K\left[A_v + A_f\left(\frac{e^{e_b} - 1}{2}\right)\right]^N. \tag{7}$$

Our experiments provide a measurement of W_{spe} which is formally equal to [1]

$$W_{\mathrm{spe}} = -\frac{\partial F}{\partial A_{\mathrm{c}}}\bigg|_{V,T} = k_B T \frac{\partial \ln Z}{\partial A_{\mathrm{c}}}\bigg|_{V,T} \tag{8}$$

where F is the free energy and A_{c} the contact area, and therefore

$$W_{\mathrm{spe}} = \frac{\frac{\alpha N}{A_0}\left(\frac{e^{e_b}-1}{2}\right) k_B T}{A_v + \frac{\alpha A_{\mathrm{c}}}{A_0}\left(\frac{e^{e_b}-1}{2}\right)} \approx \frac{\alpha \rho_v}{A_0}\left(\frac{e^{e_b} - 1}{2}\right) k_B T\,, \tag{9}$$

where $\rho_v = N/A_v$. It is worth noting from Eqs. (7) and (9) it is possible to reobtain exactly Eq. (5) [15]. By using Eq. (9) one directly obtains e_b from the measured specific adhesion energy values provided that A_0/α is known. As the same grafting technique was used for the A and T beads, A_0/α should also be the same in all the cases and is expected to be of the order of 10^3. $A_0/\alpha = 2100$ gives values that are in closest agreement with the known binding energy values [13,14] (Table II).

Since it generally takes about 15 min for lipids to diffuse around a giant vesicle, it is surprising that for each change of aspiration pressure, the equilibrium seems to be reached in less than a minute. Given W_{spe} and Eq. (5), the enrichment is around 1% at the true equilibrium obtained within 15 min. The resulting change in W_{spe} is within the experimental error and can be neglected. We have checked it experimentally by measuring for several aspiration pressures the contact angle for 20 min. In this approximation, equilibrium can be obtained locally because of the excess of sites on the vesicle and lateral diffusion does not bring significant change. The local equilibrium time is typically the one needed for a lipid to explore an area A_0, which is less than a second. This explains the fast equilibrium and also why the observed hysteresis is not stronger than for nonfunctionalized vesicles [16].

In contrast, if there were more sites on the bead or less sites on the vesicle, the diffusion of the vesicle sites towards the contact region would change noticeably the equilibrium time. It may be noted that decreasing the site density on the vesicle would decrease the adhesion energy which is already borderline; beads with a higher site density were not available. One may also note that even though global equilibrium is not achieved, the measured adhesion energy value is not affected beyond the experimental error, and therefore it is possible to obtain, through Eq. (5), the excess of sites at global equilibrium.

A_0/α is the parameter deduced from the experimental data. The values of A_0 and α cannot be determined, and we will only briefly discuss them. As a first approximation, we may assume that the bead is saturated with nucleosides which are all accessible. As discussed above, A_0 is then expected to be comprised between 50 and 180 nm^2, giving

TABLE II. Binding energy values e_b as deduced from Eq. (9) for different values of A_0/α.

A_0/α	1600	2100	2600	Literature values
Bead/vesicle				
A/T	2.9	3.2	3.4	3.6
T/A	3.1	3.4	3.6	3.6
A/A	2.0	2.3	2.5	1.9
T/T	1.5	1.7	1.9	1.5

for α a value comprised between 0.025 and 0.086 nm^2. This seems compatible with the fact that the nucleosides A and T bind through one donor and one acceptor H-bond separated by about 2 Å [17]. Since the PEG spacer arm is flexible, in a more realistic model, the sites should be accessible only for a fraction of its conformations. In this respect, A_0 may have been underestimated as well as α since only the ratio A_0/α is known.

This microcanonical description relating adhesion energy measurements to binding energies is coherent with the experiments and can be applied to other systems. The requirement of knowing A_0/α is limiting because it is not easy to obtain, but numerical simulations may provide good estimates in some cases. The precision of the technique is given by the dispersion of the adhesion energy data and is illustrated in Table II. In the case of A/T, the error is about $0.5k_BT$.

The present approach was made possible by the use of weak bonds. Functionalization by nucleosides produces a reversible adhesion and therefore enables one to measure a relevant adhesion energy which is not the case from stronger bonds [18].

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[12] A possible extraction of the lipids from the vesicle during this process cannot be ruled out in spite of a good reversibility: even if all the connected lipids were extracted, the adhesion measured in the subsequent cycle would not be modified within the experimental error, as it can easily be seen *a posteriori* from Table I by estimating the number of actual bonds in the contact zone, which is at least 10^3 times smaller than the initial quantity of lipids.
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