A Tethered Adhesive Particle Model of Two-Dimensional Elasticity and Its Application to the Erythrocyte Membrane

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ABSTRACT A new model of two-dimensional elasticity with application to the erythrocyte membrane is proposed. The system consists of a planar array of self-adhesive particles attached to nearest neighbors with flexible tethers. Stretching from the equilibrium dimension is resisted because force is required to dissociate the particle clusters and to decrease the distribution entropy. Release of the external force is accompanied by a contraction as thermal diffusion randomizes the particles and allows interparticle attachments to form again. Analysis of membrane thermodynamics and mechanics under the two-state particle assumption results in a shear softening stress-strain relation. The shear modulus is found proportional to the square root of the surface density of particles, the interparticle adhesive energy, and is inversely proportional to the tether length. Applied to the erythrocyte membrane under the assumption that band 3 tetramer represents the particle and spectrin the tether, the shear modulus predicted corresponds to the measured value when the interparticle adhesive energy is ~4.0–5.9 kT, where kT is the Boltzmann constant multiplied by the temperature. This model suggests a mechanism wherein erythrocyte membrane deformability depends on integral protein homomultimeric interactions and can be modulated from the external surface.

INTRODUCTION

The red blood cell is extraordinarily deformable, a property that allows the cell to repeatedly pass through capillaries that may have diameters as small as one third of its dimension. The deformability of the red cell is a consequence of the remarkable elasticity of the cell membrane, which corresponds to a material ~100-fold softer than a rubber sheet of comparable thickness. This elasticity is determined by a composite structure consisting of an outer fluid-like lipid bilayer coupled to the inner membrane skeletal network. It has long been assumed that the skeletal network is responsible for the membrane deformability. A widely held model for the red cell membrane elasticity is based on the assumption that spectrin, the principal protein of the skeletal network, exhibits random-walk entropic-spring behavior (Bennett, 1985, 1990; Stokke et al., 1986). In this model, the red cell membrane shear modulus is determined by a single molecular parameter, the area density of the spectrin tetramer chains. Refinement of the model by computer simulation reveals that the spectrin network can have very different properties from those of the individual chains in isolation (Boal et al., 1992, 1993; Boal, 1994). Although the entropy-spring model has been the most popular, other explanations of red cell elasticity have also been put forward (Waugh and Evans, 1979; Marchesi, 1985; Shen et al., 1986; Speicher, 1986; Steck, 1989; McGough and Josephs, 1990; Bloch and Pumplin, 1992).

According to the entropy-spring model, the membrane elasticity is due solely to the properties of spectrins, and other skeletal proteins play only a supporting role. Evidence is emerging, however, that other proteins may be more active participants than had previously been assumed. Indeed, alterations in erythrocyte shape and deformability have been directly correlated with changes in integral membrane protein organization (Smith et al., 1979; McPherson et al., 1992, 1993; Che et al., 1993). In particular, there are two quite different sets of information in the literature which show that the red cell membrane rigidity is profoundly dependent on integral membrane proteins that span the bilayer. One set pertains to a marked increase in membrane rigidity as well as lateral immobilization of the major intramembrane proteins, glycophorin A and band 3, that is induced by anti-glycophorin antibodies (Chasis et al., 1985, 1988; Golan and Agre, 1994; Knowles et al., 1994; Mohandas and Evans, 1994; Che and Cherry, 1995). The other set describes southeast Asian ovalocytosis. This disease is caused by a mutation very close to the transmembrane domain of band 3 protein, the result of which is a remarkable increase in rigidity of the cell membrane (Saul et al., 1984; Liu et al., 1990; Tilley et al., 1990, 1991, 1993; Mohandas, 1992; Mohandas et al., 1992; Moriyama et al., 1992; Schofield et al., 1992; Tilley and Sawyer, 1992; Moriyama et al., 1993). In addition, atomic force microscope images of the cytoplasmic face of the red cell membrane seem to show spectrin both looping out from the membrane surface and stretched between what may be clusters of integral membrane proteins, presumably band 3 (MacDonald et al., 1994).

The apparent influence of integral membrane proteins on the mechanical properties of the red cell membrane can be incorporated into current models of membrane elasticity but does not naturally follow from them. It seemed worthwhile,

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therefore, to examine possible mechanisms of elasticity wherein integral membrane proteins play a central role. This examination led us to formulate a novel two-dimensional model for red cell membrane elasticity wherein integral membrane proteins form a matrix in which some are associated in clusters and others are free. In the equilibrium state, the tendency for these proteins to self-associate is balanced by two-dimensional translational entropy; however, stress on the membrane disturbs this equilibrium, pulling apart the associations along the axis of strain and, given that membrane shear occurs at essentially constant area, allows new associations to form normal to the axis of strain. When the external force is released, random thermal motion and the adhesive nature of the intramembrane particles (IMPs) reestablishes the original distribution of connections. In this mechanism, spectrin plays no role in membrane elasticity except for supplying constraints on protein movement and linkages between the proteins for force transmission. That is, the spectrin is not precluded from being conformationally flexible, but in contrast to the entropy-spring model, the IMP aggregation model treats the deformation of the red cell membrane primarily as the result of the recombination and redistribution of clusters of the integral membrane proteins. We thus refer to this model as the tethered adhesive particle (TAP) model.

Since the TAP model implicates self-association of integral membrane proteins, evidence for this putative adhesive nature is particularly relevant. The literature on freeze-fracture electron microscopy in fact provides abundant examples that dissociation of spectrin from the membrane, and hence release of the associated IMPs from the restraint of the spectrin tether, leads to aggregation of IMPs, now recognized to consist of integral membrane proteins. The extent of aggregation depends on the extent of spectrin dissociation. Simple incubation to release a small proportion of spectrin leads to modest clustering, whereas treatments such as proteolysis that release a large proportion of the spectrin lead to massive IMP aggregation. The original observation on spectrin-dependent aggregation of IMPs was that low pH induces particle aggregation at low ionic strength (Elgsaeter and Branton, 1974; Cherry et al., 1976; Elgsaeter et al., 1976; Yu and Branton, 1976), but it is now clear that when ghosts are incubated at physiological ionic strength after spectrin removal, very extensive aggregation of IMPs is also observed (Elgsaeter and Branton, 1974; Weinstein et al., 1978, 1980; Gerritsen et al., 1979; Ursitti et al., 1991).

A statistical thermodynamic analysis has been carried out for this two-dimensional system of TAPs. The canonical partition function for this system is first formulated. From the thermodynamic principle that the partial change of the free energy function with respect to the strain in a principal direction should be equal to the stress in that same direction, a constitutive strain-stress relation is deduced for deformation of the particle membrane at constant area, which leads to an expression for the shear modulus as a function of the shear strain. Given realistic estimation for the main unknown parameter, namely the IMP association energy, values corresponding to the measured shear modulus are obtained.

THEORY

One-dimensional spring model of TAPs

The statistical thermodynamics and mechanics of the one-dimensional spring model of TAPs is mathematically much simpler than that of the two-dimensional model. An analytical solution of a multi-state particle model is possible for the one-dimensional model but seems intractable for the two-dimensional model. Consequently, the effect of simplifying a multi-state to a two-state particle model can be conveniently studied only in the one-dimensional case. Furthermore, there is already an alternative solution for the one-dimensional spring model of two-state particles (Ben-Naim, 1992).

We consider a one-dimensional system consisting of a string of N adhesive particles with dimension d in the one-dimensional direction that are linked together by tethers of length l. Only nearest-neighbor interactions are considered. The separation between two neighboring particles may be n steps of length a. This step length a can be treated as the diameter of the objects that are supposed to always fill the spaces between particles. For application of the TAP model to the red cell membrane, the integral membrane constellation that constitutes the primary anchor between the spectrin network and the bilayer membrane (presumably a band 3 tetramer-ankyrin complex) would correspond to the particles in the present model. The objects between particles correspond to the lipid molecules (Fig. 1). The role of the tethers is to transmit the external force, which may

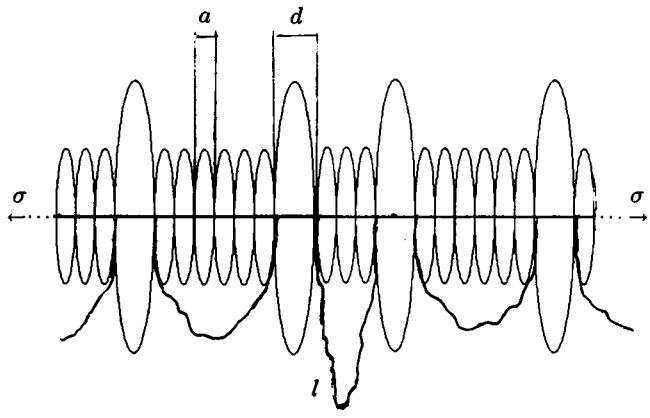

FIGURE 1 Schematic drawing of a one-dimensional model of TAPs. The system considered is a string of N particles with dimension d linked together by tethers of extended length l. The particle adhesive energy is q. Between particles are inserts of dimension a. The particle thus has multiple states of the number l/a. A particle and its associated tether are treated as a basic unit. Each basic unit thus has $n + 1$ ($n = l/a$) states, each having a unit length of $d + i(a/l)$ ($i = 0, 1, 2 \cdots, n$), respectively. When $a = l$, the particles have only two possible states: the tether is either folded with a unit length of d or fully stretched with a unit length of $d + l$. An external force σ is needed to preserve a given length L of the system.

lead to dissociation of adhesive particles, and to supply a constraint on particle displacement in the one-dimensional direction or in the membrane plane in the two-dimensional case. The interparticle adhesive energy is q. At a given temperature T, the equilibrium state is characterized by a particle distribution entropy that is at a maximum. We denote this equilibrium length of the system by L_0; it corresponds to the equilibrium external force σ_0. From this equilibrium position, the length of the system can be changed to L under the action of an external force σ. After the external force is released, the length will return to its equilibrium value by random thermal diffusion. This string of TAPs thus forms a one-dimensional spring. We suppose that this string is composed of N basic units, each containing a particle and a tether or a set of tethers (the present study is not concerned with the details of the tether-particle structure). For a multi-state particle model of object size a, there are $n = l/a$ possible values of $d + ia$ ($i = 0, 1, 2, \cdots, n$) for the length of a basic unit. The canonical (isothermal) partition function of this one-dimensional system, $Q_1 = Q_1(N, L, T)$, can be formulated as

$$Q_1 = r_+^N$$

and

$$r_+ = \exp\left(\frac{\sigma d}{kT}\right) + \sum_{i=1}^{l/a} \exp\left[-\frac{q}{kT} + \frac{\sigma(d+ia)}{kT}\right] \tag{1}$$

$$= \exp\left(\frac{\sigma d}{kT}\right)\left\{1 + \left[\frac{1-\exp(\sigma l/kT)}{1-\exp(\sigma a/kT)}\right]\exp\left(-\frac{q}{kT} + \frac{\sigma a}{kT}\right)\right\},$$

where k is the Boltzmann constant and T is the absolute temperature. The length L of this one-dimensional system can then be found as the partial change of Q_1 with respect to the force σ under constant temperature:

$$L = kT\frac{\partial Q_1}{\partial \sigma}. \tag{2}$$

The result is

$$\left(\frac{L}{Nd} - 1\right)\frac{d}{l} \tag{3}$$

$$= \frac{\left[\left(\frac{a}{l}\right)\dfrac{1-\exp\left(\frac{\sigma l}{kT}\right)}{\left(1-\exp\left(\frac{\sigma a}{kT}\right)\right)^2} - \dfrac{\exp\left(\frac{\sigma l}{kT}\right)}{1-\exp\left(\frac{\sigma a}{kT}\right)}\right] \times \exp\left(-\frac{q}{kT} + \frac{\sigma a}{kT}\right)}{1 + \left[\dfrac{1-\exp(\sigma l/kT)}{1-\exp(\sigma a/kT)}\right]\exp\left(-\frac{q}{kT} + \frac{\sigma a}{kT}\right)}.$$

We define a dimensionless length L^* and a dimensionless force σ^* as follows

$$L^* = \left(\frac{L}{Nd} - 1\right)\frac{d}{l}, \quad \text{and} \quad \sigma^* = \frac{\sigma}{q/l}. \tag{4}$$

We then plot L^* versus σ^*, for a one-dimensional array of tethered adhesive multi-state particles at a given value of l/a, for example, at $l/a = 200$, for various adhesive energies of $q = 0.2\ kT$, $1\ kT$ and $5\ kT$ in Fig. 2 *a*. Around an equilibrium position near the middle of each curve, the larger the force, the longer the length and vice versa. The system thus behaves like a spring under the action of the external force. However, the length-force relation of this TAP spring is nonlinear, i.e., the flexibility of this spring, f, which is the reciprocal of the rigidity μ_e,

$$f = \frac{1}{\mu_e} = \frac{\partial L}{\partial \sigma}, \tag{5}$$

is not constant with force σ. We define a dimensionless flexibility f^* as

$$f^* = \frac{\partial L}{\partial \sigma}\Big/\frac{Nl^2}{kT}, \tag{6}$$

and plot in Fig. 2 *b* the dimensionless flexibility-force relation, f^* versus σ^*, again at $l/a = 200$, for the association energies of $q = 0.2\ kT$, $1\ kT$, and $5\ kT$. Although not explicitly known at this point, there is a particular value of the external force, the equilibrium value σ_0, where the flexibility has its maximum. The flexibility of this nonlinear spring always decreases with either increase or decrease of the external force from its equilibrium value σ_0, a behavior called strain-hardening. The reason this one-dimensional model exhibits strain-hardening is that the distributional entropy of the system has a maximum around the equilibrium value, where the partial change of the entropy with respect to the force (or length) is zero. Any departure from the equilibrium position causes the rate of the entropy change with the force (or length) to increase and the larger the force, the larger this rate increases.

The equilibrium length L_0 can be found by solving the equation

$$\frac{\partial^2 L}{\partial \sigma^2} = 0 \tag{7}$$

for the equilibrium force σ_0, which then gives the equilibrium length L_0 from Eq. 3. L_0 can be written as:

$$L_0 = N(d + \alpha l) \quad \text{and} \quad 0 < \alpha < 1. \tag{8}$$

For a system of particles of multiple states, it is complicated to deduce explicit expressions for the flexibility $f = \partial L/\partial\sigma$ from Eq. 3 or for the equilibrium length L_0 from Eq. 7. Particular solutions for given values of l/a can be obtained, however, with a computer symbolic manipulation program such as Mathematica. For example, for the given value of $l/a = 200$ and $q = 5\ kT$, it is found that at $\sigma = \sigma_0 = 0.248\ q/l$; the flexibility has a maximum, and the equilibrium length is $L_0 \doteq N(d + 0.439l)$, i.e., $\alpha \doteq 0.439$.

In the case of $a = l$, we have the simplest two-state particle model: the tether in each basic unit of the system is either folded to have a basic unit length of d or fully stretched to have a basic unit length of $(d + l)$. In this

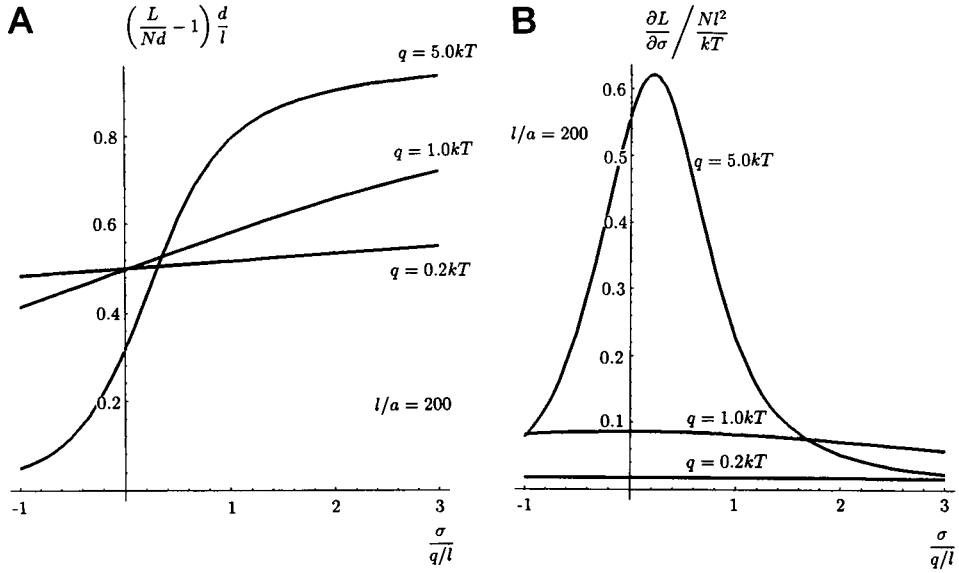

FIGURE 2 The one-dimensional elasticity model of TAPs with multiple states (l/a = 200). (*a*) The length-force relation in a dimensionless form: $(L/Nd - 1)(d/l)$ versus $\sigma/(q/l)$ for various particle association energies of q = 0.2 kT, 1 kT, and 5 kT. An equilibrium length is always observed, but the magnitude of the equilibrium length and force depends on the value of q. In all cases, the larger the extension from the equilibrium position, the larger the force. The TAP system thus behaves like a prestressed, nonlinear spring. (*b*) The flexibility-force relation in a dimensionless form: $(\partial L/\partial\sigma)/(Nl^2/kT)$ versus $\sigma/(q/l)$. At equilibrium, the system exhibits its maximum value of flexibility. This maximum value depends on the magnitude of the particle association energy q. For displacement from equilibrium, strain-hardening is observed, i.e., the flexibility depends on extensional force. The larger the external force, the smaller the flexibility.

simplest case, all of the above results are greatly simplified. The length-force relation, Eq. 3, becomes

$$\left(\frac{L}{Nd}-1\right)\frac{d}{l} = \frac{\exp(-q/kT + \sigma l/kT)}{1 + \exp(-q/kT + \sigma l/kT)}, \qquad (9)$$

which can be solved for an explicit force-length relation:

$$\sigma = \frac{q}{l} + \frac{kT}{l}\log\frac{(L/Nd - 1)(d/l)}{1 - (L/Nd - 1)(d/l)}. \qquad (10)$$

The equilibrium length is $L_0 = N(d + l/2)$, i.e., $\alpha = 0.5$. The equilibrium force is $\sigma_0 = q/l$. For $L > L_0$, $\sigma > \sigma_0$ and for $L < L_0$, $\sigma < \sigma_0$. This two-state particle system thus behaves like a spring with a prestress $\sigma_0 = q/l$.

In Fig. 3, *a* and *b*, are plotted the dimensionless length-force relation and the dimensionless flexibility-force relation, i.e., $L^* = (L/Nd - 1)(d/l)$ and $f^* = (\partial L/\partial\sigma)/(Nl^2/kT)$ versus $\sigma^* = \sigma/(q/l)$, for the two-state particle model (l/a = 1) for the particle adhesive energies q = 0.2 kT, 1 kT, and 5 kT. Comparison of Fig. 3, *a* and *b*, with Fig. 2, *a* and *b*, allows two conclusions. 1) Regardless of the magnitude of the particle adhesive energy, the equilibrium length is always $L_0 = N(d + l/2)$, i.e., α_0 always has the value 0.5, and the equilibrium force is always $\sigma_0 = q/l$; 2) The flexibility has its maximum at the equilibrium position, and this maximum is always equal to $Nl^2/4kT$. In other words, regardless of the strength of the particle adhesive energy, for the two-state particle model the rigidity for the system at equilibrium is always

$$\mu_e = \frac{4kT}{Nl^2}. \qquad (11)$$

It is interesting to compare this result to that given by the random-walk polymer model of rubber elasticity theory in which the external force is proportional to the extensional deformation for a Gaussian chain composed of N links of length l. This proportionality, i.e., the extensional rigidity, is constant with deformation and is given by $\mu = 3kT/Nl^2$ (Treloar, 1979). Like the random-walk polymer, the two-state TAP model is also an entropy spring, and whereas the random-walk model depends on the conformational entropy of the chain, the TAP model is based on the particle distribution entropy. The TAP model allows only extensional deformation, and the links do not have rotational freedom. Hence its equilibrium value of the rigidity is one third larger than that for the random-walk entropy-spring model.

The solution for a one-dimensional system consisting of a string of elements, each of which can be in one of two states (long or short), has been derived using the partition matrix element method (Ben-Naim, 1992). It can be shown in this simple case that although the partition matrix element method differs from the present treatment, its result with elasticity can lead to, and thus is equivalent, to our Eq. 10. The idea for the two-state particle model is parallel to the Lenz-Ising model for the ferromagnetization of a set of spins (Brush, 1967).

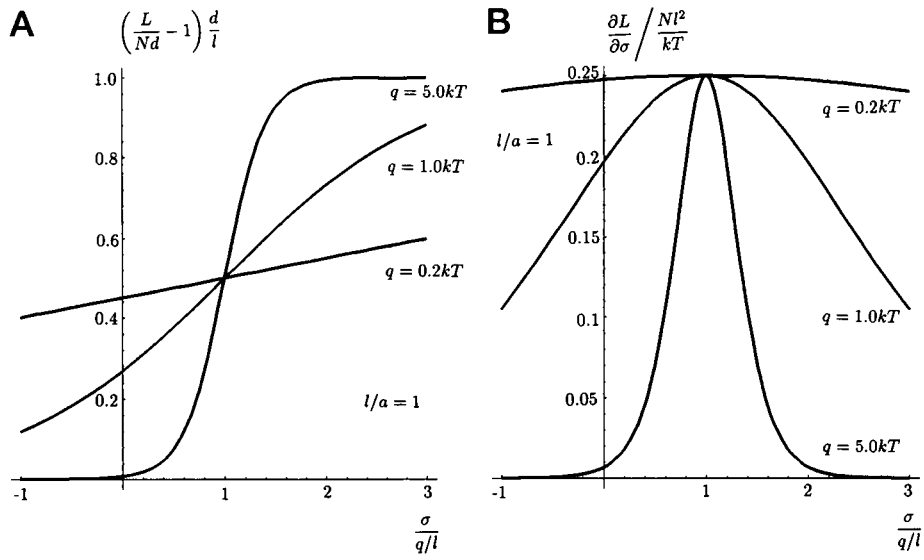

FIGURE 3 The one-dimensional elasticity model of TAPs with two states only. (*a*) The length-force relation in a dimensionless form: $(L/Nd - 1)(d/l)$ versus $\sigma/(q/l)$ for the same values of the particle association energies of $q = 0.2\ kT$, $1\ kT$, and $5\ kT$ as given in Fig. 2, *a* and *b*. Similar to the multi-state particle model, the two-state particle system behaves like a spring. The equilibrium length and force, however, are independent of the particle association energy q and always have the values of $L_0 = N(d + l/2)$ and $\sigma_0 = q/l$. (*b*) The flexibility-force relation in dimensionless form: $(\partial L/\partial\sigma)/(Nl^2/kT)$ versus $\sigma/(q/l)$. At equilibrium, the system has maximal flexibility. This maximal flexibility is independent of the magnitude of the particle association energy q and always has the value of $Nl^2/(4kT)$. The equilibrium force is equal to $\sigma_0 = q/l$, which corresponds to the equilibrium length $L_0 = N(d + l/2)$. A strain-hardening response is observed.

Two-dimensional elasticity model of TAPs

Consider a planar array of TAPs on which a coordinate system Ox_1x_2 is constructed. There are altogether N^2 adhesive particles connected by flexible tethers. The cross-section of each particle in the membrane plane is a circle of diameter d. The tethers confine the maximum separation of the particles in the membrane plane to the tether length l. The total number of tethers and the way they are organized is not relevant to the present model. The maximum and minimum areas of the membrane are $[N(d + l)]^2$ and $(Nd)^2$, respectively. Under the action of the stress σ_1 in the x_1 direction and σ_2 in the x_2 direction, the dimensions of this membrane are deformed to L_1 in the x_1 direction and to L_2 in the x_2 direction, respectively. If x_1 and x_2 are the two principal directions, a maximum shear strain occurs at an angle of 45° to the x_1 or the x_2 direction (Fig. 4). The membrane stress is defined as the force per unit length of the membrane edge. Equilibrium will be characterized by a particular area. Any displacement therefrom will cause more particles to associate or dissociate. The loss or acquisition of the adhesive energy is compensated for by the increase or decrease in the distribution entropy of the particles in the membrane plane due to the dimension change. Because the structure of the membrane in the two principal directions x_1 and x_2 should be the same, the equilibrium dimensions of the membrane in these two directions should also be the same, i.e., there should be $L_1 = L_2 = L_0$, which corresponds to the stresses $\sigma_1 = \sigma_2 = \sigma_0$ at equilibrium.

For mathematical simplicity, we consider only the two-state particle model for the two-dimensional elasticity problem. As in the one-dimensional model, in any direction x_1 and x_2, we consider one particle and an associated tether as a basic unit of the system. Each basic unit in the x_1 or in x_2

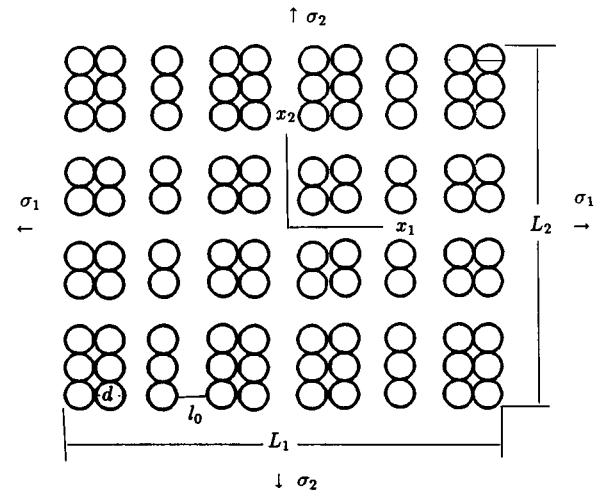

FIGURE 4 Schematic drawing of a planar membrane of TAPs. There are altogether N^2 particles within this system. The cross-section of the particles within the membrane plane is a circle of diameter d. All particles are connected by tethers of length l. The role of the tethers is only to supply constraint for the movement of the particles in the membrane plane and to transfer external force to break the particle-particle associations. The maximum and minimum area of this membrane is $[N(d + l)]^2$ and $(Nd)^2$, respectively. Under the action of the stresses σ_1 in the x_1 direction and σ_2 in the x_2 direction, the dimensions of this membrane are deformed from an equilibrium size of $L_0 \times L_0$ to $L_1 \times L_2$, respectively. In the case of an area-conserving deformation, $L_1L_2 = L_0^2$. Extension in the x_1 direction is accompanied by contraction in the perpendicular x_2 direction.

direction has only two states: the particle is either associated with its neighbor at the unit length d or dissociated from its neighbor at the unit length $(d + l)$. The canonical partition function for this two-dimensional system at the temperature T, $Q_2 = Q_2(N, L_1, L_2, T)$, can thus be written as follows

$$Q_1 = r_1^N r_2^N \tag{12}$$

with

$$r_1 = \exp\left(\frac{\sigma_1 L_2 d}{kT}\right)\left[1 + \exp\left(-\frac{Nq}{kT} + \frac{\sigma_1 L_2 l}{kT}\right)\right] \tag{13a}$$

$$r_2 = \exp\left(\frac{\sigma_2 L_1 d}{kT}\right)\left[1 + \exp\left(-\frac{Nq}{kT} + \frac{\sigma_2 L_1 l}{kT}\right)\right]. \tag{13b}$$

Following standard procedures of statistical thermodynamics, the physical forces $\sigma_1 L_2$ in the x_1 direction and $\sigma_2 L_1$ in the x_2 direction can be obtained from the partial change of the free energy with respect to the length as:

$$\sigma_1 L_2 = kT\frac{\partial \log Q_2}{\partial L_1} \quad \text{and} \quad \sigma_2 L_1 = kT\frac{\partial \log Q_2}{\partial L_2} \tag{14}$$

After some manipulation, we find the force-length relation for this TAP membrane in which the two principal forces and the two principal lengths are coupled according to:

$$\sigma_1 L_2 = \frac{Nq}{l} + \frac{kT}{l}\log\frac{(L_1/Nd - 1)(d/l)}{1 - (L_1/Nd - 1)(d/l)} \tag{15a}$$

$$\sigma_2 L_1 = \frac{Nq}{l} + \frac{kT}{l}\log\frac{(L_2/Nd - 1)(d/l)}{1 - (L_2/Nd - 1)(d/l)}. \tag{15b}$$

Under the action of stresses σ_1 and σ_2 of arbitrary magnitude, the deformation of this membrane can be decomposed into two different kinds of deformation: a pure area expansion or compression without shape distortion and a pure shear deformation at a constant area (Evans and Skalak, 1980). The area deformation is caused by the mean stress, $\bar{\sigma} = (\sigma_1 + \sigma_2)/2$, and the pure shear deformation is produced by the shear stress, which is half of the difference between the two principal stresses, $\tau = (\sigma_1 - \sigma_2)/2$. Related to the equilibrium state, any change in dimension causes more particles to associate or dissociate so that the total adhesive energy change is partially compensated for by the change in the particle distribution entropy of the membrane due to the departure from the equilibrium dimensions. The equilibrium dimension can be found from the principle that the shear deformation should be zero in the equilibrium state, because there can be no directional preference for the deformation of the membrane at equilibrium. This means the shear stress would be zero at equilibrium. Therefore,

$$\tau = \tfrac{1}{2}(\sigma_1 - \sigma_2) = 0 \tag{16}$$

is one of the equations to be solved to find the equilibrium size L_0. From Eqs. 15a and 15b, the zero shear stress state can be obtained under the condition of $L_1 = L_2 = L$. The mean force $\bar{\sigma}L$ at the equilibrium state is thus

$$\bar{\sigma}L = \frac{Nq}{1} + \frac{kT}{1}\log\frac{(L/Nd - 1)(d/l)}{1 - (L/Nd - 1)(d/l)}. \tag{17}$$

The equilibrium dimension and the equilibrium isotropic stress are then found to be

$$L_0 = N\left(d + \frac{l}{2}\right) \quad \text{and} \quad \bar{\sigma}_0 = \frac{Nq}{L_0 l}. \tag{18}$$

Note that, as for the one-dimensional model, in general the lengths can be written in the form of $L_1 = N(d + \alpha_1 l)$ and $L_2 = N(d + \alpha_2 l)$ with $0 < \alpha_1, \alpha_2 < 1$. The equilibrium value of α_1 and α_2 are both equal to 0.5. This value of $\alpha_0 = 0.5$ is the result of the oversimplification inherent in the two-state particle assumption. $\alpha = 0.5$ means that 50% of the tethers are folded and 50% are fully stretched in each of the two principle directions.

We now examine the elastic characteristics that this planar array of tethered two-state particles may have in pure shear deformation, which means that deformations of the membrane occur at constant area, i.e., where the variables L_1 and L_2 are related by the following area-conserving condition:

$$L_1 L_2 = L_0^2. \tag{19}$$

The shear strain can be computed from the extension ration L_1/L_0 as

$$\gamma = \frac{1}{2}\left[\left(\frac{L_1}{L_0}\right)^2 - \left(\frac{L_0}{L_1}\right)^2\right] \tag{20}$$

(Evans and Skalak, 1980), which can be solved to express the extension ratio in shear deformation as

$$\frac{L_1^2}{L_0^2} = \gamma + \sqrt{\gamma^2 + 1}. \tag{21}$$

The shear stress can be computed from Eqs. 15a and 15b as

$$\tau = \tfrac{1}{2}(\sigma_1 - \sigma_2) \tag{22}$$

$$= \frac{1}{2}\left[\frac{Nq}{L_0 l}\left(\frac{L_1}{L_0} - \frac{L_0}{L_1}\right) + \left(\frac{kT}{L_0 l}\right)\left(\frac{L_1}{L_0}\right)\log\frac{(L_1/Nd - 1)(d/l)}{1 - (L_1/Nd - 1)(d/l)} - \left(\frac{kT}{L_0 l}\right)\left(\frac{L_0}{L_1}\right)\log\frac{(L_0^2/L_1 Nd - 1)(d/l)}{1 - (L_0^2/L_1 Nd - 1)(d/l)}\right].$$

If particle adhesion involves only weak noncovalent bonds, the energy q may have a magnitude of only a few kT, i.e., $q/kT \sim O(1)$. Because N^2 is the total number of the particles in this two-dimensional system, N is a very large number. Therefore, $(kT/Nq) \ll 1$. Compared with the first term in the brackets on the right side of Eq. 22, the second and third terms are all very small and thus can be neglected. We denote the area density of particles for the membrane at

equilibrium by N_0, i.e.,

$$N_0 = \frac{N^2}{L_0^2}. \tag{23}$$

The stress-extension ratio relation, Eq. 22, is then approximated by

$$\tau = \frac{\sqrt{N_0}q}{2l}\left[\left(\frac{L_1}{L_0} - \frac{L_0}{L_1}\right)\right], \tag{24}$$

which, combined with Eq. 21, can be converted easily to the shear stress-shear strain relation as follows

$$\tau = \frac{\sqrt{N_0}q}{2l}[(\gamma + \sqrt{\gamma^2 + 1})^{1/2} - (\gamma + \sqrt{\gamma^2 + 1})^{-1/2}]. \tag{25}$$

The two curves in Fig. 5 are plots of the shear stress (normalized by the quantity $\sqrt{N_0}q/2l$) versus (*a*) the shear deformation γ and (*b*) the extension ratio L_1/L_0 in the principal direction x_1. We can see from *curve a* that the shear stress is not strictly proportional to the shear strain. The tethered particle system in shear deformation is a nonlinear spring.

One of the two constitutive equations of membrane thermodynamics relates the shear stress to the shear strain as

$$\tau = \mu_s\gamma, \tag{26}$$

i.e., the shear stress is proportional to the shear strain (Evans and Skalak, 1980). The proportionality coefficient, μ_s, the shear modulus, characterizes the mechanical behavior of the membrane in shear deformation. Note that μ_s is not necessarily a constant. Indeed, as we shall show later, in general it depends on the shear deformation. For the present model, the shear modulus can be found by taking the derivative of Eq. 22 with respect to γ. Noting first that

$$\mu_s = \frac{\partial\tau}{\partial\gamma} = \frac{\partial\tau}{\partial L_1}\frac{\partial L_1}{\partial\gamma}, \tag{27}$$

we find

$$\begin{aligned}\mu_s = {} & \frac{L_1/L_0}{2(L_1^2/L_0^2 + L_0^2/L_1^2)} \\ & \times\left[\left(1 + \frac{L_0^2}{L_1^2}\right)\frac{Nq}{L_0 l} + \frac{kT}{L_0 l}\log\frac{(L_1/Nd - 1)(d/l)}{1 - (L_1/Nd - 1)(d/l)}\right. \\ & + \frac{L_0 kT}{L_1^2 l}\log\frac{(L_0^2/L_1Nd - 1)(d/l)}{1 - (L_0^2/L_1Nd - 1)(d/l)} \\ & + \frac{L_1 kT/L_0 lNd}{(L_1/Nd - 1)[1 - (L_1/Nd - 1)(d/l)]} \\ & \left. + \frac{L_0^3 kT/L_1^3 Nd}{(L_2/L_1Nd - 1)[1 - (L_0^2/L_1Nd - 1)(d/l)]}\right].\end{aligned} \tag{28}$$

Compared with the first term in the bracket on the right side of Eq. 28, the remaining three terms are all small quantities of higher order of magnitude and thus can be neglected when there is weak association between particles. The shear modulus can therefore be approximated as

$$\mu_s = \frac{\sqrt{N_0}q}{2l}\left(\frac{L_1/L_0 + L_0/L_1}{L_1^2/L_0^2 + L_0^2/L_1^2}\right), \tag{29}$$

which can be related to the shear strain according to

$$\mu_s = \frac{\sqrt{N_0}q}{2l}\left[\frac{(\gamma + \sqrt{\gamma^2 + 1})^{1/2} + (\gamma + \sqrt{\gamma^2 + 1})^{-1/2}}{(\gamma + \sqrt{\gamma^2 + 1}) + (\gamma + \sqrt{\gamma^2 + 1})^{-1}}\right]. \tag{30}$$

The two curves in Fig. 6 are plots of the shear modulus μ_s (normalized by the quantity $\sqrt{N_0}q/2l$), versus (*a*) the shear deformation γ and (*b*) the extension ratio L_1/L_0 in the principal direction x_1. We see that the shear modulus is not constant with shear strain. Instead, shear softening is observed, i.e., the shear modulus decreases as the shear strain increases. It is interesting to compare the one- and two-dimensional models of TAPs; the former exhibits strain-hardening in extension whereas the latter exhibits shear softening in shear deformation. As explained above, the force needed to break the particle adhesion so as to extend the one-dimensional spring is a constant force of the magnitude q/l. The one-dimensional elasticity model undergoes strain-hardening in extension because the particle distribution entropy increases with increasing spring length. For the two-dimensional model in shear deformation, the situation is different. Shear deformation is the result of an extension in one principal direction and a corresponding contraction in

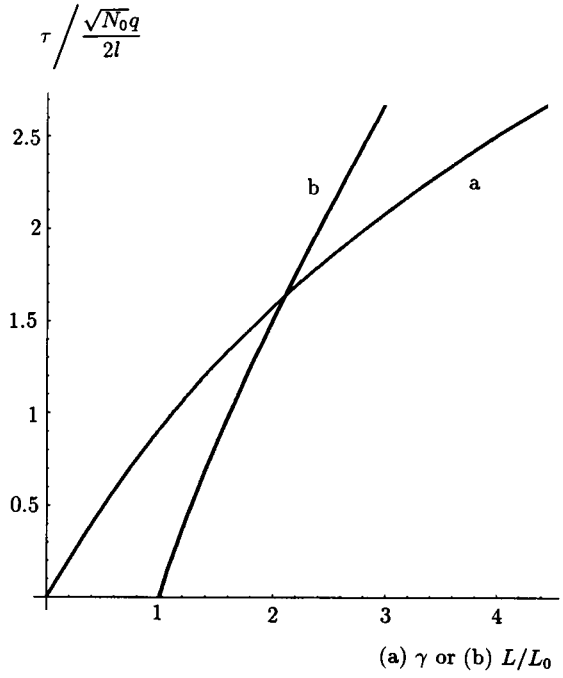

FIGURE 5 The two-dimensional elasticity of planar TAPs with two states only. The shear stress (normalized by the quantity $\sqrt{N_0}q/2l$) is plotted versus the shear deformation, γ (*curve a*) and versus the extension ratio L_1/L_0 (*curve b*). The shear stress is not exactly proportional to the shear strain; the system behaves like a nonlinear spring in shear deformation.

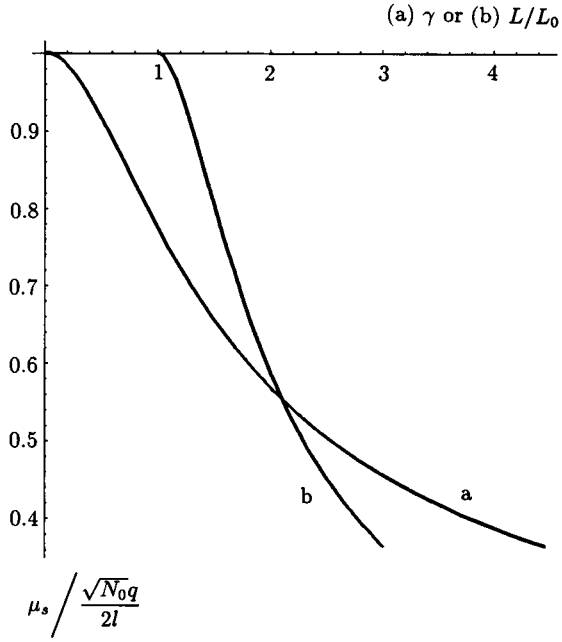

FIGURE 6 The two-dimensional elasticity of planar TAPs with two states only. The shear modulus μ_s (normalized by the quantity $\sqrt{N_0 q}/2l$) is plotted versus the shear deformation γ (*curve a*) and versus the extension ratio L_1/L_0 (*curve b*). The shear modulus is not constant in the shear strain. In this case, shear softening is observed, i.e., the shear modulus decreases as the shear strain increases. At equilibrium, i.e., when the zero shear strain $\gamma = 0$, or equivalently, when the extension ratio $L_1/L_0 = 1$, the shear modulus has a value of $\sqrt{N_0 q}/2l$, where N_0 is the area density of the particles in the membrane plane, q the particle association energy, and l the tether length. For red blood cell membranes, N_0 and q could represent the area density and the association energy of the band 3 aggregates, and l is the length of the spectrin tetramer to which the band 3 aggregate is connected via ankyrin.

the perpendicular direction at constant area. Particle dissociation in one principle dimension is thus compensated by particle adhesion in the other principle dimension.

In the equilibrium state, i.e., at the shear strain $\gamma = 0$, or equivalently, at the unit extension ratio $L_1/L_0 = L_2/L_0 = 1$, Eqs. 29 and 30 become a very simple expression for the equilibrium value of the shear modulus, i.e.,

$$\mu_s = \frac{\sqrt{N_0 q}}{2l}. \tag{31}$$

This expression reveals that, according to the two-dimensional TAP model of the shear elasticity, the shear modulus of the membrane is proportional to the square root of the area density of the particles and the particle adhesion energy strength but inversely proportional to the length of the tethers. Intuitively, these relationships are readily understood. According to membrane mechanics, an extension in a particular direction is characterized by a pure shear deformation at an inclination of 45° with respect to that direction. q/l is the force needed to break the association between the particles along a line in the direction of extension. $\sigma = \sqrt{N_0} \cdot q/l$ is therefore the stress needed to break the particle associations in the extension direction. The shear stress at 45° to the axis of extension is $\tau = \sigma/2 = \sqrt{N_0} \cdot q/2l$. This means that the shear modulus represents half the magnitude of the extensional stress. The larger the particle adhesion energy, the larger the extensional stress needed to produce enough work ($\sqrt{N_0} \cdot q$) to dissociate the particles and extend the membrane, and hence the larger the shear modulus. In contrast, the longer the tether, the smaller the extensional stress needed to produce a given amount of work ($\sqrt{N_0} \cdot q$) to dissociate the particles and extend the membrane, and hence the smaller the shear modulus. Moreover, the larger the area density of the particles, the more particles must be dissociated to extend the membrane. The square root dependence of the area density of the particles characterizes shear deformation, i.e., one-dimensional extension.

APPLICATION TO THE RED BLOOD CELL MEMBRANE

We now suggest how the TAP model can be applied to the red blood cell membrane. We also examine how the macroscopic mechanical properties of the cell membrane in shear deformation can be qualitatively explained on the basis of the membrane skeleton structure and intrinsic membrane protein behavior. Furthermore, a quantitative estimate of some critical physical parameters can be made on the basis of the model presented here.

The red cell membrane is a fluid lipid bilayer containing a number of integral membrane proteins. The major integral membrane protein is band 3, constituting ~15–20% of total membrane proteins. There are $\sim 1.2 \times 10^6$ copies of band 3 molecules per cell (Cabantchik et al., 1978; Steck, 1978). Among them, 40% are immobile and anchored to the underlying skeletal network via ankyrin (Nigg and Cherry, 1980). The maximum bonding of ankyrin to band 3 is 1:1 (Bennett and Stenbuck, 1979), but the ratio of the number of ankyrin to the number of band 3 is 1:10 ~ 1:8 (Bennett, 1985). It has been shown by various experimental means such as freeze-fracture microscopy (Elgsaeter et al., 1976; Yu and Branton, 1976; Gerritsen et al., 1979; Weinstein et al., 1980), analytical ultracentrifuge sedimentation (Dorst and Schubert, 1979; Mulzer et al., 1990; Schuck and Schubert, 1991; Wong, 1993), lipid membrane conductance measurement (Benz et al., 1984), and fluorescence photobleaching recovery (Golan and Veatch, 1980; Nigg and Cherry, 1980) that band 3 molecules aggregate in the membrane to form tetramers and/or larger oligomers (Low, 1986; Tilley and Sawyer, 1992), and the band 3 tetramers associate with each other with low affinity. The latter would decrease the diffusion constant owing to increased effective size of aggregates and would reduce the mobility of those proteins associated with an ankyrin-anchored tetramer (Bennett, 1983).

On the inner surface of the red cell is the membrane skeleton, a network of spectrins, and a number of associated proteins. Spectrin is a flexible, bead-on-a-string-like mac-

romolecule, which presents in the red cell membrane at $\sim 1.2 \times 10^5$ tetramers of contour length 200 nm (Steck, 1974). The spectrins are connected in primarily hexagonal array. Junctional complexes, representing actin and the associated proteins, are located at the center and on six corners of the hexagon (Liu et al., 1987). The skeleton network is intimately coupled to the bilayer membrane at a limited number of sites, mainly via two molecular interactions between integral proteins and spectrins. The first linkage is provided by ankyrin (band 2.1), which simultaneously interacts with band 3 in the bilayer and with spectrin in the skeleton. The second linkage between bilayer and spectrin is provided by protein 4.1, which interacts simultaneously with glycophorin C and the skeletal spectrin. However, ankyrin seems to be the primary determinant for physical linkage between the lipid bilayer and the skeletal network. (Lux et al., 1990; Nakao, 1990).

In applying the TAP model to the red cell membrane, the particle is taken to represent the band 3 tetramer/ankyrin complex and the tether to represent the flexible spectrin tetramer. The total number of ankyrins in a cell is $\sim 120{,}000$. The surface area of an erythrocyte is $\sim 140\ \mu m^2$ (Canham, 1970; Hochmuth, 1987). The area density of the membrane particles is thus $N_0 \doteq 120{,}000/140 \doteq 860/\mu m^2$. The tether length is taken to be 80 nm, i.e., $l = 1600$ Å (Liu et al., 1987). Given these values, Eq. 31 predicts the value of the shear modulus, which is a phenomenological property of the cell membrane, to be

$$\mu_s = \frac{\sqrt{860} \times 10^4 \times 4.14 \times 10^{-14}}{2 \times 1600 \times 10^{-8}} \left(\frac{q}{kT}\right) = 0.38 \times 10^{-3} \times \left(\frac{q}{kT}\right) \tag{32}$$

Eq. 32 can be used to estimate the band 3 particle adhesion energy q from the measured shear modulus μ_s, which according to micropipette experiments is $6\text{–}9 \times 10^{-3}$ mN/m (Hochmuth, 1987); the corresponding range of q is calculated to be 4.0–5.9 kT, or equivalently, 2.4–3.5 Kcal/mol at $T = 300°$ K. This energy corresponds roughly to the energy of a hydrogen bond or to that of the hydrophobic interaction of a few methylene groups.

DISCUSSION

The simple result, Eq. 31, is obtained under two significant approximations. 1) The tethers have only two states, either fully stretched or completely folded, and 2) compared with the predominant role of the interparticle adhesive energy, the effect on the membrane elasticity of the particle distribution entropy can be neglected. From the analysis for the one-dimensional model, it is clear that the two-state particle model oversimplifies the structure of the tethers in the network, resulting in a larger flexibility, i.e., a smaller rigidity. If the effect of the particle distribution entropy is neglected, however, the difference between the two-state model and the multi-state model does not appear in the result. Even for weak interaction between particles with adhesive energies in the range of a few kTs, the error produced by neglecting particle distribution entropy terms is only on the order of kT/Nq, which is $<0.3\%$ when N^2 represents the total number of integral membrane particles in the red cell membrane, i.e., $N^2 = 860/\mu m^2 \times 140\ \mu m^2 \doteq 120{,}000$ and $N \doteq 350$. Another effect of the two-state particle oversimplification is that it always results in the equilibrium size $L_0 = N(d + l/2)$, no matter how large the interparticle interaction. The reason for this is that the equilibrium size is determined by the particle distribution for which the distribution entropy is maximal. For the two-state particle model, the distribution entropy always has a maximum when the size of the membrane is $N(d + l/2)$. For the multi-state particle model, however, the equilibrium dimension for which the system has a maximum particle distribution entropy is a function of the interparticle interaction. The analysis of the one-dimensional model showed that the deviation of the equilibrium size from $L_0 = N(d + l/2)$ is not serious.

It should be pointed out that the equilibrium size is determined with the particle distribution entropy included in the formulation, although it is subsequently found to make a negligible contribution to the shear modulus. Neglect of the entropy terms in the analysis of the membrane mechanics is simply for the sake of mathematical convenience and does not imply that the particles come together without the resistance due to deviations of the particle distribution entropy from its maximum value. Moreover, the extent of the particle aggregation in the equilibrium state is limited by the tethers.

The present theory considers the particle adhesive energy and the particle distribution entropy as a possible molecular source of the membrane elasticity in deformations at constant area. Eq. 31 describes the quantitative relationship of the membrane shear modulus to the particle adhesion energy, q, the area density of particles, N_0, and the tether length, l, which are the three physical parameters relevant to membrane macroscopic properties. When considering the complexity in structure of the red cell membrane, it is to be expected that there must be many other factors that may regulate the red cell membrane properties in addition to those considered here. One such factor, which is potentially very important and has received considerable attention, is the conformational entropy of spectrin. The random-walk polymer entropy-spring model has been used to predict the membrane shear modulus. The prediction is very simple: $\mu_s = N_s kT$, where N_s is the area density of spectrin tetramers (Stokke et al., 1986). Here N_s is the only microscopic parameter of the membrane structure relevant to the macroscopic properties of the membrane. The model we present emphasizes other structural components of the membrane. Accurate representation of the membrane properties will undoubtedly require a combination of these and other considerations.

Although more factors are involved in the present theory than in the polymer entropy-spring approach, there is an obvious similarity between the two models, in that entropy is a critical thermodynamic parameter in both. In the former, however, the conformation entropy of the chain-like spectrin in the membrane skeletal network is the sole determinant of membrane elasticity. In contrast, in the TAP model, the enthalpy, in the form of the particle-particle adhesion energy, is also considered important. In this model the tethers play no role except for supplying a constraint to the particle displacement and transmitting an external force. The important difference between the two models with regard to the red cell membrane elasticity is that the TAP model allows modulation of the membrane rigidity at the level of the bilayer membrane, which also includes the external surface of the red cell. We regard this as significant, not only because there is evidence that band 3 is self-adhesive, but also because its transmembrane domain has been implicated in cell rigidity. Furthermore, because there is evidence for an association of glycophorin and band 3 (Che and Cherry, 1995), the effect of antiglycophorin antibodies on membrane elasticity could be explained as transmission of a conformational change from glycophorin to band 3, thereby affecting the magnitude of q. It would seem appropriate that models for red blood cell membrane elasticity take this factor explicitly into account. It should be recognized that the TAP model does not preclude the additional component of the red cell membrane elasticity due to the random-walk entropy of the spectrins in the skeletal network; indeed, there is enough evidence that spectrin is at least moderately flexible and that a complete statistical thermodynamic model would need to include the entropy components attributable to available configurations of the spectrin molecules in the skeletal network.

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REFERENCES

Ben-Naim, A. 1992. One-dimensional model. *In* Statistical Thermodynamics for Chemists and Biochemists. Plenum Press, New York. 191–260.

Bennett, V. 1983. The human erythrocyte as a model system for understanding membrane cytoskeleton interaction. *In* Cell Membrane, Method and Reviews, Vol. 2. E. Elson, W. Frazier, and L. Glaser, editors. Plenum Press, New York. 149–195.

Bennett, V. 1985. The membrane skeleton of human erythrocytes and its implications for more complex cells. *Annu. Rev. Biochem.* 54:273–304.

Bennett, V. 1990. Spectrin-based membrane skeleton: a multipotential adaptor between plasma membrane and cytoplasm. *Physiol. Rev.* 70: 1029–1065.

Bennett, V., and P. J. Stenbuck. 1979. The membrane attachment protein for spectrin is associated with band 3 in human erythrocyte membranes. *Nature.* 280:468–473.

Benz, R., M. T. Tosteson, and D. Schubert. 1984. Formation and properties of tetramers of band 3 protein from human erythrocyte membranes in planar lipid bilayer. *Biochim. Biophys. Acta.* 775:347–355.

Bloch, R. J., and D. W. Pumplin. 1992. A model of spectrin as a concertina in the erythrocyte membrane skeleton. *Trends Cell Biol.* 2:186–189.

Boal, D. H. 1994. Computer simulation of a model network for the erythrocyte cytoskeleton. *Biophys. J.* 67:521–529.

Boal, D. H., U. Seifert, and J. C. Shillcock. 1993. Negative Poisson ratio in two dimensional networks under tension. *Phys. Rev.* E48:4274–4283.

Boal, D. H., U. Seifert, and A. Zilker. 1992. Dual network model for red blood cell membranes. *Phys. Rev. Lett.* 69:3405–3406.

Brush, S. G. 1967. History of the Lenz-Ising model. *Rev. Mod Phys.* 39:883–893.

Cabantchik, Z. I., P. A. Knauf, and A. Rothstein. 1979. The anion transport system of the red blood cell. The role of membrane evaluated by the use of "probes." *Biochim. Biophys. Acta.* 515:239–302.

Canham, P. B. 1970. The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cell. *J. Theor. Biol.* 26:61–81.

Chasis, J. A., N. Mohandas, and S. B. Shohet. 1985. Erythrocyte membrane rigidity induced by glycophorin A-ligand interactions: evidence for a ligand-induced association between glycophorin A and skeletal proteins. *J. Clin. Invest.* 75:1919–1926.

Chasis, J. A., M. E. Reid, R. H. Jensen, and N. Mohandas. 1988. Signal transduction by glycophorin A-ligand interactions: role of extracellular and cytoplasmic domains in a modulatable process. *J. Cell. Biol.* 107: 1351–1357.

Che, A., and R. J. Cherry. 1995. Loss of rotational mobility of band 3 proteins in human erythrocyte membranes induced by antibodies to glycophorin A. *Biophys. J.* 66:1881–1887.

Che, A., R. J. Cherry, L. H. Bannister, and A. R. Dluzewski. 1993. Aggregation of band 3 in hereditary ovalocytic red blood cell membranes: electron microscopy and protein rotational diffusion studies. *J. Cell Sci.* 105:655–660.

Cherry, R. J., A. Bürkli, M. Busslinger, and G. Schneider. 1976. Rotational diffusion of band 3 proteins in the human erythrocyte membrane. *Nature.* 263:389–393.

Dorst, H. J., and D. Schubert. 1979. Self-association of band 3-protein from human erythrocyte membrane in aqueous solution. *Hoppe-Seylar's Z. Physiol. Chem.* 360:1605–1618.

Elgsaeter, A., and D. Branton. 1974. Intramembrane particle aggregation in erythrocyte ghosts. I. The effects of protein removal. *J. Cell Biol.* 63:1018–1030.

Elgsaeter, A., D. M. Shotton, and D. Branton. 1976. Intramembrane particle aggregation in erythrocyte ghosts. II. The influence of spectrin aggregation. *Biochem. Biophys. Acta.* 426:101–122.

Evans, E. A., and R. Skalak. 1980. Mechanics and Thermodynamics of Biomembranes. CRC Press, Boca Raton.

Gerritsen, W. J., A. J. Verkleij, and L. L. M. V. Deenen. 1979. The lateral distribution of intramembrane particles in the erythrocyte membrane and recombinant vesicles. *Biochim. Biophys. Acta.* 555:26–41.

Golan, D. E., and P. Agre. 1994. Action at a distance: another lesson from the red cell. *Biophys. J.* 66:1271–1272.

Golan, D. E., and W. Veatch. 1980. Lateral mobility of band 3 in the human erythrocyte membrane studied by fluorescence photobleaching recovery: evidence for control by cytoskeletal interactions. *Proc. Natl. Acad. Sci. USA.* 77:2537–2541.

Hochmuth, R. M. 1987. Properties of red blood cells. *In* Handbook of Bioengineering. R. Skalak and S. Chien, editors. McGraw-Hill, New York. 12.1–12.17.

Knowles, D. W., J. A. Chasis, E. A. Evans, and N. Mohandas. 1994. Cooperative action between band 3 and glycophorin A in human erythrocytes: immobilization of band 3 induced by antibodies to glycophorin A. *Biophys. J.* 66:1726–1732.

Liu, S. C., L. H. Derick, and J. Palek. 1987. Visualization of the hexagonal lattice in the erythrocyte membrane skeleton. *J. Cell Biol.* 104:527–536.

Liu, S. C., S. Zhai, J. Palek, D. E. Golan, D. Amato, K. Hassan, G. T. Nurse, D. Babona, T. Coetzer, P. Jarolim, M. Zaik, and S. Borwein. 1990. Molecular defect of the band 3 protein in Southeast Asian ovalocytosis. *New Engl. J. Med.* 323:1530–1538.

Low, P. S. 1986. Structure and function of the cytoplasmic domain of band 3: center of erythrocyte membrane-peripheral protein interactions. *Biochem. Biophys. Acta.* 864:145–167.

Lux, S. E., K. M. John, and V. Bennett. 1990. Analysis of cDNA for human erythrocyte ankyrin indicates a repeated structure with homology to tissue-differentiation. *Nature.* 344:36–42.

MacDonald, R. C., J. M. Mikrut, S. S. Feng, A. H. Swihart, J. A. Osladil, and J. B. Ketterson. 1994. Atomic force microscopy of the erythrocyte skeleton suggests a new mechanism of elasticity. *Mol. Biol. Cell.* 5:418*a*. (Abstr.)

Marchesi, V. T. 1985. Stabilizing infrastructure of cell membranes. *Annu. Rev. Cell Biol.* 1:531–561.

McGough, A. M., and R. Josephs. 1990. On the structure of erythrocyte spectrin in partially expanded membrane skeletons. *Proc. Natl. Acad. Sci. USA.* 87:5208–5212.

McPherson, R. A., W. H. Sawyer, and L. Tilley. 1992. Rotational diffusion of the erythrocyte integral membrane protein band 3: effects of hemichrome binding. *Biochemistry.* 31:512–518.

McPherson, R. A., W. H. Sawyer, and L. Tilley. 1993. Band 3 mobility in camelid erythrocytes: implications for erythrocyte shape. *Biochemistry.* 32:6696–6702.

Mohandas, N. 1992. Molecular basis of red cell membrane viscoelastic properties. *Biochem. Soc. Trans.* 20:776–782.

Mohandas, N., and E. A. Evans. 1994. Mechanical properties of the red cell membrane in relation to molecular structure and genetic defects. *Annu. Rev. Biophys. Biomol. Struct.* 23:787–818.

Mohandas, N., R. Winardi, D. Knowles, A. Leung, M. Parra, E. George, J. Conboy, and J. Chasis. 1992. Molecular basis for membrane rigidity of hereditary ovalocytosis: a novel mechanism involving the cytoplasmic domain of band 3. *J. Clin. Invest.* 89:686–692.

Moriyama, R., H. Ideguchi, C. R. Lombardo, H. M. Van Dort, and P. S. Low. 1992. Structural and functional characterization of band 3 from southeast Asian ovalocytes. *J. Biol. Chem.* 267:25792–25797.

Moriyama, R., C. R. Lombardo, R. F. Workman, and P. S. Low. 1993. Regulation of linkages between the erythrocyte membrane and its skeleton by 2,3-diphosphoglycerate. *J. Biol. Chem.* 268:10990–10996.

Mulzer, K., L. Kampmann, P. Petrasch, and D. Schubert. 1990. Complex associations between membrane proteins analyzed by analytical ultracentrifugation: studies on the erythrocyte membrane proteins band 3 and ankyrin. *Colloid & Polym. Sci.* 268:60–64.

Nakao, M. 1990. Function and structure of the red blood cell cytoskeleton. *In* Blood Cell Biochemistry, Vol 1. J. R. Harris, editor. Plenum Press, New York. 195–225.

Nigg, E. A., and R. J. Cherry. 1980. Anchorage of a band 3 population at the erythrocyte cytoplasmic membrane surface: protein rotational diffusion measurements. *Proc. Natl. Acad. Sci. USA.* 77:4702–4706.

Saul, A., G. Lamont, W. H. Sawyer, and C. Kidson. 1984. Decreased membrane deformability in Melanesian ovalocytes from Papua, New Guinea. *J. Cell Biol.* 98:1348–1354.

Schofield, A. E., M. J. A. Tanner, J. C. Pinder, B. Clough, P. M. Bayley, G. B. Nash, A. R. Dluzewski, D. M. Reardon, T. M. Cox, R. J. M. Wilson, and W. B. Gratzer. 1992. Basis of unique red cell membrane properties in hereditary ovalocytosis. *J. Mol. Biol.* 223:949–958.

Schuck, P., and D. Schubert. 1991. Band 3-hemoglobin associations: the band 3 tetramer is the oxyhemoglobin binding site. *FEBS Lett.* 293: 81–84.

Shen, B. W., R. Josephs, and T. L. Steck. 1986. Ultrastructure of unit fragments of the human erythrocyte membrane. *J. Cell Biol.* 102: 997–1006.

Smith, J. E., N. Mohandas, and S. B. Shohet. 1979. Variability in erythrocyte deformability among various mammals. *Am. J. Physiol.* 236: H725–730.

Speicher, D. W. 1986. The present status of erythrocyte spectrin structure: the 106-residue repetitive structure is a basic feature of an entire class of proteins. *J. Cell Biochem.* 30:245–258.

Steck T. L. 1974. The organization of protein in the human red blood cell membrane. *J. Cell Biol.* 62:1–19.

Steck, T. L. 1978. The band 3 protein of the human red cell membrane. *J. Supramol. Struct.* 8:33–46.

Steck, T. L. 1989. Red cell shapes. *In* Cell Shape, Determinants, Regulation, and Regulatory Role. W. D. Stein and F. Bronner, editors. Academic Press, San Diego. 205–246.

Stokke, B. T., A. Mikkelsen, and A. Elgsaeter. 1986. The human erythrocyte membrane skeleton may be an ionic gel. I. Membrane mechanochemical properties. *Eur. Biophys. J.* 13:203–218.

Tilley, L., M. Foley, R. F. Andre, A. R. Dluzewski, W. B. Grantzer, G. L. Jones, and W. H. Sawyer. 1990. Rotational dynamics of the integral membrane protein, band 3, as a probe of the membrane events associated with *Plasmodium falciparum* infections of human erythrocytes. *Biochem. Biophys. Acta.* 1025:135–142.

Tilley, L., G. L. Jones, G. B. Nash, and W. H. Sawyer. 1991. Decreased rotational diffusion of band 3 in Melanesian ovalocytes from Papua, New Guinea. *J. Memb. Biol.* 121:59–66.

Tilley, L., R. A. McPherson, G. L. Jones, and W. H. Sawyer. 1993. Structural organization of band 3 in Melanesian ovalocytes. *Biochim. Biophys. Acta.* 1181:83–89.

Tilley, L., and W. H. Sawyer. 1992. Rotational dynamics of human erythrocyte Band 3: monitoring the aggregation state of an integral membrane protein. *Comments Mol. Cell Biophys.* 7:333–352.

Treloar, L. R. G. 1975. The elasticity of long chain molecules. *In* The Physics of Rubber Elasticity. 3rd edition. Oxford University Press, Ely House, London. 42–58.

Ursitti, J. A., D. W. Pumplin, J. B. Wade, and R. Bloch. 1991. Ultrastructure of the human erythrocyte cytoskeleton and its attachment to the membrane. *Cell Motil. Cytoskeleton.* 19:227–243.

Waugh, R. E., and E. A. Evans. 1979. Thermoelasticity of red blood cell membrane. *Biophys. J.* 26:115–132.

Weinstein, R. S., J. K. Khodadad, and T. L. Steck. 1978. Fine structure of the band 3 protein in human red cell membranes: freeze-fracture studies. *J. Supramol. Struct.* 8:325–335.

Weinstein, R. S., J. K. Khodadad, and T. L. Steck. 1980. The band 3 protein intramembrane particle of the human red blood cell. *In* Membrane Transport in Erythrocytes. Alfred Benzon Symposium 14. U. V. Lassen, H. H. Ussing, and J. O. Wieth, editors. Munksgaard: Copenhagen. 35–50.

Wong, P. 1993. The state of association of band 3 of the human erythrocyte membrane: evidence of a hexamer. *Biochim. Biophys. Acta.* 1151:21- 27.

Yu, J. A., and D. Branton. 1976. Reconstitution of intramembrane particles in recombinants of erythrocyte protein band 3 and lipid: effects of spectrin-actin association. *Proc. Natl Acad. Sci. USA.* 73:3891–3895.