ON THE ADHESION OF VESICLES BY CELL ADHESION MOLECULES

GEORGE I. BELL AND DAVID C. TORNEY
Theoretical Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545

ABSTRACT This paper gives a detailed analysis of experiments on the kinetics of aggregation of lipid vesicles containing neural cell adhesion molecules (N-CAM). An explanation for the dependence of the "initial aggregation rate," k_{agg}, on the square of the vesicle concentration is given, accounting both for Brownian motion of the vesicles and shear effects. A model in which trimers of N-CAM are one-half of the molecular unit bridging two vesicles explains the observed dependence of k_{agg} on up to the sixth power of the lateral N-CAM concentration and corroborates electron micrographic evidence for N-CAM "triskelions."

INTRODUCTION

Hoffman and Edelman (1983) have reported experiments on the aggregation of vesicles containing neural cell adhesion molecules (N-CAM). In this system, adhesion of two vesicles is believed to take place by the binding of the N-CAM molecules on one vesicle to those on the second vesicle. Inasmuch as adhesion between cells is also mediated by N-CAM and similar molecules (Edelman, 1983), the study of such vesicle aggregation may help to delineate parameters of importance in cell adhesion.

In the experiments (Hoffman and Edelman, 1983), the appearance of aggregates having volumes exceeding a threshold was measured as a function of vesicle concentration, number, and type of N-CAM molecules per vesicle, as well as other parameters. Two salient findings were that the rate of appearance of superthreshold aggregate volume could be expressed as a constant, k_{agg}, times the square of the initial vesicle concentration, and that k_{agg} is proportional to the third to the sixth power of the number of N-CAM molecules per vesicle. One purpose of the following analysis is to clarify the conditions under which this behavior is to be expected and to estimate an upper limit to k_{agg}.

Superthreshold aggregates contain many monomers. For example, in some of the experiments, vesicles were prepared by passage through a 0.4-μm filter, and aggregates were detected when their volume exceeded 1.5 μm^3 (diameter, 1.42 μm). If we assume that the initial vesicle diameters are no larger than 0.4 μm, then a superthreshold aggregate contains at least $(1.42/0.4)^3 = 45$ vesicles. (In unreported experiments, S. Hoffman has shown that the initial vesicles in such a preparation indeed have mostly diameters somewhat less than 0.4 μm.) Analysis of such large aggregates introduces considerable uncertainty since their shape, in particular, is rather uncertain, and therefore reliable estimates of their collision rates are not possible. A further complication is that, under the conditions of the experiment, both Brownian and shear-induced collisions are significant.

In this paper, we first consider the kinetics of aggregation and show that the observed dependence on initial vesicle concentration is to be expected for any process where the state of an aggregate can be changed only by binary collisions. Estimates for an upper limit to k_{agg} for both Brownian and shear-induced collisions are obtained assuming that two colliding aggregates stick irreversibly with 100% probability. (Numerical results have been obtained for various combinations of Brownian and shear collisions [Spouge, J., and G. I. Bell, manuscript submitted for publication]). Estimates are also made of the sticking probability and of the dependence on the number of N-CAM molecules per vesicle. In addition, it is noted that the accumulation of adhesion molecules in regions of vesicle–vesicle contact will tend to render aggregates less sticky, and the extent of this effect is estimated. Model predictions are compared, where possible, with experimental results.

KINETICS OF AGGREGATION: THEORY

Consider the formation of aggregates containing k vesicles (k-mers) by the collision and sticking of smaller aggregates or by shear-induced (or spontaneous) breakup of larger aggregates. Let $n_k(t)$ be the concentration of k-mers, $c_{i,j}$ the collision rate for i-mers colliding with j-mers at unit concentration of each, $p_{i,j}$, the sticking probability for such a collision, and $s_{k\to i,k-i}$ the rate at which a k-mer breaks into an i-mer and a k–i-mer. Then the following system of kinetic equations describes the aggregation:

$$\frac{dn_k(t)}{dt} = \frac{1}{2}\sum_{i=1}^{k-1} c_{i,k-i}p_{i,k-i}n_i(t)n_{k-i}(t) - n_k(t)\sum_{i=1}^{\infty} c_{k,i}p_{k,i}n_i(t) - \frac{1}{2}\sum_{i=1}^{k-1} s_{k\to i,k-i}n_k(t) + \sum_{i=1}^{\infty} s_{k+i\to k,i}n_{k+i}(t) \quad k = 1, 2, 3, \ldots . \qquad (1)$$

As an initial condition, we take $n_k(0) = n_1(0)\delta_{k,1}$, representing a population of only monomers. Note that Eq. 1 contains on its right-hand side binary collision terms quadratic in n, together with linear terms not involving collisions.

During any collisions that may lead to sticking, there must be a first bridge formed between N-CAM molecules on two vesicles, and until a few bridges have formed, the aggregate may be viewed as tentative and prone to spontaneous or induced breakup. If it does dissociate at an early stage, let us agree to take this into account in the sticking probability, $p_{i,j}$, since the aggregate will break up into the original collision partners. For modest shear forces, we can expect that after a few bridges have formed (see below and Bell, 1978), the aggregate will be rather stable, and we will neglect the linear terms in Eq. 1. Further, let $f_k(t)$ be the fraction of vesicle mass in k-mers

$$f_k(t) = \frac{kn_k(t)}{n_1(0)}, \tag{2}$$

and define a rate constant for sticking,

$$r_{i,k-i} = \frac{c_{i,k-i}p_{i,k-i}}{i(k-i)}. \tag{3}$$

Then Eq. 1 can be rewritten as

$$\frac{1}{n_1(0)}\frac{df_k(t)}{dt} = \frac{k}{2}\sum_{i=1}^{k-1} r_{i,k-i}f_i f_{k-i} - kf_k\sum_{i=1}^{\infty} r_{k,i}f_i,\ k = 1, 2, \cdots, \tag{4}$$

with the initial condition $f_k(0) = \delta_{k,1}$.

It follows from Eq. 4 that the time for $f_k(t)$ to reach any attainable value will be inversely proportional to the initial concentration, $n_1(0)$. To see this, one may choose a new time variable, $\tau = n_1(0)t$, so that the left-hand side is $df_k/d\tau$. Thus, for any definite set of rate constants, f_k is a function of τ only.

Suppose that superthreshold aggregates have $k \geq \kappa$. Then the fraction of vesicles in superthreshold aggregates,

$$F(\kappa, \tau) = \sum_{k=\kappa}^{\infty} f_k(\tau), \tag{5}$$

will depend on time through τ, and the volume in superthreshold aggregates per unit volume of solution,

$$V(\kappa, \tau) = V_1 n_1(0) F(\kappa, \tau),$$

will have the same time dependence, where V_1 is the volume of a vesicle. Thus, the rate of appearance of volume in superthreshold aggregates will be

$$\frac{\partial V(\kappa, \tau)}{\partial t} = n_1(0)\frac{\partial V}{\partial \tau} = V_1 n_1^2(0)\frac{\partial F(\kappa, \tau)}{\partial \tau}. \tag{6}$$

This establishes that the rate of appearance of superthreshold aggregate volume is proportional to the square of the initial concentration, in agreement with experiment. Note that this result holds for any aggregation process based on binary collisions as the only process for formation (or breakup) of aggregates.

For two simple collision models representing approximately Brownian and shear collisions with certain sticking, we have derived explicit forms for $F(\kappa, \tau)$ and for $\kappa \gg 1$. Details are given in the Appendix. For Brownian collisions, we find

$$F \simeq F_B(\kappa, \tau) = \exp\left(-\frac{\kappa+1}{c\tau}\right)\left(1 + \frac{\kappa}{c\tau}\right), \tag{7}$$

where $c = 4\pi DR$, with D the vesicle diffusion constant and R its radius. The product $DR \simeq 2 \times 10^{-13}$ cm^3/s. For collisions induced by shear, we find

$$F \simeq F_S(\kappa, \tau) = 1 - erf\left(\sqrt{\frac{\kappa}{2}}e^{-\beta\tau}\right), \tag{8}$$

where $\beta = 8/3\ GR^3$, with G the shear rate, and

$$erf(x) = \frac{2}{(\pi)^{1/2}}\int_0^x e^{-u^2}du.$$

Note that both collision models predict that $F(\kappa, \tau) \to 1$ for large τ and fixed κ.

For comparison with experiment, we need to compute the maximum rate of appearance of superthreshold aggregates, and thus the maximum value of $\partial F/\partial\tau$ as τ is varied for fixed κ. From Eq. 7, we find that, for Brownian collisions,

$$\left.\frac{\partial F_B}{\partial \tau}\right|_{max} = \frac{1.34c}{\kappa}, \tag{9}$$

where the maximum is reached at $\tau = \kappa/3c$. From Eq. 8 for shear-induced collisions,

$$\left.\frac{\partial F_S}{\partial \tau}\right|_{max} = 0.48\beta, \tag{10}$$

with the maximum reached at $\tau = (\ell n\kappa)/2\beta$.

Experimental results were expressed by Hoffman and Edelman (1983) as

$$\left.\frac{dV}{dt}\right| = k_{agg}a^2, \tag{11}$$

where V is the volume of superthreshold aggregates in nanoliters per milliliter of solution, t is in minutes, and a is the initial vesicle concentration in milligrams of lipid per milliliter of solution. If in Eq. 6 we measure $n_1(0)$ in vesicles per milliliter, V_1 in nanoliters per vesicle, and τ in minutes per milliliter = minutes per cubic centimeter, then $\partial V/dt$ will appear in units of nanoliters of product per milliliter of solution per minute. For thin-walled vesicles of radius R and thickness ΔR in millimeters,

$$a \approx 4\pi R^2\Delta R\rho n_1(0)\frac{\text{mg}}{\text{m}\ell}, \tag{12}$$

with ρ the lipid density in grams per cubic centimeter = milligrams per cubic millimeter. The volume of a vesicle is

$$V_1 = 10^3\frac{4\pi}{3}R^3\,\text{n}\ell. \tag{13}$$

Inserting these expressions in Eq. 6 and taking maximum values for the derivatives, we have

$$\left.\frac{\partial V}{\partial \tau}\right|_{max} = \frac{10^3a^2}{12\pi R(\Delta R)^2\rho^2}\left.\frac{\partial F}{\partial \tau}\right|_{max}, \tag{14}$$

and, by comparison with Eq. 11,

$$k_{agg} = \frac{10^3}{12\pi(\Delta R)^2\rho^2}\left.\frac{\partial F}{\partial \tau}\right|_{max}. \tag{15}$$

Upper limits to k_{agg} can be estimated by assuming that vesicles stick on every collision and by using Eq. 9 or 10 as an estimate for $\partial F/\partial\tau|_{max}$. For

Brownian collisions, the upper limit is denoted by k_B, where

$$k_{agg} \leq k_B = \frac{5.4 \times 10^{-9}}{R(\Delta R)^2 \kappa}, \tag{16}$$

where we have taken $\rho = 1$ g/cm^3, $(DR) = 1.2 \times 10^{-11}$ cm^3/min.

For shear collisions, the corresponding upper limit to k_{agg} is denoted by k_S

$$k_{agg} \leq k_S = 0.034\left(\frac{R}{\Delta R}\right)^2 G, \tag{17}$$

where G is the shear rate in reciprocal minutes.

The combination of Brownian and shear collisions will be treated elsewhere (Spouge and Bell, 1985); the results are briefly summarized in the Appendix.

COMPARISON WITH EXPERIMENT

We have already seen in Eq. 6 that the predicted rate of appearance of superthreshold aggregates is proportional to the square of the initial concentration, in agreement with the results of Hoffman and Edelman (1983). This prediction holds for any aggregation process in which the state of aggregation changes only through binary collisions.

In the experiments, vesicles were incubated with mild agitation. From casual observation, we estimate the average shear rate to be ~ 10 s^{-1}. The vesicle radius for which Brownian and shear collision rates are about equal is given by Eq. A12, and for $G = 10$ s^{-1} is 0.36 μm. For smaller vesicles (or aggregates), Brownian collisions predominate, whereas for larger ones, shear-induced collisions are more important. The critical radius is estimated to be within the range of important aggregate sizes, so we have considered both Brownian and shear collisions.

The rate of appearance of superthreshold aggregates can be compared with k_B and k_S as given by Eqs. 16 and 17. If the initial vesicle radius is 0.2 μm $= 2 \times 10^{-4}$ mm and the thickness is 7 nm $= 7 \times 10^{-6}$ mm, then $\kappa \simeq 45$, and with $G = 600$/min, $k_B \simeq 1.4 \times 10^4$ nℓ/min, $k_S = 1.6 \times 10^4$ nℓ/min. These values are substantially larger than observed values of k_{agg} for reconstituted vesicles made of embryo brain lipids plus purified N-CAM, for which $k_{agg} \leq 200$. A possible explanation for this reduced rate of aggregation is that the sticking probability per collision is substantially less than unity. An additional explanation is that the kernels from which k_B and k_S were obtained, in their neglect of some hydrodynamic effects, overestimate the rate of occurrence of collisions that can lead to adhesion. If, on each collision, the partners stick with a probability p, the effect is to lengthen all time scales in the aggregation process by $1/p$ so that the observed values of k_{agg} may suggest $p \lesssim 10^{-2}$. Moreover, since k_{agg} is a sensitive function of the number of N-CAM molecules per vesicle, it appears that p is also a sensitive function of N-CAM surface density. Estimates of p in a later section suggest that values of $p \lesssim 10^{-2}$ are to be expected.

For aggregation of "brain vesicles" treated with neuraminidase, $k_{agg} \simeq 10^4$ was observed. In this case, the vesicle concentration was measured in milligrams of membrane protein per milliliter, which is, however, probably similar to the lipid content per milliliter. Moreover, the vesicles were prepared by filtration through a 0.8-μm filter. If their initial radii were as large as 0.4 μm, then $\kappa \simeq 6$ and the estimated values of k_B and k_S would each be increased by a factor of 4. In any case, it appears that sticking probabilities are much nearer unity for "brain vesicles" than for "reconstituted vesicles," and that hydrodynamic effects are not primarily responsible for the previously noted, smaller values of k_{agg}.

A substantial disparity between theory and experiment is found when we observe the amount of material appearing in superthreshold aggregates. Theory predicts that all of the initial vesicles should appear in superthreshold aggregates, whereas only a small percent (of the reconstituted vesicles) are observed to do so. (This assumes that the vesicle lipids are single bilayers. If the vesicles, or aggregates, are multilamellar, somewhat more mass for a given volume will be found; however, most of the mass is still not observed to appear in superthreshold aggregates.) Various explanations can be considered. The most obvious is that the aggregates are unstable, either spontaneously or against the shear forces to which they are exposed, so that the distribution of aggregate sizes approaches a limit in which formation and breakup rates are in balance. To initiate an experiment, the suspension is filtered, a process that presumably breaks up large aggregates. If, after aggregation has occurred, the suspension is filtered and allowed to reaggregate, the rate is similar to the original aggregation rate. Also, if the large aggregates are removed by centrifugation, the small aggregates in the supernatant will form large aggregates, the weight fraction at long time being approximately half of its prior value (Hoffman, S., personal communication). This is evidence in favor of the "reversibility" of the vesicle aggregation and also of a limited vesicle heterogeneity.

Shear forces during incubation appear to be too small to be effective in breaking aggregates. To see this, note that the relative velocity of different portions of an aggregate will be $\Delta v \sim GR \lesssim 10^{-3}$ cm/s. A particular vesicle in the aggregate may be subject to a force, $f \simeq 6\pi\eta R\Delta v$, tending to separate it from the rest of the aggregate, where η is the viscosity ($\simeq 10^{-2}$ g/cm · s). For $R = 2 \times 10^{-5}$ cm, $f \lesssim 4 \times 10^{-9}$ dyn, which is too weak a force to materially perturb a single bond (Bell, 1978). Larger shear rates, by at least a factor of 100, would be needed to accelerate the spontaneous breakup of aggregates.

Although larger shear rates may be present in the Coulter counter used for aggregate identification, it may be noted that any shear-induced breakup will upset the quadratic dependence of dV/dt on $n_1(0)$ (Eq. 6). Hence, the observed dependence can be taken as evidence against the importance of spontaneous or shear-induced breakup.

Another explanation for the nonappearance of vesicles

in superthreshold aggregates may be that the N-CAM molecules become sequestered in regions of contact between vesicles. In order to analyze this possibility, we consider the sticking process during a collision.

A MOLECULAR MODEL OF N-CAM–MEDIATED ADHESION KINETICS

The initial rate of appearance of superthreshold aggregates, k_{agg}, is observed to be proportional both to the square of the initial vesicle concentration and to the (lateral) concentration of N-CAM raised to the power x, where $3 < x < 6$. The latter finding is remarkable, and a theory explaining this dependence is the subject of this section.

Because an increase in the N-CAM concentration increases k_{agg}, one can infer that in an average encounter between vesicles (or aggregates), the adhesion probability is low. N-CAM molecules, which have an extracellular length of on the order of a few hundredths of a micron (Edelman, 1984), are much smaller than the vesicles, and encounters having a large contact area with a separation on the order of 10^{-2} μm are a rare event. These considerations are made quantitative in the next section.

One bond bridging two vesicles may be sufficient to initiate essentially irreversible adhesion. In any case, the initial rate of bond formation between randomly placed molecular units on two vesicle surfaces must be proportional to the product of the lateral concentrations of the molecular units in each surface. This fact will be found universally, for example, whether or not the two surfaces are in relative motion, whether or not the reaction is diffusion limited, and whether or not coloumb or hydrodynamic effects dominate the kinetics. The initial rate of N-CAM to N-CAM bonding between vesicles with the same concentration is therefore expected to be proportional to the square of the N-CAM concentration. Because the probability of adhesion of two vesicles that encounter one another is small (reaction-limited adhesion), k_{agg} is also expected to be proportional to the square of the N-CAM concentration.

The simplest explanation for the observed proportionality between k_{agg} and N-CAM concentration raised to the power x ($3 < x < 6$) is that there is an equilibrium between N-CAM and N-CAM trimers and that the trimers are the molecular units that then bond together to form a bridge between two vesicles. The equilibrium concentration of trimers could be proportional to the first through the third power of the N-CAM concentration (as the equilibrium constant decreases), and then the square of the trimer concentration would give k_{agg} proportional to the N-CAM concentration raised to the second through the sixth power. Additional evidence for the "trimer" hypothesis can be gained from electron micrographs of N-CAM molecules on solid supports clearly showing some "triskelions" or objects having a threefold rotation axis of symmetry (Edelman et al., 1983). To reiterate, we adopt the model that N-CAM trimers, which are in equilibrium with N-CAM monomers, form the molecules that bridge two vesicles, because it is the simplest model consistent with the data. Further experiments may reveal other details and other N-CAM polymers that are important in vesicle–vesicle and cell–cell bridging.

The equation for the trimer concentration in a trimerization equilibrium in which the dimer concentration is negligible is found from the equilibrium equation and molecular conservation.

$$T = KM^3 = K(M_0 - 3T)^3 . \tag{18}$$

T is the number of trimer molecules per unit area of vesicle membrane, M is the number of N-CAM monomers per unit area, and M_0 is the initial number of N-CAM molecules per unit area. K is the equilibrium constant. Eq. 18 is a cubic equation in T with one real root, which is the equilibrium trimer concentration, T_{eq},

$$T_{eq} = \frac{M_0}{3}\left(1 - \frac{3\gamma^{1/3}}{2}\{[(1+\gamma)^{1/2}+1]^{1/3} - [(1+\gamma)^{1/2}-1]^{1/3}\}\right), \tag{19}$$

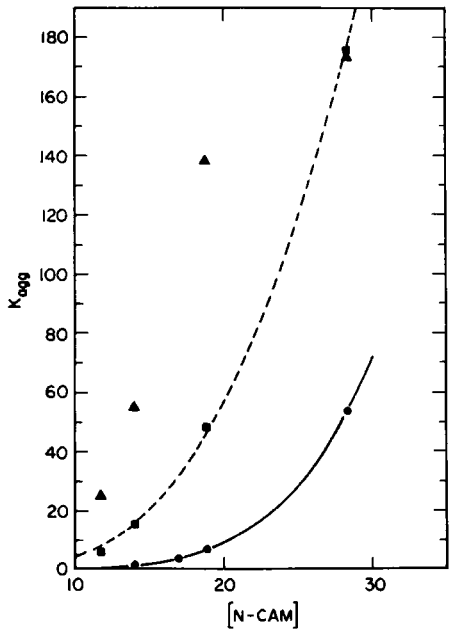

FIGURE 1 The data in this figure are from Table II of Hoffman and Edelman (1983). The x axis is the N-CAM concentration expressed in micrograms of N-CAM per milligram lipid ($10 \leq x \leq 30$). The y axis is the "initial rate" of appearance of superthreshold particles, k_{agg} ($0 \leq k_{agg} \leq 180$). The three sets of data with four points each are the untreated vesicles (circles), mock treated vesicles (squares), and neuraminidase-treated vesicles (triangles). The least-squares fit of the model, Eqs. 19 and 20, is shown as a solid curve for the first data set, for which $K = 7 \times 10^{-5}$ and $\alpha = 45$, and as a dashed curve for the second data set, for which $K = 1.4 \times 10^{-3}$ and $\alpha = 8.62$. As is explained in the text, it is not possible to obtain a reasonable fit to the third set.

with

$$\gamma = 4/(3^4 K M_0^2) .$$

Note that for large values of KM_0^2, $T_{eq} \simeq M_0/3$; that is, all N-CAM molecules are found in trimers. For small values of KM_0^2, $T_{eq} \simeq KM_0^3$, and most of the N-CAM molecules are in monomers.

The mathematical model of aggregation gives

$$k_{agg} = \alpha T_{eq}^2 , \tag{20}$$

with α a proportionality constant. Fig. 1 shows a least-squares fit of this nonlinear model to the data relating k_{agg} and N-CAM concentration (M_0) given in Table II of Hoffman and Edelman (1983, p. 5764). Their Table II presents three sets of data with four data points each; these are shown in Fig. 1. We have obtained reasonable fits to the first two sets of data.

The first set gives k_{agg} as a function of N-CAM concentration for untreated vesicles, for which we find an equilibrium constant K of 7.0×10^{-5} with dimensions $(\mu g/mg\ lipid)^{-2}$ and the proportionality constant α equal to 45 $(\mu g/mg\ lipid)^{-2} \cdot cm^{-3} \cdot s$. (The sum of residuals squared is 0.45.) The second set gives k_{agg} for vesicles put in a buffer (mock treatment) in which k_{agg} is increased when compared with the untreated vesicles. For this set of data, we find K to be 1.4×10^{-3} and α to be 8.6.[1] (The sum of residuals squared is 8.59.) Over the range of these two experiments, these equilibrium constants mean that the percent of the monomers in trimers goes from 3 to 12 and 25 to 48, respectively. The increase in the equilibrium constant with the mock treatment suggests that some element of the ionic environment can strongly modulate the equilibrium between monomers and trimers. No interpretation of the variation in α between these two experiments can be given without detailed knowledge of the structures of the monomers and trimers.

There is a scaling property of Eqs. 19 and 20. If only a fraction f of the N-CAM monomers can participate in the trimerization equilibrium, then the same fit to the data as was found with f equal to unity will be achieved with

$$K' = Kf^{-2}$$

and

$$\alpha' = \alpha f^{-2} .$$

For example, if the monomers are randomly inserted, then only half would have an "outward" orientation, and f would equal one-half. Then the data would suggest a K and an α four times larger than if f were unity. Regardless of f, the same fraction of available monomer would be predicted to be in trimers for a given value of k_{agg} in data sets one or two.

The third set, also shown in Fig. 1, gives k_{agg} for vesicles put in a buffer with neuraminidase. Because of the rapid decrease in the slope between data points, it was not possible to obtain a reasonable fit of the model to this set of data. Perhaps with the sialic acid removed, other polymers besides trimers are significant, and these polymers' concentrations vary according to mass action. Although the data are not extensive, there are clear qualitative differences in this third case. It is to be noted that both adult and embryonic N-CAM molecules have a significant content of sialic acid, and the neuraminidase treatment would presumably remove the sialic acid entirely, perhaps making this third experiment "unphysiological."

STICKING PROBABILITY

An a priori estimate of the sticking probability per collision cannot be made, since we have no knowledge of the rate of reaction of membrane bound N-CAM molecules. However, we can write the rate of formation of bridges between vesicles that are in contact (Bell, 1978, 1981) as

$$\frac{dn_b}{dt} = 4\pi\epsilon D_m T_{eq}^2 , \tag{21}$$

where n_b is the number of bridges per unit area, T_{eq} is the number of free N-CAM trimers per unit area on either vesicle, D_m is the diffusion constant for N-CAM trimer translation in the vesicle membrane, and $\epsilon \leq 1$ is a factor by which the actual bridge formation rate falls short of its diffusion limit and by which the initial rate falls with time (Torney and McConnell, 1983), averaged over collision durations. In the reconstituted vesicles, a fraction f of the N-CAM molecules may be oriented in the wrong direction and ineffective in mediating adhesion; this could be taken into account by multiplying M_0 by f in Eq. 19, thereby reducing T_{eq}, or equivalently by reducing ϵ. The total number of bridges, N_b, expected in a collision can be obtained on multiplying dn_b/dt by the collision duration τ and contact area A_c:

$$N_b = 4\pi\epsilon D_m T_{eq}^2 \tau A_c . \tag{22}$$

If a single bridge forms during a collision, this should suffice to stabilize the nascent aggregate against the small shear forces during incubation. The bridge may spontaneously break before subsequent bridges form, depending on the (unknown) dissociation rate constant. However, if a

[1] It may be useful to convert the equilibrium constant K with dimensions $(\mu g/mg\ lipid)^{-2}$ to an equilibrium constant $\tilde{K}$ with dimensions cm^4, K being the ratio of the number of N-CAM trimers per square centimeter to the number of (monomers per square centimeter)^3 on the outside of a vesicle. One N-CAM molecule has a molecular weight of $\sim 2 \times 10^5$ (Cunningham et al., 1983; Rutishauser, 1984), and a phospholipid molecule has a molecular weight of ~600. We make a reasonable assumption regarding the lipid mass density per unit area, that one such phospholipid molecule occupies 64 Å^2 on one surface of the bilayer. We further assume that the N-CAM molecules are dilute enough that they do not significantly displace lipid molecules. Then, with 14 μg N-CAM/mg lipid, there are $\sim 1.4 \times 10^{10}$ N-CAM molecules/cm^2, which corresponds to ~300 N-CAM molecules on a spherical vesicle of radius 4×10^{-1} μm. Thus, one must multiply K by $3^{-1} \times 10^{-18}$ to get $\tilde{K}$.

few bridges form during the collision, the resulting aggregate should be effectively irreversible (Bell, 1978), at least during incubation conditions.

The duration of a Brownian collision, τ_B, is estimated to be $\tau_B \simeq R^2/6D \simeq 7 \times 10^{-3}$ s (Bell, 1978), whereas the duration of a shear collision is $\tau_S \simeq G^{-1} \simeq 0.1$ s. The "area of contact" has been estimated as the area of a spherical vesicle that is within a distance Δ of a touching spherical vesicle, where Δ is approximately the length of an N-CAM bridge. This area is $A_c = \pi R\Delta \sim 6 \times 10^{-3}\ \mu m^2$ if $\Delta \sim 10^{-2}$ μm. We estimate an upper limit for T_{eq} to be $2 \times 10^9/cm^2$ (see the previous section). Finally, we take the diffusion coefficient D_m for the N-CAM trimers, 10^{-10} cm^2/s, to be slightly smaller than that observed in the mobile N-CAM fraction on chick embryo brain cells and chick embryo retina cells (Gall and Edelman, 1981). Putting these values into Eq. 22 gives for the number of bonds formed in a shear collision on the order of $10^{-2}\,\epsilon$ and in a Brownian collision on the order of $10^{-3}\,\epsilon$.

A number of conclusions can be drawn from these equations. First, Brownian collisions are expected to be appreciably less sticky than shear-induced collisions because of the shorter duration of the Brownian collisions. This suggests that the greatest difficulties may be in getting the smallest particles to stick to each other. Further, it is most likely that no bridges will form during a collision, especially a Brownian collision. Under these conditions, the values of N_B represent very nearly the probability of forming a single bridge, p_b, during a collision.

In a recent review, Edelman (1984) notes that the length of a N-CAM molecule may be as great as 4×10^{-2} μm, as visualized in electron microscopy. This suggests that the length of a bond between two vesicles could be larger than taken above, perhaps as large as $\sim 8 \times 10^{-2}$ μm. Nevertheless, the expected number of bonds formed per collision would be $\ll 1$, and the conclusions are unaffected.

REDISTRIBUTION OF N-CAM MOLECULES

At equilibrium, the number of bridges, N_b, in the contact area, A_c, between two vesicles in a dimer can be expected to be related to the concentration of N-CAM trimers, T'_{eq}, on each vesicle of area A, according to

$$\frac{N_b}{A_c} = K_b T'^2_{eq}, \tag{23}$$

with K_b an equilibrium constant for bridge formation. The trimer concentration T'_{eq} is calculated from Eq. 19, replacing M_0 with M'_0:

$$M'_0 = M_0\left(1 - \frac{3N_b}{AM_0}\right). \tag{24}$$

We can use Eqs. 19 and 23 to find the fraction b of monomers in bridges (b equals $3N_b/AM_0$). If we assume one contact area between two identical vesicles with the ratio A_c/A equal to 0.01, and if we take K_b to be 10^{-7} cm^2 (which corresponds roughly to an equilibrium constant in solution of 10^7 M^{-1}), we find for the data point with 18.8 μg N-CAM/mg lipid in data set one with $K = 7 \times 10^{-5}$ that b is 0.06, and in data set two with $K = 1.4 \times 10^{-3}$, we find that b is 0.4. (We assume $p = 1$, that is, that all N-CAM molecules are on the outside of the vesicle.) In these two cases, the equilibrium surface concentrations of unbound N-CAM trimers are, respectively, 0.9 and 0.4 of the value before adhesion. Thus, it is plausible that after one adhesion between two vesicles, a significant fraction of the bridging molecules is unavailable for sticking, and their sticking probabilities toward subsequent adhesions would be correspondingly reduced.

It remains to consider the time that is required for receptor redistribution and hence whether a significant depletion of free receptors can be achieved in the time between collisions. A lower limit to the redistribution time can be found by assuming that every receptor that diffuses to the contact area sticks there. The mean time, T_a, for absorption is then

$$T_a = \kappa_a\left(\frac{A_c}{A}\right)\frac{R^2}{D_m}, \tag{25}$$

where κ_a is a function of (A_c/A), given by Chao et al. (1981), for a circular contact area. For $A_c/A \simeq 0.01$, $\kappa_a \simeq 3.5$, and with $R = 0.2$ μm, $D_m = 10^{-10}$ cm^2/s, $T_a = 14$ s.

This redistribution time should be compared with the time between Brownian collisions that result in sticking, T_s, which is initially $T_s = [4\pi RDn_1(0)/p_b]^{-1}$. Expressing $n_1(0)$ in terms of the lipid concentration in micrograms per milliliter, a, from Eq. 12, we find $T_s = 1.4/ap_b$ s. Under the experimental conditions of Hoffman and Edelman (1983), $a \simeq 1.0$ so that if $p_b \leq 0.01$, as appears likely for synthetic vesicles, $T_s \gtrsim 10\ T_a$, and there appears sufficient time for receptor redistribution.

Thus, the redistribution of receptors remains a possibility for explaining the small proportion of vesicles that rapidly appear in superthreshold aggregates. That is, small aggregates that have sequestered a substantial fraction of their receptors will stick with smaller probability and contribute only very slowly to the formation of superthreshold aggregates. Such long-time behavior was not followed in the experiments.

DISCUSSION

We have seen that an aggregation process in which the state of aggregation changes only as a result of binary collisions will lead to the appearance of mass (or volume) in superthreshold aggregates at a rate proportional to the square of the initial monomer concentration, as observed by Hoffman and Edelman (1983). This result was derived under the assumption that the collision rate constants, $c_{i,j}$,

and sticking probabilities, $p_{i,j}$, are constants. Under the experimental conditions of Hoffman and Edelman (1983), it appears that both Brownian and shear-induced collisions may be important, and we have estimated the maximum rate of appearance of volume in superthreshold aggregates for both types of collisions and compared the results with experiment. The results are summarized by an aggregation parameter, k_{agg}. For neuraminidase-treated "E-form brain vesicles," the calculated value of k_{agg} is close to the observed value, whereas for all other experimental cases, the observed values are smaller. In particular, for reconstituted vesicles, observed values of k_{agg} are smaller than the theoretical maximum value by factors of $\sim 10^{-4}$–10^{-2}. This suggests that the sticking probability has comparable values (10^{-4}–10^{-2}). Our estimates for the probability of bridge formation during a collision are consistent with this range of sticking probabilities.

According to our theoretical model, all material should eventually appear in superthreshold aggregates, whereas in fact only a small fraction does so during the time of observation. Shear-induced disaggregation is probably responsible for this effect. This conclusion is supported by the observation of further aggregation once large aggregates have been removed, as noted previously. There is no conflict between this conclusion and the finding that the initial rate of formation of superthreshold aggregates is proportional to the square of the initial vesicle concentration, because the constant rate of formation is strong evidence that disaggregation leading to vesicle loss from the superthreshold "compartment" is negligible during this interval. One could test this conclusion by carrying the experiment to longer times to see whether there is a real plateau that has been reached and by running the experiment at several shear rates to see how the rate of aggregation and the plateau are affected.

Quantitative calculations of the rate of formation of large aggregates are scarcely possible because of the uncertainty as to the shape of the aggregates and as to rate constants for bridge formation and breakage.

We have given a theoretical model explaining the remarkable dependence of k_{agg} on the N-CAM concentration: there is an equilibrium trimerization of N-CAM molecules, and each trimer can bond with a trimer on another vesicle, forming a "bridging molecule." This model is in agreement with two of the three sets of data presented, but it does not explain the behavior seen with neuraminidase-treated vesicles. Ultimately, the validation of the theory will rest on the observation of bridges composed of two N-CAM trimers, possibly in "freeze-fracture" experiments. The observed dependence may be the manifestation of the type of regulation of N-CAM–mediated cell adhesion in vivo. When more is known about the "binding site" and the structure of the molecular units bridging two vesicles, one can ask more detailed questions about the kinetics of bridge formation in an experiment such as we have analyzed here and in vivo.

APPENDIX

Aggregation for Special Collision Kernels

Brownian. For Brownian collisions (Chandrasekhar, 1943), the collision kernel $c_{i,j}$ is

$$c_{i,j} = 4\pi D_{ij} R_{ij}, \tag{A1}$$

where D_{ij} is the mutual diffusion coefficient ($D_{ij} = D_i + D_j$), and R_{ij} is the collision radius [$R_{ij} \simeq \frac{1}{2}(R_i + R_j)$]. Since the diffusion coefficient, D_i, of a (roughly spherical) particle is inversely proportional to its radius, R_i, it is customary to set $D_i R_i = DR$, a constant $\simeq 2 \times 10^{-13}$ cm^3/s. Eq. A1 then becomes

$$c_{i,j} = 2\pi DR \frac{(R_i + R_j)^2}{R_i R_j}. \tag{A2}$$

If $R_i \simeq R_j$, then $c_{i,j}$ is a constant,

$$c_{i,j} \simeq 8\pi DR. \tag{A3}$$

Note that for spherical particles $R_i \sim i^{1/3}$, so

$$c_{i,j} \simeq 2\pi DR \frac{(i^{1/3} + j^{1/3})^2}{i^{1/3} j^{1/3}},$$

and large aggregates can accrete monomers more rapidly than small ones. If $i = 45$, corresponding to a threshold aggregate $c_{45,1} \simeq 11.7\pi DR$, only 46% larger than Eq. A3, then the approximation does not appear seriously in error. Moreover, the shape of aggregates is not spherical but irregular, so that refinements beyond Eq. A3 are unwarranted.

With the collision kernel A3, and assuming unit sticking probability $p_{i,j} = 1$,

$$n_k(\tau) = n_1(0)[(c\tau)^{k-1}/(1 + c\tau)^{k+1}], \quad k = 1, 2, \cdots \tag{A4}$$

(Smoluchowski, 1916; Chandrasekhar, 1943) so that

$$V(\kappa, \tau) = V_1 \sum_{k=\kappa}^{\infty} k n_k(\tau) \simeq \frac{n_1(0) V_1}{c\tau(1 + c\tau)} \int_{k=\kappa}^{\infty} k \left(\frac{c\tau}{1 + c\tau}\right)^k dk,$$

where, for large values of κ, we have replaced the sum by an integral. The integral is readily evaluated and, for large values of $c\tau$,

$$V(\kappa, \tau) = V_0 \exp\left(-\frac{\kappa + 1}{c\tau}\right)\left(1 + \frac{\kappa}{c\tau}\right), \tag{A5}$$

where V_0 is the initial volume of vesicles, $V_0 = n_1(0)V_1$. Note that for $c\tau \gg \kappa$, $V(\kappa, \tau) \to V_0$; i.e., for large times, all the initial material is predicted to appear in superthreshold aggregates.

Shear Flow. For uniform shear flow with shear rate G s^{-1}, the collision kernel is approximately

$$c_{i,j} = \frac{4}{3} G(R_i + R_j)^3 \tag{A6}$$

(Smoluchowski, 1916; see also Bell, 1981). (An accurate treatment including hydrodynamic effects would be much more complex; see Curtis and Hocking, 1970, and Jeffery and Onishi, 1984.) If the aggregates are roughly spherical, then it is reasonable to set $R_i = R_1 i^{1/3}$ so that

$$c_{i,j} \simeq \frac{4}{3} G R_1^3 (i^{1/3} + j^{1/3})^3. \tag{A7}$$

Since

$$f_{ij} \equiv (i^{1/3} + j^{1/3})^3 = i + 3i^{2/3} j^{1/3} + 3i^{1/3} j^{2/3} + j,$$

we see that for $i \gg j$ or $i \ll j$, it may be reasonable to set $f_{ij} \simeq f^0_{ij} \equiv i + j$. However, for $i = j, f_{ij} = 4f^0_{ij}$. Thus, a more reasonable approximation for f_{ij} is $2(i + j)$, since this is in error by no more than a factor of 2 as compared with Eq. A7 for all i,j. This approximate kernel,

$$c_{i,j} \simeq \tfrac{8}{3} GR_1^3(i + j) , \qquad \text{(A8)}$$

gives simple solutions for $n_k(\tau)$ and $V(\kappa, \tau)$. Since aggregate shapes are uncertain, we have taken Eq. A8 as a simple representation for shear-induced collisions. It has at least the needed property of letting large aggregates add monomers much faster than small aggregates can.

Trubnikov (1971) has shown that the kernel A8 gives

$$n_k(\tau) = n_1(0) \frac{k^{k-1}}{k!} \frac{f}{1 - f} \exp\{-k[1 - f - \ell n(1 - f)]\} , \qquad \text{(A9)}$$

where $f = \exp(-\beta\tau)$, and $\beta = \tfrac{8}{3} GR_1^3$. For large values of k and $\beta\tau$,

$$n_k(\tau) \simeq n_1(0)(2\pi k^3)^{1/2} f \exp(-\tfrac{1}{2} kf^2) . \qquad \text{(A10)}$$

On summing over $k \geq \kappa$ and replacing the sum by an integral, we find

$$V(\kappa, \tau) = V_0 \left[1 - erf\left(\sqrt{\frac{\kappa}{2}} e^{-\beta\tau}\right)\right] . \qquad \text{(A11)}$$

As for the Brownian case, it is predicted that for large τ, $V \rightarrow V_0$, and all material will be found in superthreshold aggregates. Whereas in the Brownian case, the required time is $\tau \sim \kappa$, for the shear kernal, $\tau \sim \ell n\kappa$ because large aggregates grow preferentially.

Numerical Results: Combined Kernels. The detailed numerical results obtained by combining Brownian and shear kernels will be published separately (Spouge, J., and G. I. Bell, manuscript submitted for publication).

For the experimental conditions of Hoffman and Edelman (1983), it appears that Brownian and shear collisions are of comparable importance. The combined kernels will promote more rapid growth because the Brownian collisions predominate for small aggregates, which, once they reach a critical size, grow much more rapidly due to shear collisions. For $i \simeq j$, the critical size can be estimated by equating the collision kernels in Eqs. A3 and A6 to obtain a critical radius, R_c,

$$R_c = \sqrt[3]{\frac{3\pi}{4}} \frac{(DR)}{G} , \qquad \text{(A12)}$$

equal to ~0.36 μm for the experimental conditions of Hoffman and Edelman (1983).

As an approximation for the combined effects of Brownian and shear motion, we can add the collision kernels given by Eqs. A3 and A8 to obtain

$$c_{i,j} \simeq 8\pi DR + \tfrac{8}{3} GR^3(i + j) . \qquad \text{(A13)}$$

In the notation of Spouge and Bell (manuscript submitted for publication), the shear fraction, δ, in this kernel is given by

$$\frac{4\delta}{1 - \delta} \simeq \frac{GR}{3\pi DR} ,$$

so that $\delta \simeq 0.01$. For this value of δ, numerical results indicate that $k_{app} \simeq 2k_B$ or approximately the sum of k_B and k_S.

More accurate treatments of the combined effects of shear and Brownian motion are available for two colliding spheres of equal size (Zeichner and Schowalter, 1977; van de Ven and Mason, 1977, and references therein), but not for more general cases.

The least-squares fits to the data shown in Fig. 1 were obtained by Dr. Richard J. Beckman of the Statistics Division at Los Alamos National Laboratory, using the NLIN procedure and the Marquardt Method of the SAS package (Ray, 1982). We are indebted to S. Hoffman for communication of unpublished results and to him, U. Rutishauser, and G. Edelman for useful discussions.

This work was supported by the United States Department of Energy.

Received for publication 15 April 1985 and in final form 5 July 1985.

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