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Theoretical Models for the Specific Adhesion of Cells to Cells or to Surfaces

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Introduction

There are many examples in which the adhesion of cell to cell or of cell to substrate is mediated by bonds between specific molecules. In some cases, the molecules are well understood. Consider, for example, a solution containing cells such as red blood cells which do not ordinarily stick to one another. Suppose that molecules, such as antibody molecules, which have two or more sites for binding to specific determinants on the cell surface are introduced into the solution. A single molecule may be able to bind simultaneously to determinants on two cells and thus a sufficient concentration of such molecules may agglutinate the cells. Such agglutination of red cells by specific antibodies is commonly used for determining blood types. In other experiments the agglutination of various cells by protein molecules called lectins[1] has been analysed. These studies generated considerable excietment about a decade ago when it was found that tumor cells are more readily agglutinated[2] than normal cells.

In these examples, both the bridging molecules and the cell surface determinants are fairly well characterized, while in many systems in vivo the adhesive interactions appear to involve specific molecules but are

less completely understood. For example, in various developing systems, such as aggregating cells of the cellular slime mold[3] or sponge cells[4] or in the developing retina[5], cell-cell adhesion seems to involve specific molecular interactions but the molecules are not completely characterized. In addition, it appears that the immune system involves cell-cell interactions mediated by antibody molecules, antigen molecules, antigen-antibody complexes, or by the interactions of mutually complementary cell surface receptors. Finally many studies have been made of the adhesion of c surfaces where the interaction appears to involve interaction between extracellular molecules such as fibronectin[6] and cell surface determinants. Again such studies have been motivated by findings that tumor cells have altered adhesive properties[6,7] which may account in some essential ways for the invasive and metastatic properties of the tumor cells.

During the past couple of years I have attempted to construc some parts of a theoretical framework for the analysis of cell adhesion mediated by specific molecular interactions. In this paper, I will review some components of this framework and describe some recent experimental and theoretical results.

The Conceptual Model

I have in mind the fluid mosaic model of the cell membrane[8] wherein the membrane is regarded as a phospholipid bilayer in which various integral membrane proteins are retained by virtue of their favorable free energy in the hydrophobic interior of the membrane.

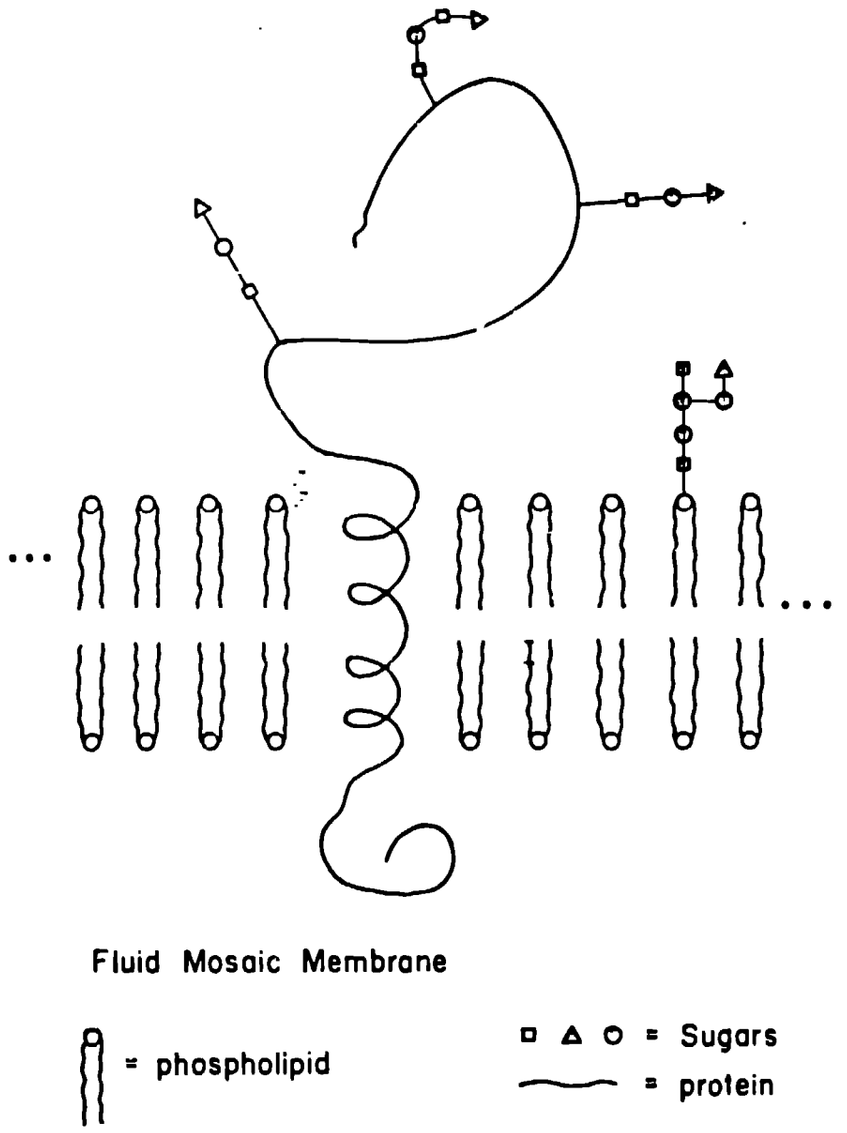

Figure 1. Fluid mosaic model of a cell membrane.

Proteins of interest for cell to cell binding have portions which extend beyond the lipid bilayer on the outside of the cell and most are glycoproteins, that is, they include covalently attached sugar moieties. The proteins are more or less free to move translationally in the plane of the membrane and to rotate about an axis perpendicular to the membrane. The translational diffusion coefficients of several integral membrane proteins have been measured and values $\sim 10^{-10}$ cm^2/sec are regarded as typical[9]. However, some surface proteins are virtually immobile[10] while others may move more freely (see following sections). By contrast the diffusion coefficients for typical proteins of molecular wieght $\sim 10^5$ in aqueous solutions are $\sim 5 \times 10^{-7}$ cm^2/sec.

For present purposes, let us designate as receptors those cell surface molecules which may mediate the adhesion of cell to cell or to substrate. Thus various membrane proteins or glycolipids might serve as receptors. For example, certain lymphocytes have antibody-like surface molecules which can serve as receptors in binding the cells to complementary soluble antigens or to antigenic determinants on the surfaces of other cells or on substrates. We shall regard such antibody-antigen interaction as a paradigm for the interaction between complementary receptors which may produce cell-cell adhesion.

In addition, soluble ligands including antibodies, antigens, lectins, and antigen-antibody complexes can mediate cell-cell adhsion. In all cases the ligand must have at least two binding sites, one of which interacts with each cell so as to form a molecular bridge between the cells.

Both receptor molecules and ligands may have multiple binding sites and any general theoretical treatment would be quite complex. In order to

clarify the physical effects, I shall emphasize the simplest situation, in which the adhesion is produced by interaction between mutually complementary receptors, each with a single binding site. Adhesion produced by bivalent ligands interacting with monovalent receptors is only slightly more complex[11]. The main complication is that the ligands may crosslink receptors on each cell as well as form bridges between cells. Although I will emphasize cell-cell interactions, it should be recognized that most of the results would hold with minor modifications for cell-substrate interactions and that some would apply to the interaction of a cell with itself, in which, for example a cell protrusion sticks to some portion of the cell surface.

Although it is possible for cells with immobile receptors to stick to each other, this generally requires a high density of surface receptors on each cell. The problem has been treated by Chak and Hart[12].

Rate and Extent of Intercellular Bond Formation

Assume that two cells with complementary mobile receptors come into contact with each other. Unless there are very many receptors per unit area on say the second cell, it is unlikely that a particular receptor on the first cell will immediately find a complementary receptor on the second cell so positioned and oriented as to permit immediate binding. However insofar as the receptors are mobile they can diffuse about until by chance they achieve positions and orientations which do permit bond formation. A theory has been developed for predicting such rates of bond formation assuming that the receptors are diffusing on two dimensional surfaces with known diffusion coefficients[13,14]. In addition, something must be known about the intrinsic rate constants

for reaction of the receptors. This information can be obtained from measured reaction rates in solution, if available.

If $N_{1f}(x,t)$ and $N_{2f}(x,t)$ are the numbers of free receptors per unit area on the two cells at position x and time t, $N_b(x,t)$ is the number of intercellular bonds, and k_+ and k_- are the forward and reverse rate constants for intercellular bond formation, we may write

$$\frac{dN_b}{dt} \quad k_+ N_{1f} N_{2f} \quad - \quad k_- N_b \tag{1}$$

According to the theory[13,14] the rate constants may be calculated from the receptor diffusion constants, D_1 and D_2, which are measurable, and solution reaction rates. In particular

$$k_+ = 2\pi E(D_1 + D_2) \quad , \tag{2}$$

where E is a factor (< 1) by which the reaction rate falls short of the diffusion limit. Moreover the equilibrium constant, $K^m = k_+/k_-$, for membrane bound reactants may be related to the equilibrium constant K^s for reactants in solution by

$$K^m = \frac{K^s}{R} \tag{3}$$

where R is some distance ~ 10 - 100 Å, relative to a lipid bilayer, within which the reactants may be localized.

From these equations we can deduce some important conclusions. First of all, the rate of bond formation can be very large. Consider for example, cells such as small lymphocytes having $\sim 10^5$ receptors on their surfaces, which is a representative value for the number of antibody like molecules. These cells have radii ~4μm and hence area ~200μm^2

and thus ~500 receptors per μm^2. When these cells first come into contact, $N_b = 0$ and $N_{if} \cong N_i$, the total number of receptors per unit area. If we take[9] $D_1 = D_2 = 10^{-10}$ cm^2/sec and E = 0.1 then $k_+ \cong 10^{-10} cm^2/sec$ $= 10^{-2} \mu m^2/sec$ and hence from equation (1) $dN_b/dt \cong 2.5 \times 10^3/\mu m^2 sec$. Thus if these two cells are in contact over an area $\sim 1\mu^2$ for a few msec, they can establish ~10 bonds, which it will be seen, may suffice to provide rather tight binding.

After the cells have been in contact for a while, $N_b(x,t)$ will increase at positions in the contact area. Bond formation will reduce N_{fi} locally but further free receptors will diffuse into the contact area. Indeed, if the contact area is only a suitably small fraction of the cell surface, and if the diffusion of <u>free</u> receptors is not impeded by the presence of bound receptors, then[15] at equilibrium $N_{fi} \cong N_i$ so that the number of bonds per unit area is, from equations (1) and (3)

$$N_b \cong K^m N_1 N_2 = \frac{K^s}{R} N_1 N_2 \qquad (4)$$

This means that the number of bonds per unit area can greatly excede the initial number of receptors per unit area. Indeed this can happen even for moderate values of K^s because of the very high <u>local</u> concentrations of receptors adjacent to the cell surface. Suppose, for example, that $K^s = 10^6\ M^{-1} = 1.7 \times 10^{-15} cm^3/molecule$ and $R = 20\mathring{A}$. Then $K^m \cong 10^{-8} cm^2$ $= 1\mu m^2$ so that for $N_i = 500/\mu m^2$, $K^m N_i = 500$. This means that these parameters could lead to a five hundred fold concentration of receptors in the contact area, i.e. $N_b \cong 500\ N_i$, where i=1,2 and j=2,1.

Another way of interpreting these results is to observe that N_i/R can be interpreted as the local concentration of receptors adjacent to the surface. The above parameters give $N_i/R = 500 \times 10^8/2 \times 10^{-7}$

$= 2.5 \times 10^{17}$ molecules/cm^3 $= 0.4 \times 10^{-3}$ M, a remarkably high concentration of specific biological macromolecules.

Note that equation (4) gives a criterion for receptor redistribution. If $K^m N_1 >> 1$, then $N_b >> N_2$ so that the receptors on the second cell will accumulate in the contact area. Similarly if $K^m N_2 >> 1$, then $N_b >> N_1$ so that receptors will accumulate in the contact area on the first cell. The time for receptor redistribution is of the order of r^2/D where r is the radius of the contact area[15] which is in the range of a few seconds to a few minutes for $0.1\ \mu m < r < 1\ \mu m$ and $D \cong 10^{-10}$ cm^2/sec.

It should be noted that the foregoing model neglects biological complications which may be essential in many cases. Obviously, we have assumed that the receptors are mutually accessible to each other. This implies that the two cells must be able to get close enough together and that there must not be intervening macromolecules masking the receptors from each other. It is predicted[16,17] that non-specific electrical forces will permit the lipid bilayers to approach to within 50-100 Å of each other and indeed will favor such equilibrium separations. On the other hand it appears that at least *in vivo* cells are often separated by such molecules as collagen, fibronectin, or mucopolysaccharides[7]. In such cases, the adhesion of cells to molecules of the extracellular matrix is important, while opportunities for direct contact between integral membrane glycoproteins may be minimal unless the matrix is disrupted.

Another problem concerns the mobility of cell receptors. For one thing, in photobleaching experiments[9] a fraction of the cell surface molecules often appear to be immobile. There are various explanations for this apparently immobile fraction, but one possibility might be

that there are local variations of receptor mobility over the cell surface such that, for example, receptors on cell protrusions such as microvilli are relatively immobile. This is believed to be the case for intestinal epithelial cells. In addition, there is evidence that the adhesion of cells to objects can reduce the mobility of receptors on the whole cell surface[18], presumably by modulating the linkage of receptors to the cytoskeleton, and that cytoskeletal connections accumulate in regions of cell-cell contact[19].

Thus under various circumstances cells can modulate the mobility of their receptors. In particular since adhesion per se may lead to mobility changes and indeed to receptor phagocytosis (eating) or excytosis (shedding), I suggest that the foregoing model may represent only the early stages of cell-cell binding and receptor redistribution. The sequelae may be complex and biologically important but are outside the scope of the model.

Strength of Specific Bonds

It is of interest to consider how effective specific bonds are in holding two cells together, or a cell to a substrate, in opposition to hydrodynamic or other forces. To this end I have estimated the force which is required in order to rapdily break a typical antigen antibody bond[13]. In general, bonding may be viewed as due to some free energy minimum, of depth $-E_o$, relative to well separated reactants, and of range r_o in some reaction coordinate. The force which is required in order to eliminate bonding is of the order of $F_o = E_o/r_o$, which for a typical antigen-antibody bond is around 1.2×10^{-5} dynes.

Of course each bond is spontaneously reversible and no force at all is required in order to break it if one is willing to wait long enough. But if two cells are stuck together by many bonds there is virtually no chance that they will all be broken at one time, unless a force is applied so as to stress the bonds (or unless the cells do something active to terminate binding). Under these circumstances, a bond lifetime τ, is expected to depend exponentially on the force per bond, f,

$$\tau \simeq \tau_o \exp[(E_o - r_o f)/kT] \qquad (5)$$

where τ_o is some natural bond frequency. τ_o can be estimated from E_o and $\tau(f=0)$, which is the reciprocal of the reverse rate constant for bond formation. In equation 5, T is the absolute temperature and k is Boltzmann's constant.

Using this approach, I have estimated[13] that a force $\simeq 4\times10^{-6}$ dynes/bond ($\simeq 1/3\ F_o$) will suffice for separating the cells. These estimates were made for particular bond parameters, E_o = 8.5 kcal/mole, r_o = 5Å, and for $K^m N_2 = 10^3$ and could vary by a factor two or more for other bond parameters of interest.

This force may be compared with other forces to which a cell may be subject. First of all there are non-specific electrical forces between cells because they are charged and polarizable objects. It has been predicted[16,17] that the long range (van der Waals) forces are attractive and that a force $\sim 10^{-5}$ dynes μm^{-2} is required to separate two cells from this attraction. Note that this is equivalent to about 2 of our specific bonds/μm^2. Since much larger receptor densities are expected, it is clear that specific bonds can be much stronger than the non specific attractive electrical forces.

The force required to hold a cell in a fluid stream can be estimated by Stokes law for laminar flow around a sphere. The result is[13], for a sphere of radius 4μm that ~13v bonds will suffice to resist a flow of v cm/sec. If, for example we are considering the adhesion of a lymphocyte to an endothelial cell in the venule of a lymph node[20] where v ≅ 0.3 cm/sec, this tells us that around 4 bonds should suffice to attach the cell.

Such estimates assume that all of the bonds are equally stressed. This may be a reasonable approximation for attachment of a lymphocyte to an endothelial cell, where a single microvillus on the lymphocyte appears to stick to a local pit on the endothelial cell[21], but it greatly overestimates the strength of attachment in other cases. For example, experiments have been performed[22] with cells which have been permitted to adhere to the surface of a circular disc. The disc is then spun in a fluid at an angular velocity such that cells near the periphery are stripped off while those near the axis are unperturbed. At some intermediate radius the cells are just barely removed.

The fluid flow near the rotating disc is nearly laminar and analysis shows[23] that the drag force or stress per unit area on the disc is

$$F_D = 0.8\ \mu\ r\ \omega^{3/2}\ \nu^{-1/2} \qquad (6)$$

where μ is the fluid viscosity (in dyne-seconds per cm^2), r is the radius under consideration, ω is the angular velocity and ν is μ/ρ with ρ the fluid density. In water μ = ν = 0.01 so that

$$F_D = 0.08\ r\omega^{3/2}\ \text{dynes/cm}^2. \qquad (7)$$

This force acts on the disc parallel to its surface and if there is a flattened cell on the surface this stress will tend to remove the cell from the surface. At some radius r there will be a critical sheer stress F_{DC} which is just sufficient to remove the cells. The force on

a cell of area A, prior to removal is then $F_{bc}A$ which is nearly balanced by the stressing of bonds which hold the cell to the surface. In an example, a critical stress of ~50 dynes/cm^2 was measured[22] and if the cell area were ~200μm^2 = 2×10^{-6}cm^2 the force per cell would be ~10^{-4} dynes. This could be sustained by ~25 of our typical bonds.

In this example, the force is parallel to the surface and many of the cell-substrate bonds may be ineffective in opposing such motion. The only ones that can be clearly effective are those near the trailing edge of the cell which must break in order to permit cell motion. If further bonds were attached to the cell cytoskeleton they might also impede cell motion but the facts are not clear. It is observed that cells subject to near the critical stress are strongly deformed but details of their attachment are unclear. Nevertheless this example illustrates that as in all theories of adhesion, it is not straight-forward to predict macroscopic yield stresses from theoretical microscopic bond strengths.

In our estimate of bond strength, it was assumed that as a receptor-receptor bond is stressed, this will be the weakest link in the chain. It is easy to see that covalent bonds in a receptor are much stronger for they have about tenfold larger values of E_o and threefold smaller values of r_o. However it is less clear that the receptor will not pull out of the lipid bilayer. For a particular integral membrane protein, glycophorin, I have estimated[13] that the force required to uproot this receptor is about 1.0×10^{-5} dynes. For a ganglioside (lipid molecule with attached sugars) the corresponding force was estimated to be near 5×10^{-6} dynes. It thus appears that the competition between receptor uprooting and bond breaking will depend on the precise nature the receptor and strength of the bond.

Some Recent Experiments

The foregoing theoretical framework may be used to design and interpret a wide range of experiments. Consider, for example a surface which has been coated with some ligand to which certain cell receptors can bind. The cell can attach to the surface by means of receptor-ligand interactions and receptors on the top surface may, if still mobile, diffuse to the bottom surface and stick there. This motion has been exploited by S. Silverstein and colleagues[24] to measure the diffusion constant of Fc receptors on macrophages. These receptors are protein molecules which bind to the Fc or stem portion of certain classes of antibodies. The experiment is performed such that the cells are first allowed to spread on the surface which is then coated with antibodies in the cold. The cells are then warmed and a rapid disappearance of Fc receptors from the top surface is observed while other receptors on top do not disappear. From the observed rate of disappearance and assuming that receptors which diffuse off the top never return, it is possible to deduce for the receptors in the cell membrane, a diffusion coefficient $\gtrsim 10^{-9} cm^2/sec$. This is a much larger value of D than expected from photobleaching experiments on other receptors and other cells. In the photobleaching experiments, fluorescent ligands are first bound to the receptors and then bleached by a pulse of laser light. The question is thus raised whether in such experiments the mobility of the receptor may be reduced by either ligand attachment or the light pulse. In this context it is of interest to note[25] that hybrid antibodies, one arm of which binds to a receptor and the other arm to ferritin or to a virus, are capable of inducing receptor clustering, a reaction normally associated with the crosslinking of receptors by bivalent ligands. These results raise the possiblity that fluorescein

labeled ligands might cause unexpected receptor-receptor and/or receptor-cytoskeleton interactions and reduce receptor mobilities.

A quite different set of experiments[26] has concerned the fusion of intracellular granules with the cell membrane, which is the common mechanism whereby cells secrete hormones, neurotransmitters, and other specialized biochemicals. These granules are lipid bilayers, with imbedded proteins, inside of which are the molecules to be secreted. The secretory event seems to be triggered by a transient increase in the concentration of cations, usually Ca^{++}, in the vicinity of the granule which facilitates adhesion of the granule to the cell wall. The two lipid bilayers then break down in the vicinity of the adhesive contact allowing the contents of the granule to be released outside the cell. All of these events can take place in a fraction of a second.

New light may have been shed on these secretory processes by studying the adhesion between chromaffin granules from cells of the adrenal glands[26]. It was found that in the presence of sufficient cations, which are presumably required to neutralize charges on the granule surfaces, the granules will stick together on nearly every collision. If however, the proteins are removed, the sticking probabilities are reduced by around two orders of magnitude. The interpretation[27] is that protein-protein interactions facilitate efficient sticking.

After two granules are firmly stuck together, freeze-fracture electron microscopy suggests that proteins are systematically excluded from the contact area. It has been suggested[27] that this is because certain lipids become concentrated in the contact area. That is, the protein mediated contact facilitates a phase separation of lipids which in turn excludes proteins, save around the periphery of the contact area.

This example suggests that some of our model considerations may be applicable to rather different and important biological situations. It also points out that other phenomena such as, in this case, the requirement for cations and the phase separation of lipids may be involved.

I have sketched elsewhere[15] some of the applications which may be expected when adhesion involves two different kinds of interacting receptors on each cell. These considerations were developed as a possible model for the genetic restriction of immune responses, but more generally, I suggest that this example and those cited above show that diverse and complex interactions may be expected to result from cell-cell contact mediated by specific receptors and/or ligands.

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