EQUILIBRIUM THEORY FOR THE CLUSTERING OF BIVALENT CELL SURFACE RECEPTORS BY TRIVALENT LIGANDS

Application to Histamine Release From Basophils

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ABSTRACT For certain cell types, the cross-linking of bivalent cell surface receptors by multivalent ligands is an important biochemical step in the transmission of information across the cell's membrane to its interior. The formation of cell surface receptor-ligand aggregates has been shown to "turn on" and "turn off" particular cell responses. It has been hypothesized that very large aggregates generate signals that small aggregates cannot. This hypothesis has not been rigorously tested as yet, in part because of a lack of quantitative information about aggregate sizes. Here we develop a general equilibrium theory for the clustering of bivalent receptors by trivalent ligands. In addition to predicting the concentrations of receptor-ligand aggregates of all possible sizes, we show that a range of ligand concentrations exists at which extremely large aggregates, i.e., superaggregates, form on the cell surface. The formation of a superaggregate corresponds to a sol-gel phase transition, and we study this transition in some detail. For the biologically interesting case of histamine release by basophils, we show, using realistic parameter values, that such transitions should occur when the cells are from highly allergic individuals. We prescribe in detail experimental conditions under which such transitions should occur. These conditions can be used as a guide to test whether or not large aggregates provide signals to cells that small aggregates do not.

INTRODUCTION

The aggregation of receptors on cell surfaces triggers significant physiological responses in a variety of biological systems. For example, the clustering of the epidermal growth factor (EGF) receptor on human epidermoid carcinoma cells leads to the aggregation of these receptors in coated pits and the induction of DNA synthesis (1). Anti-EGF receptor antibodies (2) which are capable of cross-linking EGF receptors, initiate many of the same biological events that EGF does. Similarly, bivalent antibodies raised against the insulin receptor mimic many of the effects of insulin (3,4). Aggregation of the gonadotropin releasing hormone receptor into microaggregates, possible dimers, leads to the release of pituitary luteinizing hormone from pituitary cultures (5,6).

One of the best worked out examples of the role of receptor aggregation in initiating cellular responses is the immunoglobulin E– (IgE-) mediated activation and desensitization of basophils and mast cells. In the presence of calcium, clustering of cell surface IgE antibodies, or the Fc_ϵ receptors that IgE binds to, triggers mast cells and basophils to release histamine (7–9). Simply forming IgE dimers is sufficient to trigger both histamine release (10) and specific desensitization (11). The binding of monomeric ligands to IgE triggers neither release nor desensitization (12,13). As expected, trimers of IgE also trigger release, but, surprisingly, the trimer release signal appears to be qualitatively different than that of the dimer (14,15). Whether very large aggregates can generate signals that small aggregates cannot is unknown. It is still an open question, for example, whether the nonspecific desensitization of basophils (16) is triggered by large aggregates of IgE or by large numbers of cross-linked IgE, independent of the size of the aggregate in which they occur (17).

When basophils and mast cells are exposed to a bivalent ligand, the only surface aggregates that can form are linear and circular chains of IgE and ligand. If the ligand's valence is three or greater, networks of IgE and ligand can be built up, and, at least for some parameter values, both aggregates and "superaggregates" can form on the basophil surface. These superaggregates, which correspond to the gel phase in a system capable of undergoing a sol-gel transition, can span a large fraction of the cell surface. Although the valence of almost all naturally occurring allergens (ligands capable of triggering an allergic reaction culminating in histamine release from basophils and mast cells) is unknown, the expectation is that many are highly

multivalent. Thus, it is worth while investigating if, for the parameters that characterize the basophils, sol-gel-like phase transitions can occur.

If the receptor and ligand each has a valence of at least two and either the ligand or receptor has a valence of three or greater, the possibility exists that gelation can occur on the cell surface. Here we consider the simplest case where gel-like states can form. We present an equilibrium theory for the binding of a trivalent ligand to a bivalent cell surface receptor such as IgE. We derive conditions for the onset of the sol-gel transition and discuss under what experimental situations this transition can be observed on basophils. From our results for this simple model system we discuss possible ways to test if superaggregates are capable of generating unique biological signals.

THEORY

We consider a dilute suspension of cells that have a homogeneous population of bivalent receptors on their surfaces. The medium surrounding the cells also contains trivalent ligand at an equilibrium concentration C. The ligand can bind to the bivalent receptors, but we assume the concentration of cells is so dilute that ligands cannot simultaneously bind to receptors on different cells. We assume that there are X_T receptors per cell and that during the time it takes for equilibrium to be established X_T remains constant, e.g., both internalization or shedding of receptors and insertion of newly synthesized or recycled receptors into the membrane is negligible.

At equilibrium, we characterize a trivalent ligand binding to cellular receptors by three constants (see Fig. 1): K, the intrinsic affinity, which describes the binding of a single site on a ligand in solution to a single cell surface receptor site; K_x, the first cross-linking constant, which describes the binding to a single receptor site of a site on a ligand that already has one of its sites bound to a receptor; and K_{xx}, the second cross-linking constant, which describes the binding to a single receptor site of a site on a ligand that already has two sites bound to two other receptors. We assume that a ligand cannot have two of its sites bound simultaneously to the two sites on a single receptor. To present the theory it is useful to introduce the following nondimensional parameters:

$$c = 3KC \tag{1a}$$

$$\alpha = 4K_xX_T \tag{1b}$$

$$\beta = K_{xx}X_T. \tag{1c}$$

Linear Chains

Our procedure for characterizing the system is to enumerate and assign the correct statistical weight to all possible receptor-ligand complexes. We begin by considering linear chains. In Fig. 2 all linear chains that begin and end with a free receptor site are depicted. If we let X be the equilibrium concentration of free receptors (both sites unoccupied) on the cell surface, then, from Fig. 1 and Eqs. 1a and 1b, the equilibrium concentration of a complex of two receptors cross-linked by a single ligand is $12KK_xCX^2 = \alpha cX^2/X_T$ and the equilibrium concentration of a linear chain of n receptors cross-linked by $n - 1$ ligands is $(\alpha cX/X_T)^{n-1}X$. We now define certain quantities referred to in statistical mechanics as partition functions. A partition function is simply the sum of the concentrations of all aggregates or of a subclass of all aggregates. For example, we define Q_f, the partition function for linear chains with both ends free, as shown in Fig. 2, to be the sum $Q_f(X) = X\Sigma_{n=1}^{\infty}(\alpha cX/X_T)^{n-1} = X/(1 - \alpha cX/X_T)$.

In general each end of a linear chain can be in one of two states, free or bound. Taking this into account, Q_0, the

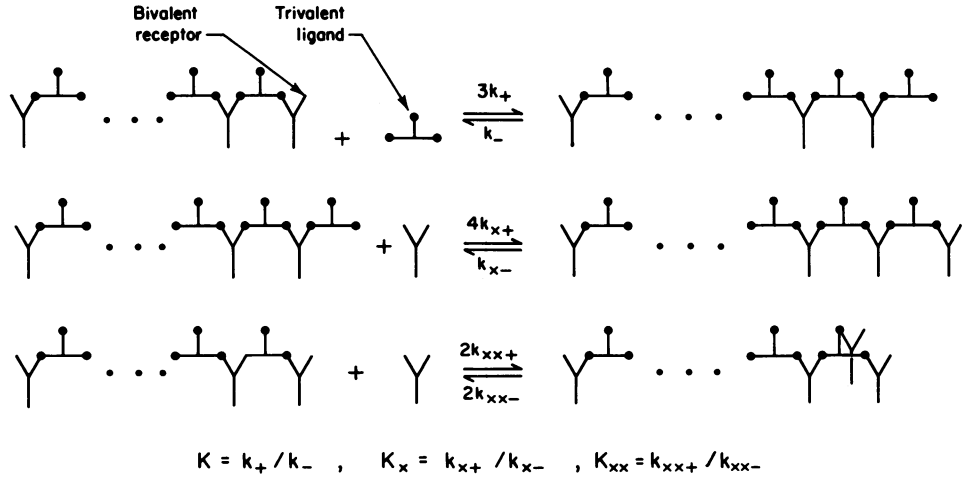

FIGURE 1 The three classes of reactions that occur between a bivalent receptor and a trivalent ligand: the binding of a single site on the ligand to a receptor site with equilibrium constant K, the first cross-linking step with equilibrium constant K_x, and the second cross-linking step with equilibrium constant K_{xx}. In the figure we have assumed the linear chain is planted at its left end and growth only can occur at the right. The statistical factors multiplying the rate constants indicate the number of ways each reaction can occur.

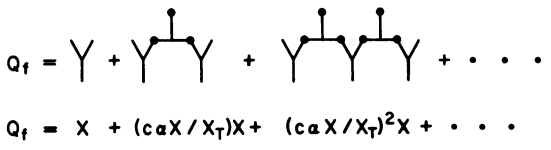

FIGURE 2 The partition function for linear chains which both begin and end with a free receptor site.

partition function for all linear chains becomes

$$Q_0(X) = (1 + c)^2X/(1 - \alpha cX/X_T). \quad (2)$$

We will work with the nondimensional partition functions $q_f = Q_f/X_T$ and $q_0 = Q_0/X_T$, and the nondimensional receptor concentration $x = X/X_T$. In terms of these quantities

$$q_0(x) = (1 + c)^2q_f = (1 + c)^2x/(1 - \alpha cx). \quad (3)$$

An important biological quantity is x_{poly}, the fraction of receptors in aggregates, which we define as

$$x_{poly} = 1 - w, \quad (4)$$

where

$$w = (1 + c)^2x. \quad (5)$$

The quantity w is the fraction of receptors in un-cross-linked states.

The nondimensional partition function for all linear chains can now be expressed as

$$q_0(w) = w/(1 - \delta w) \quad (6)$$

where

$$\delta = \alpha c/(1 + c)^2. \quad (7)$$

Dembo and Goldstein (18) previously analyzed the equilibrium binding of bivalent ligands to bivalent receptors and obtained results identical in form to Eq. 6. The only change is that for a bivalent ligand the statistical weights that give the number of ways a ligand can bind to a receptor are different, in particular $c = 2KC$ and $\alpha = 2K_xX_T$.

Branched Chains

To obtain the partition function, which includes both linear chains and branched structures, we follow a procedure for counting aggregates used by Gō in the theory of helix-coil transitions (19) and Wiegel and Perelson in their study of red cell aggregation (20). We shall only consider structures that do not contain loops. In graph theory, such structures are called trees. A subclass of trees that plays a central role in our counting method is "planted plane trees" whose nodes are of degree three or less. A planted tree is a tree with one end, its root, distinguished from all other ends (21). We begin our enumeration procedure at the planted end of a tree. A planted plane tree is a planted tree that can be drawn in the plane in such a way that no branches intersect except at nodes (21). The degree of a node is the number of branches incident with it (21). One can show by direct construction that a way to count and assign the correct statistical weight to molecular aggregates that contain no loops is to first count and weight planted plane trees and then to correct for the degeneracy that arises by having chosen one particular end at which to begin the counting. After we derive the partition function for branched aggregates, we will show by example that we have assigned precisely the same statistical weights as one would have obtained by considering a sequence of chain elongation and chain branching reactions that lead to the final structure. Gordon and Temple (22) and Gordon and Torkington (23) discuss in general the relationship between tree counting and the statistical weights in a partition function. In their discussion, they choose to map molecular aggregates into trees differently than we do and hence utilize a different tree counting procedure to obtain statistical weights. Tutte (24) and Gordon and Torkington (23) derive different methods of counting planted plane trees that give results that are equivalent to the ones we derive below. However, our method, in addition to counting the number of planted plane trees, automatically assigns a statistical weight to the tree and is thus more useful for our purposes.

To begin our counting, we define a planted plane tree of order 0 as a linear chain. We define a planted plane tree of order 1 to be a linear chain or a planted plane tree with one branch point (Fig. 3*a*). To obtain the partition function for trees with one branch point we begin assigning statistical weights at the root. All planted plane structures start with a linear chain whose rooted end may be free or bound. This initial chain contributes a term $(1 + c)q_f$ to the partition function. This chain terminates in a receptor with one free site. To form a branch point, a ligand must first bind to this site. This binding contributes a term $3KC$ to the partition function. A receptor must now bind to each of the two remaining free ligand sites. The binding of a receptor to the first ligand site contributes a term $4K_xX$ to the partition function, while the binding of the second receptor contributes a term $K_{xx}X$ (see Fig. 1). Thus, for a linear chain with a branch point leading to two receptors one obtains the nondimensional partition function $(1 + c)q_f\, c\alpha\beta x^2$. To count all planted plane trees with a single branch point we

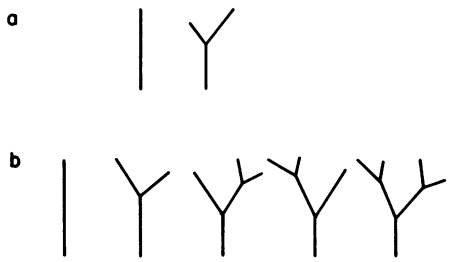

FIGURE 3 The planted plane trees of (*a*) order 1 and (*b*) order 2.

need to allow the two arms that bifurcate from the linear planted chain to be any length. Formally, we do this by replacing x with $q_f(1 + c)$ in the partition function. Consequently, q_1, the partition function for a tree of order 1 is given by $q_1 = q_0 + (1 + c)q_f c\alpha\beta\, q_f^2(1 + c)^2$. Using Eq. 3 this simplifies to

$$q_1 = q_0(1 + \gamma q_0^2), \tag{8}$$

where we have introduced the branching parameter γ,

$$\gamma = \frac{c\alpha\beta}{(1 + c)^3}. \tag{9}$$

We now define a planted plane tree of order k to be either a linear chain or a planted plane tree that begins with a linear chain and then bifurcates into two planted plane trees each of order $k - 1$. By analogy with the arguments given above, the partition function for a planted plane tree of order k is

$$q_k = q_0(1 + \gamma q_{k-1}^2). \tag{10}$$

So, for example, a planted plane tree of order 2 is either a linear chain or a linear chain that bifurcates into two planted plane trees of order 1. From Eq. 10 we find that the partition function

$$q_2 = q_0(1 + \gamma q_1^2). \tag{11}$$

When the expression for q_1, Eq. 8, is substituted into Eq. 11, the expression for q_2 becomes

$$q_2 = q_0 + \gamma q_0^3 + 2\gamma^2 q_0^5 + \gamma^3 q_0^7. \tag{12}$$

From the picture of planted plane trees of order 2 shown in Fig. 3 *b*, one sees that such trees may have any of five possible structures, with each structure contributing one term to the right side of Eq. 12.

As the index k is increased, planted plane trees of higher and higher complexity are generated. In the limit as $k \to \infty$, all possible planted plane trees are generated. We define q_p, the partition function for all planted plane trees, by

$$q_p = \lim_{k\to\infty} q_k.$$

From Eq. 10 we find q_p must satisfy the equation $q_p = q_0(1 + \gamma q_p^2)$. Of the two solutions only one approaches q_0 as $\gamma \to 0$. This solution, which is the one of interest, is given by

$$q_p = \frac{1 - (1 - 4\gamma q_0^2)^{1/2}}{2\gamma q_0}. \tag{13}$$

To obtain q, the partition function for branched molecular structures, we must correct q_p for certain degeneracies. First, when we counted all linear chains we ignored the fact that there are two possible choices for the root, the left or right end. In our subsequent counting of planted plane trees, we always assumed the tree began with an initial linear chain, i.e., its "trunk." This choice placed no constraint on possible tree structures, since we allowed the trunk to be as small as a single ligand or a single receptor. Because one can choose either the left or right end of the trunk chain as a possible root we multiply q_p by a factor of 2 to correct for the degeneracy in this choice. Second, a given molecular structure planted at any of its ends corresponds to the same molecular structure. We correct for this degeneracy by dividing the statistical weight for a planted plane tree with m branch points by $m + 2$, the number of free ends. To determine the statistical weight of a planted plane tree with m branch points, we note that if we expand q_p in a power series in γ, the coefficient of γ^m is the desired statistical weight. To obtain q, we could, in the power series for $2q_p$, replace γ^m by $\gamma^m/(m + 2)$. This means that

$$\frac{1}{2\gamma}\frac{d}{d\gamma}(\gamma^2 q) = q_p$$

where q_p is given by Eq. 13. Solving this differential equation, we find

$$q = \frac{1}{\gamma q_0}\left[1 - \frac{1 - (1 - 4\gamma q_0^2)^{3/2}}{6\gamma q_0^2}\right]. \tag{14}$$

As a check we note that in the limit of zero branching ($\gamma \to 0$) q reduces to q_0. As a further check, we compare below the concentration of aggregates of a particular size as predicted from q; the prediction was obtained by the usual chemical procedures.

Concentration of Aggregates Containing n Receptors

From the partition function q, we can obtain an expression for the concentration of ligand-receptor aggregates of different sizes and shapes (i.e., with different degrees of branching). Let w_n be the nondimensional concentration of aggregates containing n receptors. The previously introduced variable w, the fraction of receptors in un-cross-linked states, is w_1. From the definition of q_0 [Eq. 6], we note that w_n is composed of a weighting factor q_n multiplied by w^n, i.e.,

$$q = \sum_{n=1}^{\infty} q_n w^n \equiv \sum_{n=1}^{\infty} w_n. \tag{15a}$$

From Eqs. 14 and 6, we obtain the leading terms in this expansion:

$$\begin{aligned} q(w) = {} & w + \delta w^2 + \tfrac{1}{3}(3\delta^2 + 2\gamma)w^3 + (\delta^3 + 2\gamma\delta)w^4 \\ & + (\delta^4 + 4\gamma\delta^2 + \gamma^2)w^5 + \tfrac{1}{3}(3\delta^5 + 20\gamma\delta^3 + 15\gamma^2\delta)w^6 \\ & + (\delta^6 + 10\gamma\delta^4 + 15\gamma^2\delta^2 + 2\gamma^3)w^7 \\ & + (\delta^7 + 14\gamma\delta^5 + 35\gamma^2\delta^3 + 14\gamma^3\delta)w^8 \ldots \end{aligned} \tag{15b}$$

It is easy to see where each term that contributes to q_n comes from. In Fig. 4 we illustrate this for $n = 5$. The

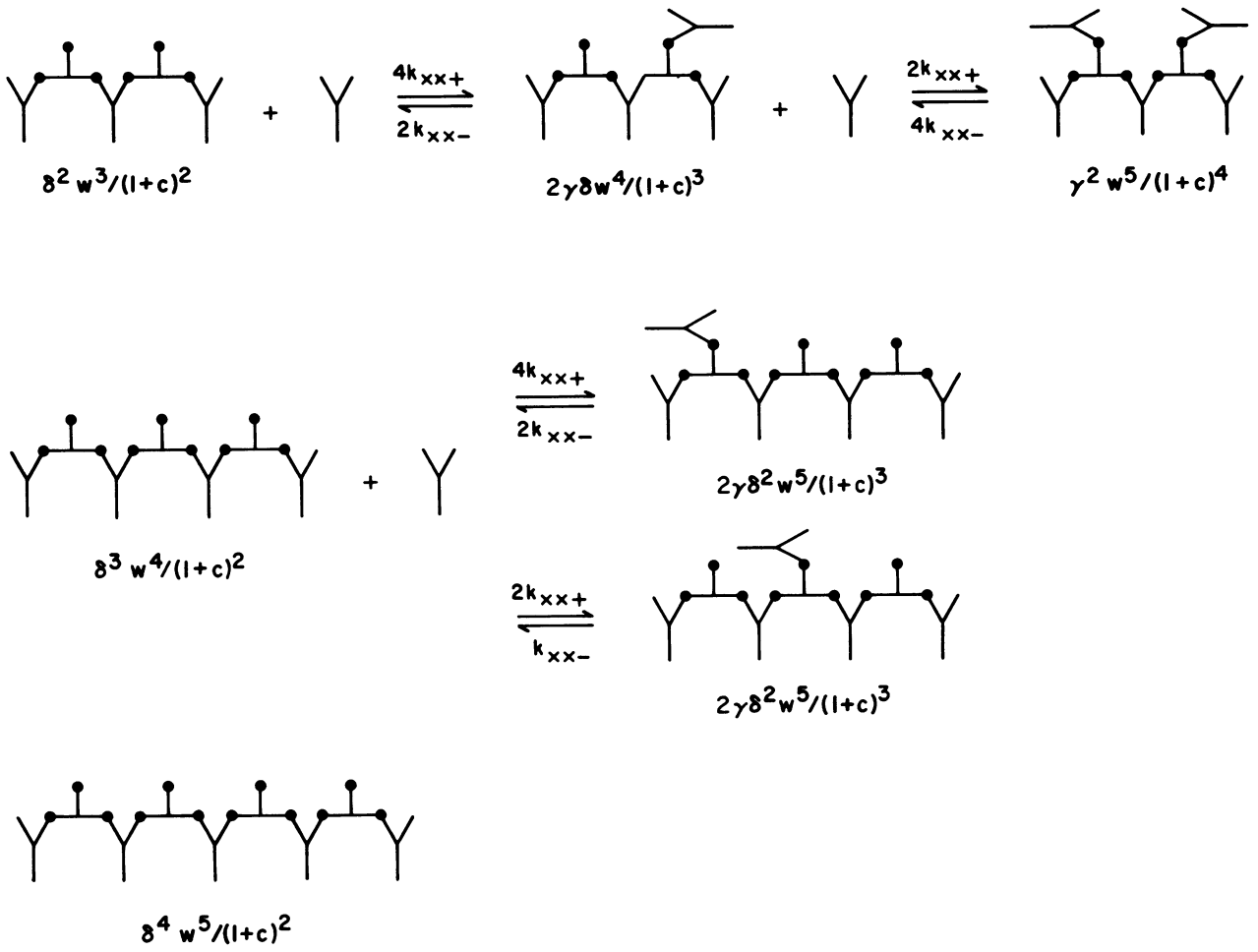

FIGURE 4 The statistical weights of aggregates with five receptors as constructed directly from the reactions agree with those predicted by Eq. 15b. The weighting factor q_5 in Eq. 15b is a sum of three statistical weights corresponding to the three types of aggregates five receptors can form: aggregates with two, one, or no branch points. We show how to obtain these weights from the law of mass action, starting from the known statistical weights for linear chains (Fig. 2). We show only aggregates whose ends are free. To obtain the statistical weight for all aggregates involving five receptors, i.e., each end being either free or bound to a ligand, we must multiply each statistical weight by $(1 + c)^e$ where e equals the number of ends. When this is done we find that $q_5 = (\delta^4 + 4\gamma\delta^2 + \gamma^2)w^5$.

example shown in Fig. 4 also demonstrates that our tree-counting procedure gives rise to the usual statistical weights as derived directly from chemistry.

We can find an analytic expression for the general term in the expansion of q as follows. Let $w_{n,m}$ be the nondimensional concentration of aggregates containing n receptors and having m branch points (i.e., $m + 2$ ends). Then q can be expressed as

$$q = \sum_{n,m} q_{n,m}\gamma^m w^n = \sum_{n,m} w_{n,m}. \tag{16a}$$

Using techniques discussed by Wiegel and Perelson (20), one can show that

$$w_{n,m} = \frac{2\delta^{n-2m-1}\binom{n-1}{2m}\binom{2m}{m}\gamma^m w^n}{(m+1)(m+2)}. \tag{16b}$$

Comparing Eqs. 15a and 16a one finds

$$w_n = \sum_m w_{n,m} = 2\delta^{n-1}w^n \sum_m \frac{\binom{n-1}{2m}\binom{2m}{m}(\gamma/\delta^2)^m}{(m+1)(m+2)}. \tag{17}$$

Unfortunately, a closed form expression for this summation cannot be obtained. (The term $\binom{2m}{m}/[m + 1]$ is a Catalan number [25] and the sum over m in Eq. 17 when $\gamma = \delta^2$, seems to be a new generalization of the Motzkin number [25]. This is not surprising since the Motzkin numbers determine the number of planted plane trees with n branches [26].)

Conservation Laws

The fraction of aggregates containing n receptors, w_n, is expressed in Eq. 17 in terms of w, the fraction of receptors in un-cross-linked states. To find w we note that the total

number of receptors on the surface is constant. Thus w must obey the following conservation law:

$$1 = \sum_{n=1}^{\infty} nw_n = w\frac{\partial q}{\partial w}. \tag{18}$$

Using Eqs. 6 and 14, we write the conservation law in the following form

$$1 = \frac{1}{\gamma w U(w)}\{2 - U(w) - 2[1 - U(w)]^{1/2}\}, \tag{19}$$

where

$$U(w) \equiv 4\gamma q_0^2 = 4\gamma w^2/(1 - \delta w)^2. \tag{20}$$

Note that if $U(w)$ is ever >1, Eq. 19 breaks down, i.e., there are no longer real solutions for w. As we shall see, the critical case, $U(w) = 1$, will indicate the onset of a phase transition, and, hence, $U(w)$ will play an important role in our theory.

Rewriting Eq. 19 with only $[1 - U(w)]^{1/2}$ on the right side, squaring the resulting equation, and substituting in Eq. 20 transforms Eq. 19 into

$$16\gamma^2 w^3[\gamma^2 w^3 + (2\gamma - \delta^2)w^2 + (1 + 2\delta)w - 1] = 0. \tag{21}$$

From Eq. 9 $\gamma \neq 0$ as long as $c \neq 0$. Further $w = 0$ only when all receptors are cross-linked, but such an equilibrium state can not be attained with finite cross-linking constants. Hence we assume $\gamma w \neq 0$ and reduce Eq. 21 to the cubic

$$\gamma^2 w^3 + (2\gamma - \delta^2)w^2 + (1 + 2\delta)w - 1 = 0. \tag{22}$$

Because Eq. 22 was derived by squaring the conservation law, extraneous roots have been introduced. In fact, if one takes Eq. 19 and changes the term $-2(1 - U)^{1/2}$ to $+2(1 - U)^{1/2}$, so the equation reads

$$1 = \frac{1}{\gamma w U(w)}\{2 - U(w) + 2[1 - U(w)]^{1/2}\} \tag{23}$$

and then operates on it in a manner analogous to the conservation law, one again derives Eq. 22. Real roots of Eq. 22 may therefore correspond to a solution of either Eq. 19 or Eq. 23. This is shown explicitly in Fig. 5 for a particular choice of γ and δ. For each value of c there is only one real root of Eq. 22. Substituting this value of w into Eq. 19, one finds the conservation law is only satisfied for $c < c_-^*$ or $c > c_+^*$, where c_-^* and c_+^* are positive concentrations whose values are determined below. We also show below that in the region $c_-^* < c < c_+^*$ (for which this procedure does not yield a value of w satisfying the conservation law), the system has undergone a phase transition and we predict that an infinite-sized aggregate, a gel, coexists with finite-sized aggregates, the sol.

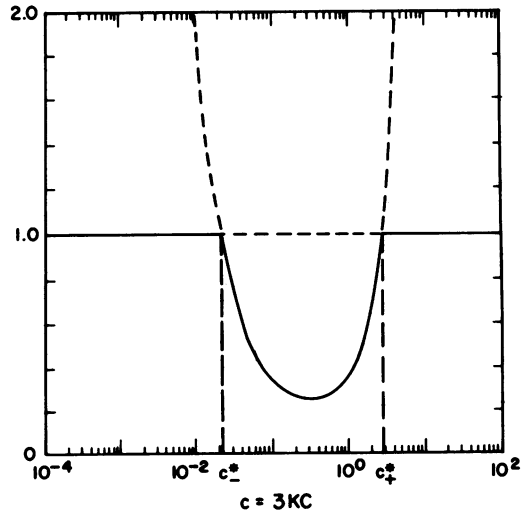

FIGURE 5 With $\alpha = 32, \beta = 8$, and $r_x = 1$, the cubic Eq. 22 was solved for w. For each value of c there was only one real root. $U(w)$ was formed according to Eq. 20 and substituted into Eq. 19, the conservation law, or Eq. 23. The right-hand sides of Eq. 19 (——) and Eq. 23 (---) are plotted. Note that w found by solving the cubic (Eq. 22) only satisfies the conservation law for $c < c_-^*$ and $c > c_+^*$. In the region $c_-^* < c < c_+^*$, w satisfies the pseudoconservation law, Eq. 23.

Moments of the Aggregate Size Distribution

At any external ligand concentration, one finds that on the cell surface there is a distribution of receptor-ligand aggregate sizes. Here we characterize that distribution by the number of receptors in aggregates. Let M_0, M_1, and M_2 be the zeroth, first, and second moment of the aggregate size distribution, where size is measured in terms of the number of receptors per aggregate. Then

$$M_0 \equiv \sum_{n=1}^{\infty} w_n = q, \tag{24a}$$

$$M_1 \equiv \sum_{n=1}^{\infty} nw_n = w\frac{\partial q}{\partial w}, \tag{24b}$$

$$M_2 \equiv \sum_{n=1}^{\infty} n^2 w_n = w\frac{\partial}{\partial w}\left(w\frac{\partial q}{\partial w}\right). \tag{24c}$$

The zeroth moment is the total number of finite-sized aggregates divided by the total number of receptors, X_T. The first moment is the total number of receptors in finite-sized aggregates divided by X_T. Thus, it all aggregates are finite, $M_1 = 1$, as indicated by the conservation law, Eq. 18. We define the value of a summation running from $n = 1$ to $n = \infty$ as the limit of $N \to \infty$ of finite sums ranging from $n = 1$ to $n = N$. If an infinite-sized aggregate, or gel, appears, then M_1 will be < 1 and $1 - M_1$ will correspond to the fraction of receptors in the gel phase.

During the course of a polymerization reaction, a point may be reached at which the probability of having an infinite-sized aggregate changes from zero to a positive value. This point is called the gel point. At the gel point, M_1 is still one, but because large aggregates have become prevalent, one finds $M_2 \to \infty$. Although we know of no

general proof that M_2 must become unbounded at the gel point, this in fact is the case in all well-studied examples.

The second moment, M_2, is related to a common measure of aggregate sizes in polymer chemistry, the weight average degree of polymerization,

$$DP_w = M_2/M_1. \tag{25}$$

At the gel point, M_2, and therefore DP_w, $\rightarrow \infty$. Because DP_w can be easily measured (e.g., by light scattering), the divergence of DP_w has become the practical criteria for having reached the gel point. From Eqs. 18–20 and 24c, one finds that

$$M_2 = -1 - \frac{2}{(1 - \delta w)}\{1 + [1 - (1 - U)^{-1/2}]/\gamma w\}. \tag{26}$$

Generation of Superaggregates

When $U = 1$ we see from Eqs. 25 and 26 that DP_w becomes unbounded, indicating the presence of infinite-sized aggregates, i.e., a gel. In a finite system, such as a cell surface, infinite-sized aggregates can not appear, but one finds that a single super-large aggregate forms, which is substantially bigger than any other aggregate (27–29). We shall show that the condition $U = 1$ can be met for biologically realistic values of C, γ, and δ in the well-studied system of IgE-mediated histamine release from human basophils. Hence we predict that sol-gel transformations can occur on cell surfaces in systems involving the binding of trivalent ligands to bivalent receptors.

No Superaggregates Form in Systems Containing Only Linear Aggregates

Before studying the occurrence of cell surface sol-gel transformations, we review the situation when only linear aggregates can occur (18). In this case $\gamma = 0$ and hence from Eq. 20, $U = 0$ if $q_0(w)$ is finite. When $\gamma = 0$, $q = q_0$, and the conservation law reduces to

$$1 = w\frac{\partial q_o}{\partial w} = \frac{w}{(1 - \delta w)^2}. \tag{27}$$

Eq. 27 is a quadratic equation for w that has the explicit solution,

$$w = \frac{1 + 2\delta - \sqrt{1 + 4\delta}}{2\delta^2}. \tag{28}$$

(The solution with the positive square root is not physical since w goes to infinity rather than one as the ligand concentration, and therefore $\delta \rightarrow 0$.) For all values of δ, i.e., $0 \leq \delta \leq \infty$, the solution for w, given by Eq. 28, satisfies the conservation law, Eq. 27, and has the properties that $w \leq 1$ and $\delta w < 1$. This means that $q_0(w) = w/(1 - \delta w)$ is real, nonnegative, and finite for all nonnegative values of δ. Thus $U = 0$, and linear chains never show a phase transition.

For linear chains, one can explicitly calculate w_n, the moments of the aggregate size distribution, and DP_w:

$$w_n = \delta^{n-1}w^n = \frac{1}{\delta}\left[\frac{1 + 2\delta - \sqrt{1 + 4\delta}}{2\delta}\right]^n,$$

$$M_0 = \frac{w}{1 - \delta w} = \frac{1 + 2\delta - \sqrt{1 + 4\delta}}{\delta(\sqrt{1 + 4\delta} - 1)}, \tag{29a}$$

$$M_1 = \frac{w}{(1 - \delta w)^2} = 1, \tag{29b}$$

$$M_2 = DP_w = \frac{w(1 + \delta w)}{(1 - \delta w)^3} = \frac{1 + \delta w}{1 - \delta w}$$

$$= \frac{1 + 4\delta - \sqrt{1 + 4\delta}}{\sqrt{1 + 4\delta} - 1}, \tag{29c}$$

where the expressions on the right were obtained by replacing w by its value in Eq. 28. Because $M_1 = 1$, $DP_w = M_2$.

When $\gamma > 0$ Branched Superaggregates Can Form

When cell surface aggregates can be branched structures, the situation changes dramatically. As shown in Fig. 6 for parameter values that characterize the cross-linking of IgE on the surface of a basophil, $U = 1$ in a biologically relevant range of ligand concentrations. In what follows, we shall first show that there are two values of c, c_-^*, and c_+^*, for which $U = 1$ for given values of the parameters γ and δ. Then we shall argue that for ligand concentrations between c_-^* and c_+^* a phase-transition occurs with an infinite-sized aggregate (a gel) coexisting with finite-sized aggregates (the sol). It is for concentrations between c_-^* and c_+^* that the conservation law, Eq. 19, breaks down.

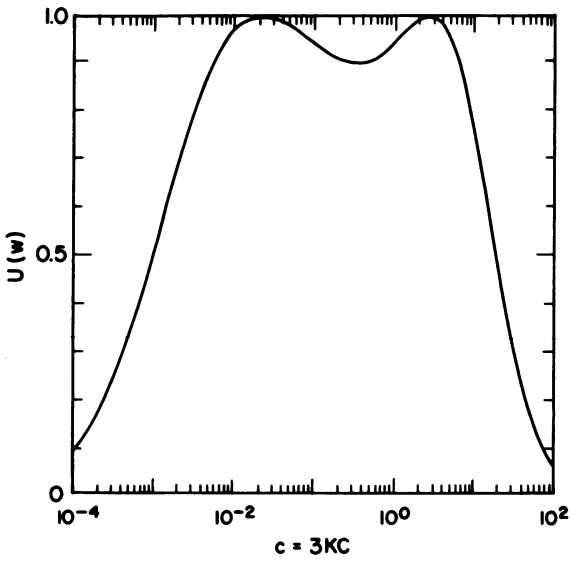

FIGURE 6 A plot of $U(w)$ vs. c for $r_x = 1$ and $\beta = 8$. For each value of c, Eq. 22 was solved for w and $U(w)$ calculated from Eq. 20. Note that $U = 1$ at c_-^* and c_+^*.

To determine the critical values of c that delinate the two-phase region, we set $U = 1$. From Eq. 19, the conservation law, we observe that when $U = 1$,

$$w = w^* = 1/\gamma^*, \tag{30}$$

where we use the superscript * to denote a "critical value," i.e., the value assumed when $U = 1$. Because w is the fraction of receptors in monomer, w^* must be <1 or, equivalently,

$$\gamma^* > 1. \tag{31}$$

Evaluating U at w^*, we see from Eq. 20 that only for parameters γ and δ that satisfy

$$1 = \frac{4\gamma^*}{(\gamma^* - \delta^*)^2}, \tag{32}$$

can $U = 1$. Because γ and δ are functions of c, Eq. 32 implies that only at particular values of c, designated c^*, can $U = 1$. Substituting Eqs. 7 and 9 for γ and δ and using the definitions in Eq. 1, Eq. 32 can be rewritten as

$$1 = \frac{K_x}{K_{xx}} \frac{c^*}{1 + c^*} \left(\frac{\beta}{1 + c^*} - 1 \right)^2. \tag{33}$$

Thus c^* is determined as the solution to the following cubic equation

$$(r_x - 1)c^3 + (3r_x - 2 + 2\beta)c^2 + (3r_x - 1 + 2\beta - \beta^2)c + r_x = 0, \tag{34}$$

where

$$r_x = K_{xx}/K_x. \tag{35}$$

In addition to being a solution to Eq. 34, c^* must obey the inequality Eq. 31 which, for $r_x > 0$, can be written in the form

$$4\beta^2 c^* > r_x(1 + c^*)^3. \tag{36}$$

Numerical simulations indicate that there are at most two real nonnegative values of c^* which solve Eq. 34 and obey this inequality. When $r_x = 1$ we can explicitly find these two values of c^*.

Special Case: Symmetric Trivalent Ligand ($r_x = 1$)

When $r_x = 1$, the first and second cross-linking constants are equal, i.e., $K_x = K_{xx}$. For this case Eq. 34 becomes quadratic in c with solutions

$$c_\pm^* = \frac{\beta^2 - 2(1 + \beta) \pm [\beta^3(\beta - 4)]^{1/2}}{2(1 + 2\beta)}. \tag{37}$$

For values of $\beta < 4$, there are no real solutions to Eq. 37. Thus, when $r_x = 1$, the possibility of a phase transition exists only for $\beta \geq 4$. When $\beta = 4$, we have from Eq. 37 that $c_+^* = c_-^* = 1/3$. This value of c^* obeys Eq. 36. As β is increased above 4, for example, by increasing the concentration of receptors on the cell surface, c_+^* and c_-^* separate and the concentration range in which a gel phase exists increases (see Fig. 7). For $\beta > 4$ we can show by tedious algebra that c_+^* and c_-^* obey Eq. 36.

For ligand concentrations corresponding to values of c between c_+^* and c_-^*, the sol and gel phase coexist. To make predictions about the compositions of these two phases (e.g., the fraction of receptors in sol) the fraction of receptors in gel, the distributions of aggregate sizes in sol, we must introduce an equation to replace the conservation law, Eq. 19, since it is no longer obeyed in the sol-gel region. We now examine one obvious choice for this equation, $U(w) = 1$.

$U(w) = 1$: An Equation of State in the Sol-Gel Region

In the sol-gel region, $c_-^* \leq c \leq c_+^*$; a fraction f_s of the receptors will be in the sol phase and a fraction $1 - f_s$ will be in the gel phase. Because there are X_T receptors per cell

$$X_s = f_s X_T, \tag{38}$$

where X_s is the concentration of receptors in the sol phase.

We define, for $c_-^* \leq c \leq c_+^*$,

$$\alpha_s = 4K_x X_s = \alpha f_s, \tag{39a}$$

$$\beta_s = K_{xx} K_s = \beta f_s, \tag{39b}$$

and from Eqs. 7 and 9

$$\delta_s = f_s \delta, \tag{40a}$$

$$\gamma_s = f_s^2 \gamma. \tag{40b}$$

We assume that in the sol-gel region the description that

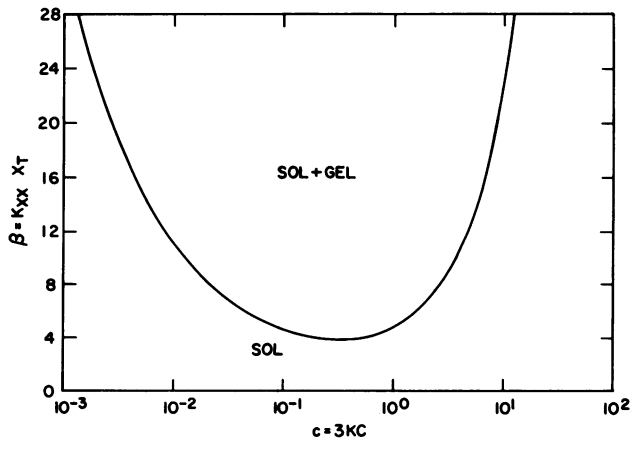

FIGURE 7 Phase diagram plotted for $r_x = 1$. The solid line is the locus of c_-^* and c_+^* values for different values of β. Note that when $\beta > 4$ sol and gel phases coexist for the region of c values between c_-^* and c_+^*.

we have developed still applies, but only to those receptors in the sol phase. The conservation law, which now holds only for these receptors, has the same form as Eq. 19, but with γ and δ replaced by γ_s and δ_s, i.e.,

$$1 = \gamma_s \frac{1}{w_s U(w_s)} \{2 - U(w_s) - 2[1 - U(w_s)]\}. \quad (41)$$

We designate w_s as the fraction of receptors in the sol that are not in aggregates. To calculate w_s, we need to know X_s or equivalently f_s, i.e., we need an additional equation that describes the coexistence of sol and gel. The equation that we choose is

$$U(w_s) = 1, \qquad c_-^* \le c \le c_+^*. \quad (42)$$

We have used the condition $U(w) = 1$ coupled with the conservation law to obtain c_+^* and c_-^*. When $U(w) = 1$, M_2 and all higher moments blow up indicating that the size of some aggregate has become unbounded. We now assume that $U(w) = 1$ holds throughout the sol-gel region, not simply the end points. Elsewhere we show that Eq. 42, when applied to the polycondensation of trivalent monomers is equivalent to Stockmayer's condition (30) for describing the sol-gel region (Perelson and Goldstein, in preparation).

Because $U(w_s) = 1$ and the conservation law, Eq. 41, both hold, γ_s and δ_s satisfy Eq. 32. Substituting δ_s and γ_s for δ^* and γ^* in Eq. 32 and solving for f_s, we find

$$f_s = \frac{(1 + c)}{\beta} [r_x^{1/2}(1 + 1/c)^{1/2} + 1]. \quad (43)$$

We can predict all the properties of the sol because when $U(w_s) = 1$, $w_s = 1/\gamma_s$. Thus, for $c_-^* \le c \le c_+^*$, one finds from Eqs. 40b, 30, and 9 that

$$w_s = (1 + c)^3/(cf_s^2\alpha\beta). \quad (44)$$

Now, for example, from Eq. 17 we can calculate the concentration of any size aggregate in the sol-gel region by replacing γ, δ, and w by γ_s, δ_s, and $w_s = 1/\gamma_s$.

We now study some of the characteristics of the sol-gel region. Eq. 43 predicts that f_s is inversely proportional to $\beta = K_{xx}K_T$, and therefore inversely proportional to X_T. Thus, for a fixed concentration between c_-^* and c_+^*, increasing X_T should decrease f_s in such a way that $X_T f_s$ remains constant. Thus, cells with large numbers of receptors should have a more prominent gel phase with a smaller fraction of their receptors remaining in the sol. For X_T fixed, $f_s = 1$ at $c = c_-^*$. As c is increased f_s first decreases to a minimum and then increases until $f_s = 1$ again at $c = c_+^*$ (see Fig. 8).

To determine the dimensionless concentration between c_+^* and c_-^* at which f_s is a minimum, c_{min}, we differentiate Eq. 43 and set $df_s/dc = 0$. We find that c_{min} is a solution to the equation

$$0 = -1 + 3c + 4(1 - 1/r_x)c^3, \quad (45)$$

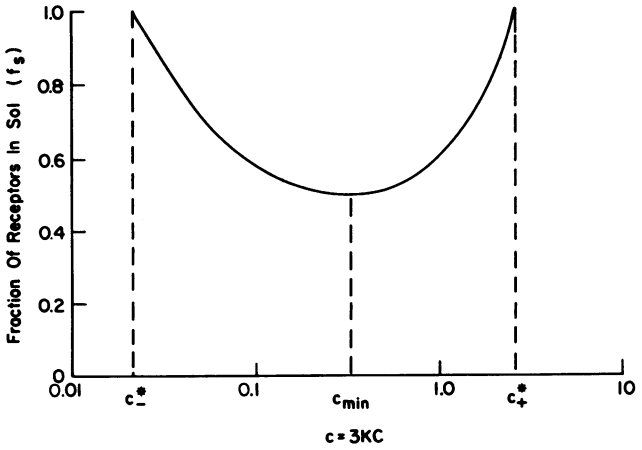

FIGURE 8 A plot of the fraction of receptors in the sol, f_s, vs. log c for $r_x = 1$ and $\beta = 8$. The minimum in the curve occurs at $f_s = 0.5$ and $c_{min} = 1/3$.

which can be expressed in the following form (31):

$$c_{min} = \sinh(\phi/3)/\sinh\phi \quad (46a)$$

where

$$\sinh\phi = (1/r_x - 1)^{1/2}. \quad (46b)$$

Because $c \equiv 3KC$, we see from Eq. 46 that the ligand concentration, C_{min}, at which f_s is a minimum depends only on the ligand-receptor single-site equilibrium constant K and $r_x = K_{xx}/K_x$, the parameter that measures the asymmetry in the ligand's ability to branch. Thus, for example, C_{min} does not depend on the cell surface receptor concentration nor on any properties of the cells themselves.

For the special case $r_x = 1$, $c_{min} = 1/3$ ($C_{min} = 1/9$ K), and $f_s = 4/\beta$. (Recall that for $r_x = 1$, a phase transition can only occur for $\beta \ge 4$.) If $r_x < 1$, the minimum is shifted to lower concentrations, but if the asymmetry in cross-linking constants is not too large the shift will be quite small, for example for $r_x = 0.5$, $c_{min} = 0.298$.

APPLICATION TO HISTAMINE RELEASE FROM BASOPHILS

Basophils are leucocytes that play a central role in allergic reactions. Stored in granules within these cells are potent mediators of anaphylaxis such as histamine and serotonin, while on the surface of basophils are Fc_ϵ receptors which bind IgE with high affinity (32,33). When IgE specific for a known antigen is bound to these receptors the basophil is said to be sensitized. Cross-linking of surface IgE in the presence of calcium can trigger sensitized basophils to degranulate (7,10–12).

When the ligand used for cross-linking is a simple bivalent antigen that rapidly equilibrates, the equilibrium distribution of cross-linked IgE determines what signals are generated by the ligand. Theory shows that cross-linking will be a maximum, i.e., the maximum number of IgE antibodies will be in aggregates, when the bivalent

ligand concentration $C = 1/2$ K (18). Further, the cross-linking curve, a plot of x_{poly} vs. log C, will be symmetric about the maximum concentration. When the number of surface IgE antibodies per basophil is relatively small ($\leq$5,000), the histamine release curve for these cells, i.e., the percentage of the total histamine contained in the cells that is released when the cells are exposed to a ligand concentration C vs. log C, has the same general shape as the cross-linking curve. For example, the maximum of both curves occurs at $C = 1/2$ K. Thus, by determining the concentration at which maximum histamine release is obtained with a bivalent antigen, one can determine the single site ligand-IgE equilibrium constant (34).

When there are large amounts of specific IgE on the basophil surface, the histamine release curve no longer follows the cross-linking curve. In the concentration range where cross-links are still rising, histamine release decreases (35). Large numbers of cross-links or possibly large aggregates appear to turn the cell off (desensitize) rather than on (16,17,36).

Parameters Characterizing Aggregate Formation on Basophils

The equilibrium constant, K, for the binding of a single IgE receptor site to a binding site on an antigen can vary over many orders of magnitude, typically $10^4\ M^{-1} - 10^9\ M^{-1}$. The binding of a simple synthetic bivalent penicillin hapten, $(BPO)_2$, to human anti-penicillin IgE has been extensively studied (12,34,36,37,39). Although K depends on the particular antiserum from which the IgE is obtained, usually $K \simeq 10^7\ M^{-1}$ (34). Further, for the human sera studied, the binding of $(BPO)_2$ to surface IgE equilibrates in seconds (37).

There are now available a number of monoclonal IgE antibodies. A murine monoclonal IgE that is being widely used in the study of histamine release binds DNP-lysine at 37°C with $K = 7.1 \times 10^7\ M^{-1}$ (38).

Cross-linking constants on cell surfaces have not been directly measured. The cross-linking constant, K_x, for the bridging of two IgE molecules by $(BPO)_2$, has been estimated to be $\sim 8 \times 10^{-10}$ cm^2/molecule from fitting a model to histamine release data (39). This agrees with theoretical calculations of K_x, which estimate that $K_x \simeq 10^{-9}$ cm^2/molecule for bivalent haptens between 30 and 40 Å in length and with equilibrium constants of $10^7\ M^{-1}$ (18).

The parameter $r_x\ K_{xx}/K_x$ is a measure of the asymmetry in the ligand's ability to cause chain branching relative to its ability to cause chain elongation. We expect that in general $r_x < 1$. Before the first cross-linking step, the ligand is anchored at only one site, whereas before the second cross-linking step, it is anchored at two sites. Thus, we expect $K_x > K_{xx}$, i.e., we expect it is easier for a free site on a ligand with one site bound to randomly search and find a free IgE site than for a free site on a ligand with two sites bound. In the latter case the free sites' motions are more severely restricted.

The number of IgE molecules per basophil on cells from allergic individuals range from $\sim 5 \times 10^4$–5×10^5 (40). However, the amount of this IgE that is specific for a particular antigen is usually much less. For example, 5×10^2–5×10^4 IgE molecules per basophil specific for antigen E, the major ragweed antigen, were found on basophils from a group of hay fever patients (41). In our theory, X_T refers to the number of specific IgE molecules per basophil.

By using basophils with free Fc_ϵ receptors (usually from nonallergic donors) and incubating them with IgE against a known antigen, the amount of specific IgE on the cell surface can be controlled (42). Using this passive sensitization technique, MacGlashan and Lichtenstein (36) varied the number of anti-BPO IgE per basophil from $8 \times 10^2 - 1.4 \times 10^4$ molecules. Much higher numbers of specific IgE per cell are difficult to achieve with human basophils from nonallergic donors, since these cells tend to have $\leq 5 \times 10^4$ Fc_ϵ receptors, with a fraction already filled with IgE. Rat basophilic leukemia cells, however, have $\sim 5 \times 10^5$ free Fc_ϵ receptors (43) and higher values of X_T may be obtainable using these cells.

We can now estimate the range of values of the parameters α and β. Recall $\alpha = 4K_xX_T$. For $K_x = 10^{-9}$ cm^2 and $X_T = 10^4$ specific IgE/basophil, and using the measured surface area of a basophil, 4.7×10^{-6} cm^2 (37), so that $X_T = 2.1 \times 10^9$ specific IgE/cm^2, we find $\alpha = 8.5$. Thus for $10^2 - 10^5$ receptors/cell, $0.085 \leq \alpha \leq 85$. The parameter $\beta = K_{xx}X_T\ r_x\alpha/4$. Thus, for the same parameters, $0.021r_x \leq \beta \leq 21r_x$. In Table I we summarize the ranges of the parameters we have discussed.

Can Superaggregates Form on Basophils?

We have shown (see Eq. 37) that for $r_x = 1$, superaggregates can only form if $\beta \geq 4$, or equivalently if $\alpha \geq 16$. In general, however, we expect $r_x < 1$. In Fig. 9, we show the coexistence curves, the values of c_-^* and c_+^* as a function of α or, equivalently, X_T, for particular values of r_x between 0.1 and 1.0. From Fig. 9, which was based on numerical studies, we conclude that for all values of $r_x \leq 1$, $\beta \geq 4$ to have superaggregate formation. Further, we conclude that for a fixed value of α, decreasing r_x favors the formation of aggregates with long linear chains over highly branched structures. This can be overcome, and highly branched aggregates can be obtained by increasing α or, equivalently, X_T, the amount of specific IgE on the cell surface. When $r_x = 1$, to obtain a phase transition, $\alpha \geq 16$, while when $r_x = 0.1$, $\alpha \geq 85.1$. Because for a typical small ligand with $K = 10^7\ M^{-1}$, we estimate that α values as high as 85 can occur on basophil surfaces (Table I), we predict that superaggregates can form when highly sensitized basophils ($X_T > 2 \times 10^4$ receptors/cell) are exposed to the appropriate concentration of trivalent ligand.

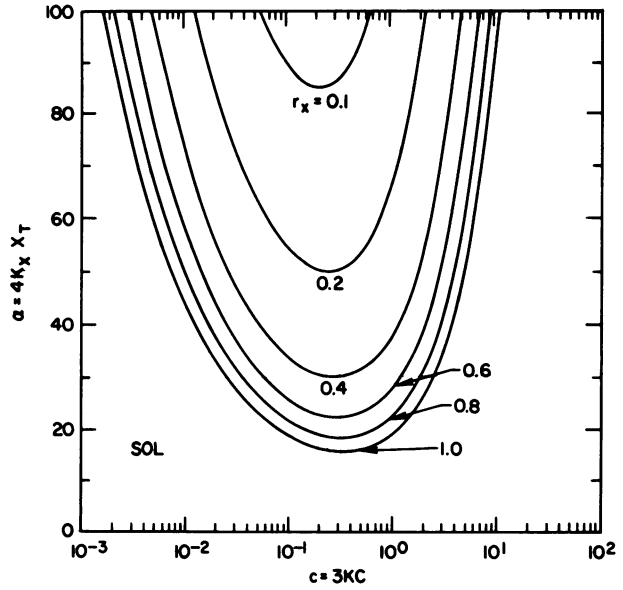

FIGURE 9 The locus of points dividing the sol region from the sol-gel coexistence region for different values of α and r_x. The solid lines indicate the values of c_-^* and c_+^* for each value of α and r_x.

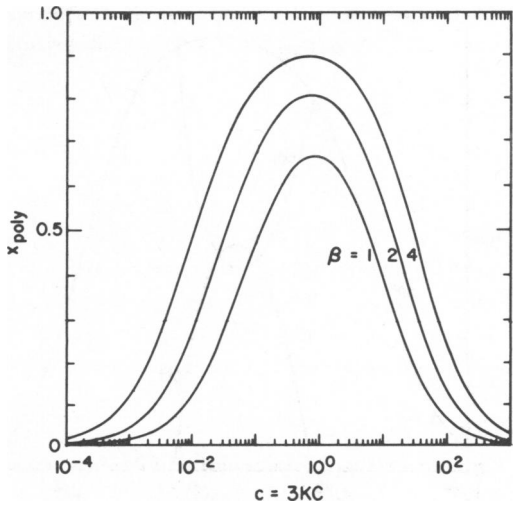

FIGURE 10 Cross-linking curves, i.e., the fraction of receptors cross-linked, x_{poly}, vs. log c, for $r_x = 1$ and $\beta = 1$, 2, and 4.

Theoretical Cross-Linking Curves

In studying histamine release from basophils, it is useful to compare measured histamine release curves with predicted cross-linking curves. As we pointed out, for bivalent ligands, the cross-linking curve is a symmetric bell-shaped curve with its maximum at $C = 1/2K$, or, equivalently, $c = 1$. For trivalent ligands, when no superaggregates from, i.e., $\beta < 4$, the cross-linking curves look similar to those obtained with a bivalent ligand. The maximum of the cross-linking curve however, is no longer at $c = 1$, but is now between $c = 0.5$ and 1.0. (A proof of this is given in the Appendix.) The curve appears to be symmetric about its maximum, but we have not proven this is so.

In Fig. 10, we show three cross-linking curves for $r_x = 1$, and $\beta = 1$, 2, and 4. As β is increased, for example by increasing the number of specific IgE molecules per cell, the cross-linking curve increases. This is because for any ligand concentration, increasing X_T increases the cross-linking and branching parameters γ and δ and therefore the fraction of IgE molecules that are in aggregates.

For the sol phase, the fraction of receptors in aggregates, $x_{poly} = 1 - w$. In Fig. 10, x_{poly} was calculated from this formula by solving Eq. 22 for w. When the sol and gel phases coexist there are contributions from both phases to x_{poly}. All the receptors in the gel phase and $(1 - w_s)$ receptors in the sol phase are cross-linked, so that

$$x_{poly} = (1 - f_s) + (1 - w_s)f_s = 1 - w_s f_s. \quad (47)$$

From Eqs. 43 and 44, therefore,

$$x_{poly} = 1 - \frac{(1 + c)^2}{\alpha c[1 + r^{1/2}(1 + 1/c)^{1/2}]}, \; c_-^* \le c \le c_+^* \quad (48)$$

By differentiating x_{poly} and setting $dx_{poly}/dc = 0$, we find for $r = 1$ that Eq. 48 is maximum when $c = (7 + \sqrt{17})/16 \simeq 0.70$. Thus in the sol-gel region x_{poly} is a maximum at $c \simeq 0.70$ provided $r_x = 1$ and $\beta > 4.3$. (For $\beta < 4.3$, $c \simeq 0.70$ is not between c_+^* and c_-^*.)

In Fig. 11, $r_x = 1$ and $\beta = 8$, so there is a range of concentrations where sol and gel coexist. From Eq. 37, we

TABLE I
TYPICAL PARAMETER VALUES FOR SENSITIZED BASOPHILS

Parameter	Symbol	Typical range	Units
Single site equilibrium binding constant	K	10^4–10^9	M
First equilibrium cross-linking constant	K_x	10^{-12}–10^{-7}	cm^2
Second equilibrium cross-linking constant	K_{xx}	$K_{xx} \le K_x$	cm^2
Cross-linking asymmetry parameter	$r_x = K_{xx}/K_x$	0–1	—
Specific IgE concentration	X_T	$1 \times 10^2 - 5 \times 10^5$	IgE/cell
Dimensionless ligand concentration	$c = 3KC$	10^{-4}–10^4	—
Dimensionless first cross-linking constant	$\alpha = 4K_x X_T$	$10^3 - 4 \times 10^{2*}$	—
Dimensionless second cross-linking constant	$\beta = K_{xx}X_T = r_x\alpha/4$	10^{-4}–10^{2*}	—

*The range for α and β was computed for $K_x \simeq 10^{-9}$ cm^2.

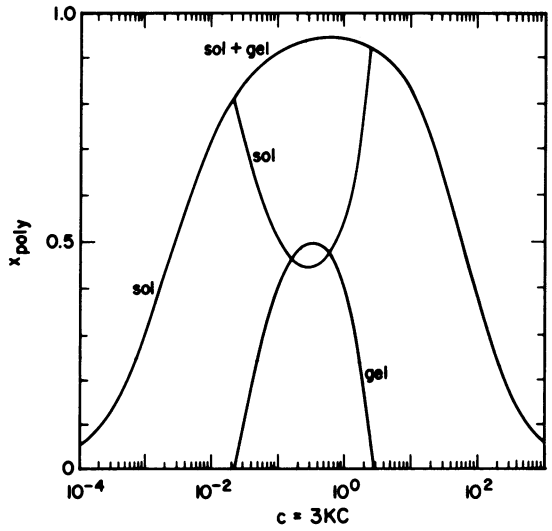

FIGURE 11 The fraction of receptors cross-linked, x_{poly}, vs. log c, for $r_x = 1$ and $\beta = 8$. For $c < 0.022$ and $c > 2.68$ there is only one phase (sol) and all cross-linked receptors are in finite sized aggregates. For $0.022 < c < 2.68$ there are two phases and x_{poly} is the sum of the fraction of receptors that are in finite aggregates (sol) plus the fraction of receptors that are in infinite aggregates (gel).

determine that this range corresponds to $0.022 \leq c^* \leq 2.68$. For the ligand concentration range that gives rise to sol-gel coexistance, we calculated x_{poly} from Eq. 47, while outside this range we calculated x_{poly} as before, from Eq. 47 with $f_x = 1$ and $w_s = w$. Note that the maximum in x_{poly} occurs at $c = 0.68$ as predicted.

Even though at $c = c_-^* = 0.022$ and $c = c_+^* = 2.68$ there is a phase transition, the cross-linking curve in Fig. 11 is a smooth function of c. Thus, any biological signal that is a function only of the number of receptors in aggregates will also be a smooth function of the ligand concentration and will not "detect" the phase transition. However, if a biological signal exists that is triggered by very large aggregates, then there should be a dramatic change in this signal as c is increased, say from a value slightly less than c_-^* to a value slightly greater than c_-^*.

CONCLUSIONS

When trivalent ligands are exposed to bivalent cell surface receptors, the possibility arises that superaggregates, i.e., very large ligand-receptor aggregates that span major portions of the cell surface, can form. We have shown that for a small, trivalent ligand with a single-site equilibrium binding constant $K = 10^{-7}\,M^{-1}$, superaggregates can form, provided there are a sufficient number ($\sim 2 \times 10^4$ or more) of specific IgE molecules (bivalent receptors) on the cell surface. Irrespective of the receptor density, at very low and high ligand concentrations there will be no superaggregate formation. When the amount of specific IgE is high enough, superaggregate formation will occur in a ligand concentration range centered about $C \cong 1/9K$, an easily obtainable ligand concentration.

Because some experiments have already been performed in which highly sensitized basophils have been exposed to highly multivalent ligands (36, 44, 45), the question arises as to why no dramatic change in the histamine-releasing properties of these cells have been reported. One possible explanation is that histamine release in the systems studied depends strongly on the number of IgE molecules in aggregates, but only weakly or not at all on the size of the aggregate. Our theory shows that the cross-linking curve for all IgE concentrations is a smooth function of the ligand concentration. When the ligand concentration is raised to the critical value where superaggregates form, some IgE molecules that are in small aggregates become part of the superaggregate, but there is no abrupt change in the total number of IgE molecules that are cross-linked. Only if a biological signal that depends on very large aggregates exists, will a dramatic change be seen. (Nonspecific desensitization of basophils has been suggested as a candidate for such a signal [46], but it is still an open question.) An alternative explanation is that for the ligands used, the kinetics of superaggregate formation is so slow that histamine release is over before any large aggregates form. For example, the kinetics of large aggregate formation was observed to be slow compared with histamine release when basophils were exposed to fluorescein-conjugated heterogeneous anti-IgE (47). Because the anti-IgE was heterogeneous, the receptor (IgE) had an effective valence greater than 2 for the ligand (anti-IgE). The possibility of slow aggregate formation can be eliminated by allowing binding to occur over a long time period at 4°C. At this temperature histamine release and desensitization are suppressed. The cells can then be warmed, and histamine release measured.

Another difficulty in observing a sol-gel transition by studying basophils arises because in general the basophil cell surface IgE concentration is heterogeneous. If different cells in the basophil population being studied have different amounts of cell surface IgE, the ligand concentration range over which superaggregates form will differ from cell to cell. This heterogeneity will therefore tend to blur the effects of the phase transition.

The equilibrium theory we have presented has certain limitations. We have neglected loop formation and only considered aggregates composed of branched treelike structures. Including loops in the theory is a difficult, unsolved problem. It has been included in the theory of bivalent receptor cross-linking by bivalent ligands (18,48) and only approached in approximate ways in polymer chemistry for branched aggregates (49,50). For solution-phase, polycondensation reactions, such as antibody-antigen reactions, it is generally believed that loop formation is not a significant process until the gel point is reached, unless the system is very dilute (51–53). This is because a free site on a molecule can either react intermolecularly with a free site on another molecule or intramolecularly with a free site on the same molecule. In the pre-gel

state, the number of free sites on any given molecule is generally small compared with the total number of free sites available in solution. Once a superaggregate forms, loop formation may become significant because the number of free sites on a superaggregate can be an appreciable fraction of all the free sites in the system (51, 52). On a cell surface the possibility of intramolecular reactions is greatly increased, first, because, unlike as in the solution case, the number of free sites available for intermolecular reaction is always limited and less than the total number of free sites on the cell surface. Second, a free site on an aggregate can find another free site on the same molecule by undergoing a random walk with a much higher probability in two dimensions than in three dimensions. For example, for a linear chain of n links, the probability of one end finding the other end goes as n^{-1} in two dimensions, and as $n^{-3/2}$ in three dimensions (18, 53). We know of no published data addressing the question of loop formation in cell surface aggregates. However, if loop formation is important on cell surfaces, its effect will be to make it more difficult to achieve superaggregate formation. This is because loops reduce the number of free sites available for increasing the size of any aggregate. Much higher values than 2×10^4 specific IgE per cell will be needed to overcome loop formation and cause superaggregate formation. Thus it is possible for loop formation to prevent the occurrence of superaggregates on basophils.

We have also assumed that a single ligand cannot bind to both sites on a single receptor. It is straightforward to take such monogomous bivalent attachments into account. An additional equilibrium constant must be introduced and an additional state added to Q_0, Eq. 2, as was done for the bivalent ligand case (18).

A very useful tool in the study of histamine release from basophils is a monovalent ligand that competes for the same receptor sites as the cross-linking agents being used to trigger release. Adding monovalent ligand always decreases the concentration of cross-links and therefore monovalent ligand can be used to manipulate in a known way the cross-linking curve and the concentration range over which superaggregates can form. If both trivalent ligand and monovalent ligand with an equilibrium constant K_m and concentration C_m are present, our theory can be modified to include monovalent ligand simply by replacing $(1 + c)$ by $(1 + c + c_m)$, where $c_m = K_m C_m$.

The theory we have presented provides a new method for finding the critical conditions (i.e., ligand concentration, values of α, β, and K) at which a cell surface sol-gel phase transition occurs. For the special case, $r_x = 1$, Macken and Perelson (54), using branching processes, have derived the gelation condition. Both methods give the same result. The branching process method also gives for $r_x = 1$, the concentration of receptor-ligand aggregates of all possible sizes (54). While the branching process method in principle can be generalized to cases in which $r_x < 1$, in practice it is not yet clear whether explicit solutions can be found. DeLisi (55) and Perelson (56) have previously studied the binding of multivalent ligands to cell surface receptors. Their work complements ours in that they predicted the total concentration of ligand bound to the surface, and the concentration of f-valent ligand bound to the surface at $i = 1, 2, \ldots f$ sites, quantities not predicted here. Perelson (56) also considered the inhibition of cross-linking by monovalent hapten. DeLisi (55) identified the sol-gel phase transition, but neither his nor Perelson's model is valid in the coexistence region. Perelson (56) noted that in certain parameter ranges, his model gave spurious results, but failed to identify this parameter regime as one in which a phase transition occurred.

Although we have concentrated on the basophil, our results are clearly applicable to other cell and model systems. Recently Peacock and Barisas (57), while studying the binding of multivalent ligands to bivalent immunoglobulin on liposomes, obtained results indicating the occurrence of a sol-gel phase transition.

APPENDIX

Here we show that c_{max}, the dimensionless concentration at which x_{poly} is a maximum, is between 0.5 and 1.0, when $\beta \leq 4$, i.e., when there is no gel region. From the sum rule, Eq. 18, and the expansion for q, Eq. 15b, we have that

$$x_{poly} = 1 - w = 2\delta w^2 + (3\delta^2 + 2\gamma)w^3 + 4(\delta^3 + 2\gamma\delta)w^4 + \ldots \quad \text{(A1)}$$

Using a prime to indicate a derivative with respect to c we have that when $c = c_{max}$, $x'_{poly} = w' = 0$. From Eq. A1 therefore, when $c = c_{max}$,

$$0 = 2\delta' w^2 + 2(3\delta\delta' + \gamma')w^3 + 4(3\delta^2\delta' + 2\gamma'\delta + 2\gamma\delta')w^4 + \ldots \quad \text{(A2)}$$

Because w is nonzero (for any ligand concentration there are always a finite number of receptors not in aggregates) the right side of Eq. A2 can only be zero if either all the coefficients of each w^n term are simultaneously zero or some coefficients are negative and some positive. We show below that all the coefficients are not simultaneously zero. Therefore some coefficients must differ in sign. For this to occur δ' and γ' must be of different signs. Because

$$\delta' = \alpha(1 - c)/(1 + c)^3, \quad \text{(A3)}$$

and

$$\gamma' = \alpha\beta(1 - 2c)/(1 + c)^4, \quad \text{(A4)}$$

this can only occur when $1 > c > 1/2$.

To find the numerical value of c_{max} when $\beta < 4$, we note that in general w is given by the cubic Eq. 22. As we noted above, at $c = c_{max}$, $x'_{poly} = w' = 0$. We find by differentiating Eq. 22 that $2\gamma\gamma^2 w_{min}^2 + (2\gamma' - 2\delta\delta')w_{min} + 1 + 2\delta' = 0$, where w_{min} is the value of w at $c = c_{max}$. This equation can be solved for w_{min} as a function of α, β, and c, since γ and δ are themselves functions of these variables. Substituting w_{min} into Eq. 22, one obtains a nonlinear equation for c_{max} as a function of α and β, that can be solved numerically.

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