Modeling Concurrent Binding of Multiple Molecular Species in Cell Adhesion

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ABSTRACT Cell adhesion provides not only physical linkage but also communication between the cell and its environment. As such, it is important to many cellular functions. Recently, the probability distribution of forming a low number of specific adhesive bonds in a short-duration contact has been described (Chesla et al., *Biophys. J.*, 1998, 75:1553–1572). This model assumes that binding occurs between a single receptor species and a single ligand species. However, cell adhesion molecules rarely work alone in physiological settings. To account for these in vivo situations, we extended the previous model to include concurrent interactions of multiple receptor–ligand species, introducing the concept of independent binding. Closed-form solutions have been obtained for cases where competition is absent or can be neglected. In two companion papers (Williams et al., *Biophys. J.*, 2000, 79:1858–1866; 2000, 79:1867–1875), the model developed herein has been applied to analyze two sets of experiments designed such that the validity of the theory was also tested.

INTRODUCTION

Short-duration contacts among circulating leukocytes and other cells are common and essential occurrences. The adhesions produced by such contacts are an integral part of many biological functions, such as inflammatory response, lymphocyte homing, and immune reaction (Springer, 1995). To predict the outcome of a contact, i.e., whether it results in a specific adhesion and if so, how many receptor–ligand bonds are formed, requires molecular information. This includes the kinetic rate constants of the interacting molecules and their respective numbers in the contact area. The densities of cell surface proteins can be measured by several conventional techniques. By comparison, not until recently have measurements of kinetic rate constants of membrane-bound receptor–ligand binding (the so-called two-dimensional kinetic parameters) become experimentally possible (Kaplanski et al., 1993; Tees et al., 1993; Alon et al., 1995; Chesla et al., 1998).

Previously, we developed a micropipette method for measuring two-dimensional kinetics (Chesla et al., 1998). It consists of an experimental assay to measure the probability of forming a specific adhesion in a short-duration contact between two cells and a mathematical model to express this probability in terms of the kinetic rates. A limitation of this model is that it only treats a simple reaction scheme, i.e., single-step binding is assumed to occur between a single receptor species and a single ligand species. Although various cell adhesion molecules can be (and often are) functionally isolated in vitro to simplify analysis, they rarely work alone in vivo. Instead, several adhesion pathways usually participate in binding and signaling simultaneously. The mathematical treatment of cell adhesion can be greatly complicated by the concurrent actions of multiple receptor–ligand species, because they render the potential for competition, cooperation, and cross-regulation. For example, T lymphocytes express, among others, T cell receptor (TcR), leukocyte function-associated antigen 2 (LFA-2 or CD2) and LFA-1 (CD11a/CD18), which, respectively, bind to major histocompatibility complex class I (MHC-I) molecule, LFA-3 (CD58), and intercellular adhesion molecules (ICAMs) on the surface of target cells. There are at least three ICAMs, ICAM-1, -2, and -3, that can interact with LFA-1. LFA-1 on resting T cells in the peripheral blood is in a low-affinity state. The engagement of TcR with MHC-I on the target cell activates the T cell, triggering a conformational change in LFA-1 that converts it from a low- to a high-affinity state. The CD2–LFA-3 interaction is also regulated by T-cell activation, although by a different mechanism (Springer, 1990).

As a first step toward developing a framework for exploring the action and interplay of multiple receptor–ligand species, we describe here a model for their concurrent binding without cross-species regulation. The present concurrent binding model is an extension of our previous single-species model. We start with the coexistence of multiple adhesion pathways but assume that there is no coupling among them. We show that solutions to this case can be constructed from the previously obtained solutions of the single-species model. We then discuss a more involved kinetic scheme allowing for overlapping binding specificity of multiple receptor (or ligand) species for the same ligand (or receptor) species. This simultaneous binding situation affords the possibility of competition among the receptors from different species for a single ligand species, as recently discussed by Li et al. (1999). Here we elucidate the condi-

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tions under which the competitions can be neglected and, consequently, simple solutions can be obtained. We show that these conditions are the same as those that warrant the use of the small system kinetic formulation of McQuarrie (1963) and under which the micropipette adhesion-frequency assay is designed to work. These lead to the concept of concurrent but independent binding. The same idea is also applied to treat nonspecific binding and to discuss the notion of additivity. We also discuss under what conditions treating multispecies binding with a single-species model is a valid approach. The theory developed herein was applied to analyze data from two dual-species interaction systems of particular interest, with results presented in companion papers (Williams et al., 2000a,b). In addition to generating new information about the biological systems involved, these experiments were also designed to provide a rigorous test of the concurrent binding model presented here. The use of two separate dual-species systems permitted us to compare, contrast, and confirm key observations.

THEORETICAL DEVELOPMENT

Independent concurrent binding

Let us first consider the monovalent reactions,

$$M_{r_i} + M_{\ell_i} \underset{k_{ri}}{\overset{k_{fi}}{\rightleftharpoons}} M_{b_i} \quad i = 1, 2, \ldots, I, \tag{1}$$

where M_r, M_ℓ, and M_b, designate, respectively, receptor, ligand, and bond. k_f and k_r stand for the forward and reverse 2D rate constants, respectively. The subscript i denotes the ith receptor–ligand pair, or species, that mediates the adhesion between two cells. Setting the total number of species I to one reduces Eq. 1 to the single-species case discussed by Chesla et al. (1998), whereas $I > 1$ represents multispecies concurrent binding. An example of the latter is the selectin and integrin adhesion pathways. Each of these adhesion receptors binds specifically to its own ligands, but they work together to slow down and arrest circulating leukocytes to the blood vessel walls of the inflamed tissue (Lawrence and Springer, 1991; von Andrian et al., 1991). A recent research focus in leukocyte biology is the cross-talk between two adhesion pathways, e.g., how binding of the selectins activates the integrins (Cooper et al., 1994; Simon et al., 1995). This situation can be modeled by allowing the kinetic rates of integrins to be functions of the number of selectin bonds and the time elapsed from the moment these bonds were formed to the current instant. Mathematically, such a treatment results in coupling between the two species, which can be quite complicated. As an initial step in this direction, it is assumed, in this first concurrent binding model, that distinct species are independent, i.e., bond formation does not alter the likelihood of other receptors forming or retaining bonds. As such, the kinetics of each species is governed by its own equations without any cross-species coupling.

To analyze the stochastic binding mediated by a low number of receptor–ligand bonds, as those observed in the micropipette adhesion-frequency assay (Chesla et al., 1998), the probabilistic kinetic formulation of McQuarrie (1963) is required. For a second-order forward, first-order reverse monovalent interaction, the master equation is (Piper et al., 1998; Chesla et al., 1998):

$$\begin{aligned}\frac{dp_{n_i}}{dt} = {} & \frac{k_{fi}}{A_c}[A_c m_{r_i} - (n_i - 1)][A_c m_{\ell_i} - (n_i - 1)]p_{n_i - 1} \\ & + k_{ri}(n_i + 1)p_{n_i + 1} \\ & - \left[\frac{k_{fi}}{A_c}(A_c m_{r_i} - n_i)(A_c m_{\ell_i} - n_i) + k_{ri} n_i\right] p_{n_i},\end{aligned} \tag{2}$$

where p_{n_i} denotes the probability of having n_i bonds of the ith species. A_c (in μm^2) is the contact area and t (in seconds) is the contact duration between the two interacting cells. m_{r_i} and m_{ℓ_i} (in molecules/μm^2) are the respective densities of the ith receptor and ligand species. n_i ranges from 0 to $A_c \tilde{m}_i$ where $\tilde{m}_i = \min(m_{r_i}, m_{\ell_i})$ and p_{n_i} is zero when n_i is outside of this range. For simplicity, the kinetic rates k_{fi} and k_{ri} are assumed to be constants. This is consistent with the assumption of neglecting cross-species regulation. It is also consistent with the experiments to be presented in the two companion papers (Williams et al., 2000a,b), which were specifically designed to test the concurrent binding model, because those micropipette experiments measured the probability of adhesion at zero force (Chesla et al., 1998). However, such an assumption is only used to obtain some of the analytical solutions (e.g., Eqs. 7 and 9) but not required for the general independent binding formulation (e.g., Eq. 3). Dependence of the kinetic rates on the bond number n_i in a manner similar to that given by Piper et al. (1998) and Long et al. (1999) can and should be assumed when the bonds are subject to an externally applied force.

We express the kinetic law in terms of the *probability* of having bonds instead of a deterministic bond density, because we are concerned with discrete rather than continuously distributed bonds. A low number of bonds are expected in short-duration cell–cell encounters before the spatially separated point contacts can be connected into a continuous area by cell spreading. The law of conservation of mass is thus expressed in the form of conservation of probability (McQuarrie, 1963). According to the Markovian assumption, the rate of change in the probability at a point (n_i) on the left-hand side of Eq. 2 is caused by the probability influxes from and effluxes to only the immediate neighbors ($n_i - 1$) and ($n_i + 1$). The influxes are represented by the first two terms on the right-hand side of Eq. 2, whereas the effluxes are represented by the last term. As a result, Eq. 2 is a tridiagonal system. This allows for a

closed-form exact solution for the steady state even in the general cases when the kinetic rates are functions of n_i (Piper et al., 1998) and when the kinetic mechanism is of higher order than that given by Eq. 1 (Zhu et al., 1999).

It follows from the independent binding assumption that, for multispecies concurrent binding, the joint probability distribution of having n_1 bonds of species 1, n_2 bonds of species 2, . . . , and n_I bonds of species I is

$$p_{n_1,n_2,\ldots,n_I} = \prod_{i=1}^{I} p_{n_i}. \tag{3}$$

In other words, the joint probability distribution is the product of the individual probability distributions of all single species. Solutions to the single species model, p_{n_i}, have been obtained in our previous work (Piper et al., 1998; Chesla et al., 1998). They can therefore be used to construct solutions to the multispecies concurrent binding model, $p_{n_1,n_2,\ldots,n_I}$, according to Eq. 3 (e.g., see Eqs. 7 and 9).

Competitive concurrent binding

We next consider the reaction

$$\sum_{i=1}^{I} M_{r_i} + IM_\ell \underset{k_{ri}}{\overset{k_{fi}}{\rightleftharpoons}} \sum_{i=1}^{I} M_{b_i}. \tag{4}$$

This reaction scheme is not the same as simply summing Eq. 1 from $i = 1$ to $i = I$, because here M_ℓ is identical for all i values. In other words, Eq. 4 allows I (≥ 2) different receptor species to concurrently bind the same ligand species, thereby affording the possibility of competition. Obviously, the indices for receptor and ligand can be exchanged in Eq. 4, thus the results presented below can also be applied to the case of multiple ligand species competitively binding to the same receptor species. Take again, as an example, the case of leukocyte adhesion to the vessel wall mediated by selectins and integrins. Different members of the selectin family of adhesion molecules, L-, E-, and P-selectin, are known to have overlapping carbohydrate ligands, e.g., P-selectin glycoprotein ligand 1 (McEver and Cummings, 1997). Also, leukocyte integrins can bind to ICAM-1, -2, and -3 (Springer, 1995). In the companion papers, two cases similar to these will be discussed. The first case concerns two human immunoglobulin G (IgG) isotypes 1 and 2 binding to the same human Fcγ receptor type III (FcγRIII or CD16) (Williams et al., 2000a). The second case deals with two human Fcγ receptor types II (FcγRII or CD32) and III binding to total human IgG (Williams et al., 2000b).

Without losing any generality, let us develop the theory for the case of $I = 2$, i.e., two receptor species simultaneously binding to the same ligand species. The more complicated equations for an arbitrary I value can be obtained along a similar line of derivation. Extending from Eq. 2, the master equation for dual-species competitive binding can be written as

$$\begin{aligned}\frac{dp_{n_1,n_2}}{dt} = {} & \frac{k_{f1}}{A_c}[A_c m_{r_1} - (n_1 - 1)][A_c m_\ell - (n_1 - 1) - n_2]p_{n_1-1,n_2} \\ & + k_{r1}(n_1 + 1)p_{n_1+1,n_2} \\ & + \frac{k_{f2}}{A_c}[A_c m_{r_2} - (n_2 - 1)] \\ & \cdot [A_c m_\ell - n_1 - (n_2 - 1)]p_{n_1,n_2-1} \\ & + k_{r2}(n_2 + 1)p_{n_1,n_2+1} \\ & - \left\{\left[\frac{k_{f1}}{A_c}(A_c m_{r_1} - n_1) + \frac{k_{f2}}{A_c}(A_c m_{r_2} - n_2)\right]\right. \\ & \left. \cdot (A_c m_l - n_1 - n_2) + k_{r1}n_1 + k_{r2}n_2\right\}p_{n_1,n_2}\end{aligned} \tag{5}$$

In contrast to Eq. 2 the dependent variable of which is a vector $\{p_{n_i}\}$ (for a fixed i value), the dependent variable of Eq. 5 is a matrix $\{p_{n_1,n_2}\}$. As such, each point is specified by a pair of numbers (n_1, n_2), instead of just one number, with four immediate neighbors ($n_1 - 1$, n_2), ($n_1 + 1$, n_2), (n_1, $n_2 - 1$), and (n_1, $n_2 + 1$). Consequently, the probability fluxes enter and leave in two directions, one along n_1 and the other along n_2, instead of just one direction. As a result, the tridiagonal system of Eq. 2 becomes two perpendicular tridiagonal systems, one for each direction, which share a common center term.

It should be pointed out that Eq. 5 cannot be obtained by simply differentiating the product of p_{n_1} and p_{n_2} and then substituting dp_{n_i}/dt ($i = 1, 2$) using the right-hand side of Eq. 2. The differences are twofold. First, there is only one ligand species in Eq. 5 instead of two. Second, binding by a receptor of one species to a ligand prevents that ligand from binding a receptor of the other species. It is this second aspect that provides the potential for competition between the two receptor species. It follows from the above discussion that the exact solution to Eq. 5 will not have the form of Eq. 3, because Eq. 3 requires independent binding to be valid. Competitive binding is not independent by definition.

Relation to deterministic kinetic equations

The coupling between the two species becomes apparent when Eq. 5 is reduced to its deterministic counterpart. This can be done by multiplying both sides by n_1 (or n_2) and summing over both n_1 and n_2 to obtain two equations for the

mathematically expected, or mean, numbers of bonds, $\langle n_1\rangle$ and $\langle n_2\rangle$, for each receptor species.

$$\frac{d\langle n_1\rangle}{dt} = \frac{k_{f1}}{A_c}[A_c m_{r_1} - \langle n_1\rangle][A_c m_\ell - \langle n_1\rangle - \langle n_2\rangle] - k_{r1}\langle n_1\rangle + \frac{k_{f1}}{A_c}[V(n_1) + V(n_1, n_2)], \tag{6a}$$

$$\frac{d\langle n_2\rangle}{dt} = \frac{k_{f2}}{A_c}[A_c m_{r_2} - \langle n_2\rangle][A_c m_\ell - \langle n_1\rangle - \langle n_2\rangle] - k_{r2}\langle n_2\rangle + \frac{k_{f2}}{A_c}[V(n_2) + V(n_1, n_2)], \tag{6b}$$

where $V(n_i)$ is the variance of n_i and $V(n_1, n_2)$ is the covariance of n_1 and n_2. The variance and covariance are measures of statistical fluctuations. As such, their contributions to Eq. 6 diminish as $\langle n_1\rangle$ and $\langle n_2\rangle$ become large. After neglecting $V(n_1)$, $V(n_2)$, and $V(n_1, n_2)$, Eq. 6, a and b can readily be identified as the deterministic kinetic equations for the simultaneous binding of two receptor species to the same ligand species. These equations, when used to describe binding of soluble molecules to cell surface counter-molecules, are the basis for the competitive inhibition binding method for measuring low affinity interactions (Cheng and Prusoff, 1973; Horovitz and Levitzki, 1987). By comparison, the deterministic counterpart of Eq. 2 would not have $\langle n_2\rangle$ in Eq. 6a or $\langle n_1\rangle$ in Eq. 6b, nor would it have the covariance $V(n_1, n_2)$ (Chesla et al., 1998).

Bond number distribution solutions

Case 1: Binomial solutions for excess ligand

Previously, we found two approximate solutions to the single-species model (Chesla et al., 1998). Here we show that these results can be extended to the multispecies models, not only Eq. 2 but also Eq. 5. The first case is when the ligands greatly outnumber the bonds in the contact area, which allows Eq. 2 to be simplified by neglecting $(n_i - 1)$ in the $[A_c m_{\ell_i} - (n_i - 1)]$ term and n_i in the $(A_c m_{\ell_i} - n_i)$ terms. The solution to the simplified Eq. 2 is a binomial distribution (Chesla et al., 1998). This is expected because, when the ligands are excessively available, binding of one receptor will not reduce the chance for another receptor to bind. For any given adhesion pathway (e.g., the ith species), all $A_c m_{r_i}$ receptors in the contact area can then be assumed to act independently and identically as they make their attempts to bind ligands with the same likelihood of success, p_i. As a result, the probability p_{n_i} of forming n_i bonds obeys the binomial distribution of parameters p_{n_i} and $A_c m_{r_i}$. According to Eq. 3, the solution to the dual-species model can be obtained as the product of two independent binomial distributions.

$$p_{n_1,n_2} \approx \prod_{i=1}^{2} \binom{V_i}{\kappa_i} [p_i(t)]^{\kappa_i}[1 - p_i(t)]^{V_i - \kappa_i}. \tag{7a}$$

where

$$\nu_i = A_c m_{r_i}, \quad \kappa_i = n_i$$

The probability for a receptor to bind a ligand to form a bond of the ith species is

$$p_i(t) = \frac{1 - \exp[-(m_{\ell_i} k_{fi} + k_{ri})t]}{1 + (m_{\ell_i} K_{ai})^{-1}}, \tag{7b}$$

where $K_{ai} \equiv k_{fi}/k_{ri}$ is the 2D binding affinity of the ith species, which is solved from

$$\frac{dp_i}{dt} = m_{\ell_i} k_{fi}(1 - p_i) - k_{ri} p_i.$$

Here the kinetic law is applied to the probability of a receptor being in the bound state rather than to the density of bonds because only one receptor is being considered. The rate of reverse reaction is proportional to the probability p_i itself. The rate of forward reaction is proportional to the probability for the receptor to be in the free state, which is $1 - p_i$. It is also proportional to the density of ligands. Here, the total instead of the free ligand density is used, consistent with the assumption of negligible bond density. The initial condition is $p_i(0) = 0$, because no bond can be formed instantaneously upon the contact of the two cell surfaces.

Next we turn from Eq. 2 to Eq. 5, which describes a different reaction scheme, that of Eq. 4 instead of Eq. 1. Using a stronger condition, that the ligands excessively outnumber both bond species combined allows one to neglect $n_i + (n_j - 1)$ in the $[A_c m_\ell - n_i - (n_j - 1)]$ terms and $n_i + n_j$ in the $(A_c m_\ell - n_i - n_j)$ term (i and $j = 1$ or 2, $i \neq j$), which results in

$$\begin{aligned}\frac{dp_{n_1,n_2}}{dt} \approx\ & k_{f1} m_\ell [A_c m_{r_1} - (n_1 - 1)] p_{n_1-1,n_2} \\ & + k_{r1}(n_1 + 1) p_{n_1+1,n_2} \\ & + k_{f2} m_\ell [A_c m_{r_2} - (n_2 - 1)] p_{n_1,n_2-1} \\ & + k_{r2}(n_2 + 1) p_{n_1,n_2+1} \\ & - \{m_\ell [k_{f1}(A_c m_{r_1} - n_1) + k_{f2}(A_c m_{r_2} - n_2)] \\ & \qquad + k_{r1} n_1 + k_{r2} n_2\} p_{n_1,n_2}.\end{aligned} \tag{8}$$

The approximation given by Eq. 8 ignores the competition in Eq. 5, effectively allowing the two species to become independent. In essence, the same approximation that neglects intraspecies competition to enable the binomial solu-

tion of Eq. 2 also neglects interspecies competition when it is applied to Eq. 5. One would therefore expect the solution of Eq. 8 to be the product of two binomial distributions. This is indeed the case, as shown below.

Let p_{n_i} be given by Eq. 7, which satisfies the simplified Eq. 2. Differentiating $p_{n_1} \cdot p_{n_2}$ and substituting the resulting dp_{n_i}/dt by the right-hand side of the simplified Eq. 2 yields

$$\begin{aligned}\frac{d}{dt}(p_{n_1}p_{n_2}) &= \frac{dp_{n_1}}{dt}p_{n_2} + \frac{dp_{n_2}}{dt}p_{n_1}\\ &\approx \{k_{f1}m_{\ell_1}[A_c m_{r_1} - (n_1 - 1)]p_{n_1-1}\\ &\quad + k_{r1}(n_1 + 1)p_{n_1+1}\\ &\quad - [k_{f1}m_{\ell_1}(A_c m_{r_1} - n_1) + k_{r1}n_1]p_{n_1}\}p_{n_2}\\ &\quad + \{k_{f2}m_{\ell_2}[A_c m_{r_2} - (n_2 - 1)]p_{n_2-1}\\ &\quad + k_{r2}(n_2 + 1)p_{n_2+1}\\ &\quad - [k_{f2}m_{\ell_2}(A_c m_{r_2} - n_2) + k_{r2}n_2]p_{n_2}\}p_{n_1}.\end{aligned}$$

Setting $m_{\ell_1} = m_{\ell_2} = m_\ell$, the above equation can readily be recognized as identical to Eq. 8, provided that the joint probability is equal to the product of two individual probabilities. Thus, Eq. 7 is also an approximate solution to Eq. 5. The only difference is that, when it is used as a solution to Eq. 5, the two ligand densities in Eq. 7b must be equated because there is only one ligand species that binds both receptor species. By contrast, when used for solution to Eq. 2, m_{ℓ_1} and m_{ℓ_2} represent densities of distinct ligands that only bind specifically to their own receptors.

When the receptors greatly outnumber the ligands, Eq. 7 (after exchanging the subscripts r and ℓ for receptors and ligands) is still an approximate solution to Eq. 2. However, this is no longer true for Eq. 5, because the condition that allows neglecting intraspecies competition among ligands for either receptor species does not automatically ensure the validity of ignoring interspecies competition between two receptor species for the same ligands. To the contrary, the latter competition may even be enhanced, especially when binding is limited by a low ligand density instead of a low binding affinity. Mathematically, neglecting $(n_i - 1)$ in the $[A_c m_{r_i} - (n_i - 1)]$ terms and n_i in the $(A_c m_{r_i} - n_i)$ terms ($i = 1, 2$) alone would not eliminate the competition terms, $[A_c m_\ell - (n_i - 1) - n_j]$ and $(A_c m_\ell - n_i - n_j)$ (i and $j = 1$ or 2, $i \neq j$) in Eq. 5. Therefore, the solution to Eq. 5 cannot be approximated by a product of two binomial distributions in this case.

Case 2: Poisson solutions for low number of bonds

The binomial type of master equations can be further simplified by dropping $(n_i - 1)$ and n_i from the $[A_c m_{r_i} - (n_i - 1)]$ and $(A_c m_{r_i} - n_i)$ terms ($i = 1, 2$). This applies to the situation in which, for each species, the number of the bonds is sufficiently low that the numbers of the receptors and ligands remain essentially constant in the contact area. This corresponds to a low affinity reaction, or the low binding transient phase of a high affinity reaction before a large number of molecules are converted into the bound state. The solution, which is a Poisson distribution as previously shown for the single-species case (Chesla et al., 1998; Long et al., 1999), can be obtained for the multispecies case in the following way. Under the given condition, the receptor number in the contact area, $A_c m_{r_i}$, can be considered infinitely large and the probability of forming a bond, p_i, becomes vanishingly small. However, the product of the two, $A_c m_{r_i} p_i$, which is the average number of bonds of the ith species, $\langle n_i \rangle$, remains finite and unchanged. Neglecting n_i from the $A_c m_{r_i} - n_i$ terms in Eq. 7a reduces it to a joint Poisson distribution.

$$p_{n_1,n_2} \approx \prod_{i=1}^{2} \frac{\langle n_i \rangle^{n_i}}{n_i!} \exp(-\langle n_i \rangle) \tag{9a}$$

$\langle n_i \rangle$ can be expressed as

$$\langle n_i \rangle = m_{r_i} m_{\ell_i} A_c K_{ai}[1 - \exp(-k_{ri}t)] \tag{9b}$$

using Eq. 7b and the condition that $A_c K_{ai} \ll 1$. This condition is derived from the requirement for the above simplification to be valid, namely, $\langle n_i \rangle \ll A_c m_{r_i}$.

The distinction between the bond number n_i and its mathematical expectation should be noted. $\langle n_i \rangle$ is determined by averaging over a large number of cell–cell contacts; and as such, it usually takes a noninteger value. By comparison, n_i is the actual number of bonds present during a single contact, and hence it can only assume an integer value.

The Poisson approximation requires a stricter assumption than in Case 1, and, consequently, eliminates competition in the binding of two receptor species even if they share overlapping specificity for the same ligand species. As such, Eq. 9 is an approximate solution for both Eqs. 2 and 5 regardless whether the receptors are more or less than the ligands. That this is the case can also be seen from the symmetry of m_{r_i} and m_{ℓ_i} in Eq. 9b ($m_{\ell_1} = m_{\ell_2} = m_\ell$ when applied to Eq. 5).

From bond number distribution to adhesion

We have shown how neglecting inter- and intraspecies competitions among individual receptor–ligand pairs allowed us to derive simple solutions for the probability distribution of having bonds, $p_{n_1,n_2,\ldots,n_I}$. Here we show that the derivation can be made much simpler if one is only interested in the probability of adhesion, P, regardless of the number of bonds. This is useful because it is P, not $p_{n_1,n_2,\ldots,n_I}$, that is directly measured experimentally (see Williams et al., 2000a,b). The latter has to be inferred from the former, using Eq. 7 or 9. Also, for the purpose of determining kinetic rates of receptor–ligand interactions, it

is sufficient to measure P, because its expression includes all the necessary parameters.

Adhesion is defined as the collective state of having at least one bond no matter to which species it belongs. From Eqs. 3 and 9a, the probability of adhesion is

$$P = 1 - p_{0,0,\ldots,0} \tag{10a}$$

$$= 1 - \prod_{i=1}^{I} \exp(-\langle n_i \rangle) \tag{10b}$$

$$= 1 - \exp\left(-\sum_{i=1}^{I} \langle n_i \rangle\right), \tag{10c}$$

where $\langle n_i \rangle$ is given by Eq. 9b. This result can also be obtained without solving the multispecies master equations. The starting point is the solution of the single-species model, i.e., the $I = 1$ case in Eq. 10, which has been previously proven (Chesla et al., 1998). For the concurrent binding of $I (\geq 2)$ independent species, Eq. 10 follows directly from De Morgan's Law (Hines and Montgomery, 1990) in that an adhesion can result from forming a bond of any species.

It follows from Eq. 10 that the average number of total bonds has to be fairly small for the adhesion probability to be appreciably less than 100%. Thus, any assay to which the present theory is applicable must be capable of detecting a low number of bonds (Chesla et al., 1998; Piper et al., 1998). However, for the purpose of evaluating kinetic rate constants, it is not necessary to know the number of bonds formed in any particular test cycle of the micropipette adhesion-frequency assay (Williams et al., 2000a,b). Furthermore, the presumption that only single-bond events occur in the assay is not needed because the contributions of multiple-bond events to the distribution in Eq. 9a are retained in the adhesion probability expression given here (Eq. 10).

Nonspecific adhesion

The idea of independent binding can also be applied to treat nonspecific adhesion mediated by unidentified agents. Here, we do not seek the probability of having nonspecific "bonds" because it is not clear whether these interactions should be described by discrete molecular entities using the same kinetic framework as Eq. 2 or 5. Nevertheless, the probability of nonspecific adhesion can often be measured in the absence of specific receptors or ligands, and the mechanism causing the nonspecific adhesion often retains its action in the presence of specific receptors and ligands. As such, the total adhesion probability (P_T) includes both specific (P_S) and nonspecific (P_N) contributions, which are most likely independent of each other. Thus,

$$P_T = \text{Prob}(S \cup N) \tag{11a}$$

$$= 1 - \text{Prob}(\overline{S \cup N}) \tag{11b}$$

$$= 1 - \text{Prob}(\bar{S})\text{Prob}(\bar{N}) \tag{11c}$$

$$= 1 - (1 - P_S)(1 - P_N), \tag{11d}$$

$$= P_S + P_N - P_S P_N \tag{11e}$$

where S and N designate the respective specific and nonspecific adhesion events, overbars represent the complement events, and Prob(·) denotes the probability of the argument. The probability of specific adhesion can be solved from Eq. 11e:

$$P_S = \frac{P_T - P_N}{1 - P_N}. \tag{12}$$

To relate the measured adhesion probability to kinetic rates of specific receptor–ligand interaction, the nonspecific component can be removed before analysis of specific effects using Eq. 10. This can be done using Eq. 12 point-by-point where discrete data are available. Alternatively, a phenomenological equation,

$$P_N = 1 - \exp[-a(1 - e^{-bt})], \tag{13}$$

where a and b are two non-negative parameters, can be fit to the nonspecific binding data alone prior to their removal using Eq. 12. For convenience, Eq. 13 assumes that the nonspecific binding has a time-dependent structure similar to the specific binding, growing from zero at $t = 0$ and asymptotically approaching an equilibrium level. This assumption has proven reasonable in the micropipette assays (Williams et al., 2000a,b). By removing the fitted mean rather than the discrete nonspecific adhesions, this approach reduces the impact of outliers at individual time points. Yet another approach is to concurrently fit the nonspecific and specific binding data using the expression for total binding,

$$P_T = 1 - \exp\left[-a(1 - e^{-bt}) - \sum_{i=1}^{I} \langle n_i \rangle\right], \tag{14}$$

where Eqs. 10c, 11e, and 13 have been applied. All data, whether total or nonspecific, is treated similarly in the subsequent search for the best overall fit. The added advantage here is that simultaneous fitting allows the algorithm to assign error to the nonspecific curve if this provides a net improvement to the fitting of the entire data set. A pre-fit nonspecific curve is unchangeable during the specific data-fitting stage.

DISCUSSION

Additivity in concurrent binding

Determination of the specific component of the total adhesion following Eq. 12 is in contrast to the common practice of simply subtracting the nonspecific adhesion from the total adhesion. In probability terms, simple subtraction im-

plies the unlikely assumption that specific and nonspecific adhesions are mutually exclusive, where $P_T = P_S + P_N$. For independent binding, $P_T = P_S + P_N - P_S P_N$, and so these two methods yield similar P_S values only when $P_S P_N \ll \min(P_S, P_N)$. Otherwise, simple subtraction underestimates the specific component.

To this end, it is interesting to note that additivity (or the lack thereof) is often used as a criterion to determine whether or not multispecies concurrent binding is cooperative. The preceding discussion indicates that binding by each component species has to be mutually exclusive for P_S to be additive (i.e., $P_S = P_{S_1} + P_{S_2} + \cdots$). For independent binding, $\langle n \rangle$ will be additive ($\langle n \rangle = \langle n_1 \rangle + \langle n_2 \rangle + \cdots$) if the conditions for the Poisson approximation are satisfied. P_S will be approximately additive when it is small, as $P_S \approx \langle n \rangle$ if $P_S \ll 1$. Clearly, for two independent interactions of equal probability of, say, 60% each, concurrent binding of both will not lead to a probability of 120%. It follows from Eq. 10c that doubling the average number of bonds will only increase binding to 84%. In one of the companion papers (Williams et al., 2000b), these arguments will be used to exclude the cooperation between Fcγ receptors IIa and IIIb at the level of affinity modulation.

Homogenized ligands and receptors

In our previous work (Chesla et al., 1998, 2000), the micropipette technique was used to measure interactions between Fcγ receptor IIIa and total serum IgG. The adhesion probability data were analyzed using the single-species binding model. However, human serum IgG is actually a nonhomogeneous mixture of four subclasses, denoted IgG1–IgG4, with a typical composition of 60%, 30%, 6%, and 4%, respectively. Measurements of affinity to total IgG therefore reflect the character of an imaginary, homogenized ligand—one whose kinetics would generate approximately the same data as the true nonhomogeneous ligand population does. This is a useful abstraction for making relative assessments of receptors. Treatment of a ligand population as homogeneous, even with knowledge that it is otherwise, is therefore acceptable as long as the treatment is consistent across experiments.

It is still of interest to examine the distinctions between the P_S versus t curve of the single-species model and that of the multispecies model. The ideal shape of a multispecies binding curve differs, in general, from that of a single-species binding curve. If the models were equivalent, then

$$\langle \bar{n} \rangle = \sum_{i=1}^{I} \langle n_i \rangle, \tag{15}$$

where each term in the sum has the same functional form as Eq. 9b but with its own parameter values. Overbars indicate a homogenized value, defined herein as the results of fitting the single-species model to multispecies data. The left-hand side denotes the average number of homogenized bonds, and it is assumed to also be given by Eq. 9b with apparent kinetic parameters $\overline{K_a}$, $\overline{k_r}$, and $\overline{k_f} = \overline{K_a} \cdot \overline{k_r}$. Eq. 15 can never be satisfied exactly except in the special case where all k_{ri} are equal. However, in most practical cases, the inequality is negligible, suggesting that detection of the presence of multiple species from observations of the total binding curve is unlikely. Large differences among the k_{ri} have the most impact, but, in fact, differences must be at least an order of magnitude and both species must form a significant portion of the bonds at equilibrium for the shape of the multispecies curve to noticeably deviate from a single-species curve (Williams, 1998). With the exception of such cases, the homogenized ligand model should match the multispecies model (and the data) reasonably well. The three apparent kinetic rate and binding affinity constants describing the homogenized ligand binding can be roughly related to the $3 \times I$ constants describing the true multispecies binding by analytically forcing Eq. 15 to be true at $t \to \infty$ and matching the derivative of each side of the equation (slope of the binding curve) at $t = 0$. This gives

$$\overline{K_a} \approx \frac{\sum_{i=1}^{I} m_{\ell_i} K_{ai}}{\sum_{i=1}^{I} m_{\ell_i}}, \tag{16a}$$

$$\overline{k_f} \approx \frac{\sum_{i=1}^{I} m_{\ell_i} k_{fi}}{\sum_{i=1}^{I} m_{\ell_i}}, \tag{16b}$$

and

$$\overline{k_r} \approx \frac{\sum_{i=1}^{I} m_{\ell_i} K_{ai} \cdot k_{ri}}{\sum_{i=1}^{I} m_{\ell_i} K_{ai}}. \tag{16c}$$

From Eq. 16, it is seen that the single-species binding parameters for the homogenized ligands are approximately the population-weighted average parameters from all individual species. The reverse rate is also weighted by affinity.

CONCLUSION

The adhesion probability model has been extended from single-species to multispecies, a case more relevant to most biological applications. The concurrent binding treated in this work includes the cases of independent and competitive binding. Closed-form approximate solutions have been obtained for the former case. Conditions for these solutions to be applicable to the latter case have been elucidated. The availability of the multispecies model provides new experimental designs and tools for studying multispecies phenomena commonly seen physiologically. It also offers a starting point for the next level of modeling where more complex phenomena such as cross-species modulation may be considered.

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