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Analysis of the Rate-Limiting Step in a Ligand–Cell Receptor Interaction: The Immunoglobulin E System†

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ABSTRACT: Theory predicts that the kinetics of simple interactions between a ligand and a receptor bound on the surface of a cell will be affected by the occupancy of receptors on the same cell. In a diffusion-limited reaction the effect will be on the rate of dissociation but *not* on the rate of association until the cell is virtually saturated with ligand. If the rate of reaction is not diffusion limited, then the opposite holds; i.e., the forward velocities will be proportional to the concentration of vacant receptors, but the reverse reactions will not be. We examined the kinetics of reaction between immunoglobulin E (IgE) and its receptor and clearly demonstrated that the reaction is not diffusion controlled. The substantial ($\simeq$30-fold) increase in the forward rate constant observed for the reaction of IgE with solubilized receptors as opposed to cell-bound receptors is therefore not an artifact of calculation. Since the reverse rate constants show little difference, we postulate that the presence of other surface components (rather than conformational differences in the receptor) affects the reaction with the cells. As an aid to the analysis, the theory has been extended so that not only the rate constants but also the entire course of the reaction of ligand with cell receptors can be predicted for diffusion-limited vs. non-diffusion-limited interactions.

Recent theories of ligand–receptor interactions make a number of predictions bearing on cellular recognition that have not been previously tested. For example, if the reaction between ligand and cell surface receptors is diffusion controlled, the rate of binding is predicted to be the same as it would be if the surface were completely covered by receptors (Berg & Purcell, 1978), even when as little as a few tenths of a percent of the surface is in fact covered. An experimental consequence is that the forward reaction rate will be independent of the number of free receptors per cell throughout almost the entire reaction. Again, under such diffusion-limited conditions and with $\gtrsim 10^4$ unoccupied receptors, these theories indicate that the dissociation of labeled ligand from the cell surface will very likely be followed by rebinding to some other empty receptor

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Table I: Protocol for Analyzing Effect of Receptor Concentration on Rate of Binding of IgE

procedure	concn of reactants: cells/mL × 10^{-6}	M × 10^8: ^{131}I-IgE	^{125}I-IgE[a]	unlabeled IgE	procedure used for stopping reaction	time (min)
(1) tagging	25	0.5			dilution/wash	1
(2) partial saturation	20			3.0	dilution/wash	0–10
(3) analysis (a) residual receptors	2.25		3.3		addition of unlabeled IgE	45
(b) association kinetics	2.25		0.94		addition of unlabeled IgE	0–11

[a] The concentrations of ^{125}I-IgE have been corrected to reflect the bindability (91%) of IgE.

on the same cell (DeLisi, 1980). An experimental consequence is that a large excess of unlabeled ligand will accelerate the dissociation of label.

In this paper, we describe experiments designed to test the consequences of the theory for the reaction of immunoglobulin E (IgE)[1] with the receptor on basophilic leukemia cells (RBL cells). This receptor specifically binds the Fc region of monomeric IgE with high affinity. It is an attractive system to investigate because it can be studied rigorously with relative ease: We have available almost unlimited amounts of relatively homogeneous reactants, the cells contain large numbers of receptors ($\geq 3 \times 10^5$/cell), and while the binding constant is high, the reaction follows the simple kinetics expected for a straightforward bimolecular reaction (Kulczycki & Metzger, 1974). In addition, we have developed an assay by which the same reaction can be studied with equivalent rigor with receptors solubilized from the cells with nonionic detergents (Rossi et al., 1977). This then permits a direct comparison between the binding by receptors bound to cells and by receptors in solution.

In the discussion of our results, we also extend the theoretical aspects of the ligand–receptor interaction. In a previous paper, the effect of cell size and receptor density on the reaction rate constants was formulated (DeLisi, 1981). We here develop additional equations that describe the entire time course of the reaction.

Experimental Procedures

Cells. RBL cells from the 2H3 subline were maintained in stationary tissue culture flasks and harvested as described elsewhere (Taurog et al., 1979). Cells were assayed for receptor number as described previously (Kulczycki & Metzger, 1974) and found to have approximately (3–4) × 10^5 receptors/cell routinely.

Cell Extract. Cells were washed twice with phosphate-buffered saline (6.7 mM phosphate, 154 mM NaCl, pH 7.4). They were subsequently suspended at 5 × 10^7 cells/mL for 20 min at 4 °C in 0.5% Triton X-100 in borate-buffered saline (0.2 M $NaBO_3$, 0.16 M NaCl). The suspension was spun at 25000*g* at 4 °C for 1 h and the pellet discarded. The supernatant was diluted with borate-buffered saline to a final concentration of 0.25% Triton X-100.

IgE. Rat monoclonal IgE (IR 162) was purified and iodinated with ^{131}I and ^{125}I in separate preparations, and the bindability was assessed as described in Kulczycki & Metzger (1974). The bindability of ^{125}I-IgE was ≃90%. The specific activities of the IgE's were $\geq 6 \times 10^8$ cpm/mg.

Binding to Cells. (*A*) *Association Kinetics.* Kinetic analyses to determine the forward rate constants for the binding of IgE to cells were performed as described previously (Kulczycki & Metzger, 1974) except that in the final assays the cells were centrifuged through cushions of a mixture of phthalate oils [60% dibutylphthalate–40% bis(2-ethylhexyl)phthalate] (Matthysens et al., 1975) instead of serum.

Cells were "tagged" by saturating a small percentage of their receptors with ^{131}I-IgE in order to be able to correct the final kinetic data for small variations in cell number that resulted from the multiple manipulations. The washed cells were then preincubated with unlabeled IgE for various times, rewashed, and then incubated with a low concentration of ^{125}I-IgE for kinetic analysis or with a higher concentration for assessment of total unoccupied receptors. In each case where labeled IgE was added, a duplicate group of tubes was set up to which a 100-fold excess of unlabeled IgE had been added prior to the addition of the labeled IgE. The details of the protocol are given in Table I.

For the calculation of the initial velocity, V_0, the average velocity at each time point was plotted vs. time and linearly extrapolated to zero time.

(*B*) *Dissociation Kinetics.* Cells were suspended at 3 × 10^7 cells/mL and incubated with ^{125}I-IgE at a ratio of IgE:receptor of ≃0.3 in order to achieve partial saturation. The cells were then washed free of unbound IgE. Half of the cells was suspended at 2 × 10^4 cells/mL in 500 mL of medium in a spinner flask. The other half was incubated with excess unlabeled IgE prior to suspending the cells in a spinner flask at 2 × 10^4 cells/mL in 500 mL of medium containing 1 μg/mL of IgE. The spinner flasks were incubated at 37 °C, and samples of 100 mL were taken at times t = 0, 3.5, 10.5, and 21.5 h. The cells in each sample were washed twice and suspended in 2.5 mL. Samples of 0.5 mL were taken in triplicate for determination of cell concentration and the amount of labeled IgE bound per cell.

Studies with Extracts. The rate of association of IgE with receptors solubilized by treating cells with 0.5% Triton X-100 was studied exactly as described by Rossi et al. (1977) by an assay that capitalizes on the change in the solubility of IgE in 40% saturated $(NH_4)_2SO_4$ when it becomes bound to the receptor. The incubation of the receptors with IgE was performed at a concentration of 5 × 10^{-9} M receptors and either 5.4 × 10^{-9} M bindable IgE (for the kinetic studies) or 2.8 × 10^{-8} M bindable IgE (for determining the total number of receptors).

Results

Association Kinetics. (*A*) *Cells.* Cells were tagged by partially saturating their receptors with ^{131}I-IgE. Under the conditions used (Table I) about 4% of the 4 × 10^5 receptors/cell became occupied. The cells were then reacted with an ≃2-fold excess of unlabeled IgE for various lengths of time (Table I); up to 75% of the receptors were occupied under the conditions used (Table II). When such cells were studied with respect to the rate with which IgE bound to the residual receptors, a marked change in the initial velocity was observed

[1] Abbreviations: IgE, immunoglobulin E; RBL, rat basophilic leukemia.

Table II: Number of Receptors and Rate of Binding of ^{125}I-IgE by Cells Preincubated with Unlabeled IgE

sample	time of preincubation (min)	^{125}I-IgE at saturation (mol/cell $\times 10^{-5}$)	R_0[a] ($M \times 10^9$)	V_0 ($M\ s^{-1} \times 10^{12}$)
A	0	4.14	1.55	1.94
B	1	3.16	1.18	1.25
C	2	2.71	1.01	0.972
D	4	2.01	0.753	0.856
E	7	1.30	0.487	0.519
F	10	0.99	0.370	0.322

[a] For 2.25×10^6 cells/mL.

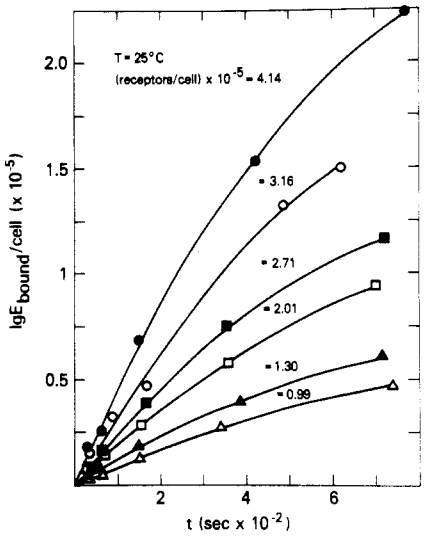

FIGURE 1: Kinetics of binding of IgE to cells bearing variable numbers of unoccupied receptors. Each point is the mean of duplicate values. The cell concentration of cells was 2.25×10^6 mL^{-1} and of IgE 9.4×10^{-9} M.

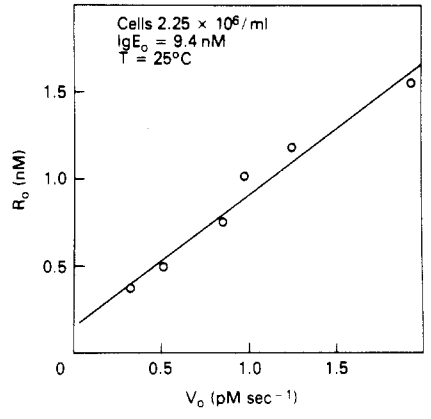

FIGURE 2: Initial velocity of binding of IgE to cells vs. receptor concentration. The initial velocities were calculated from the data shown in Figure 1 as described under Experimental Procedures.

(Table II, Figures 1 and 2). Figure 1 shows the quality of the data. Each point is the mean of duplicates that varied on the average less than 5% from the mean. The relationship between receptor concentration in moles/liter and the initial velocity of IgE binding is plotted in Figure 2. It is clear that there is a linear relationship, the two parameters having a correlation coefficient of 0.98. Using the least-squares line plotted in Figure 2, we calculated a k_f of 1.17×10^5 M^{-1} s^{-1}.

(B) Solubilized Receptors. Figure 3 illustrates the results of a study in which the rate of binding of IgE was determined in the presence or absence of 22.5% metrizamide (w/v) (see Discussion). The latter increased the viscosity of the solution

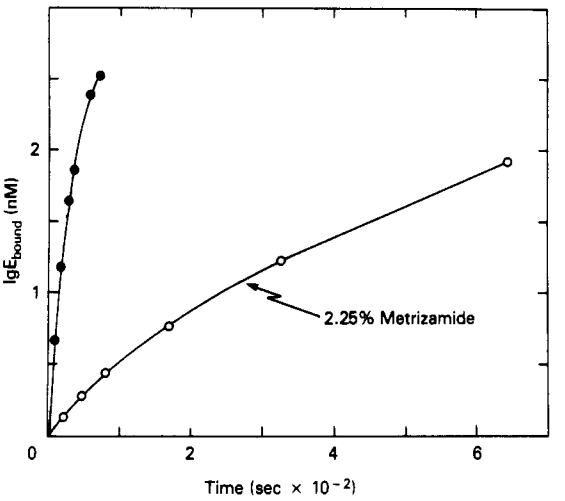

FIGURE 3: Kinetics of binding of IgE to receptors in a solution of nonionic detergent in the presence or absence of metrizamide. The receptors were at a concentration of 5 nM and the IgE was at 5.4 nM at the start of the reaction.

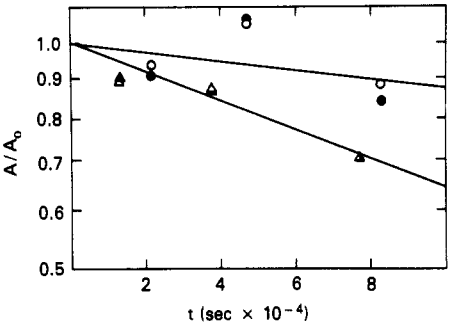

FIGURE 4: Rates of dissociation of ^{125}I-IgE from cells in the presence and absence of unlabeled IgE: (circles) 24 °C; (triangles) 37 °C; (open symbols) no IgE; (filled symbols) with unlabeled IgE. The ordinate is a log scale that shows the bound ^{125}I-IgE as a fraction of the amount bound at time zero.

about 1.6-fold relative to the solvent alone. The rate of binding showed a much greater depression. In the absence of metrizamide a k_f of $(3.7 \pm 1.7) \times 10^6$ M^{-1} s^{-1} was calculated in two separate experiments, whereas with metrizamide present k_f was reduced 15-fold (Figure 3).

Dissociation Kinetics for Cells. Cells were incubated with labeled IgE in order to achieve partial saturation of cell receptors. This resulted in ≃30% occupancy of cell receptors (82000 receptors/cell). After the cells were washed they were divided into two groups. Group A was incubated in a spinner flask with plain medium. Samples were withdrawn periodically to determine the amount of dissociation of labeled IgE. Group B was handled similarly, except that the cells were incubated with IgE prior to incubating in the spinner flask and the incubation medium contained unlabeled IgE. Both groups of cells were maintained at either 24 or 37 °C for nearly 22 h with greater than 95% viability and without a change in the cell number. The rate of dissociation for both groups followed first-order kinetics (Figure 4), and these rates were nearly identical. Each point used to construct the linear regression plots in Figure 4 represents the mean of triplicate samples. On the average, each sample varied from the mean by less than 10% for cell concentrations and less than 5% for IgE bound per cell. Both groups have a half-time of dissociation of 45 h at 37 °C and 152 h at 24 °C, yielding reverse rate constants of 4.3×10^{-6} s^{-1} and 1.3×10^{-6} s^{-1}, respectively. The kinetic constants for the cells and detergent extracts are collected in Table III.

Table III: Rate Constants for Binding of IgE to Receptors on Cells or in Detergent Extracts at 25 °C

	k_f (M^{-1} s^{-1} × 10^5)	k_r (s^{-1} × 10^6)
cells	1.2	1.3
extract	37	0.64[a]

[a] Data from Rossi et al. (1977).

Discussion

Theory. As has been described previously (DeLisi, 1981), the reaction between ligand and receptor can be considered as a two-stage process: the formation of an encounter complex L···R governed by forward and reverse diffusive rate constants k_+ and k_- and the formation of a reaction product LR governed by the reactive rate constants k_1^* and k_{-1}, i.e.

$$L + R \underset{k_-}{\overset{k_+}{\rightleftharpoons}} L\cdots R \underset{k_{-1}}{\overset{k_1^*}{\rightleftharpoons}} LR \qquad (1)$$

Here k_1^* is an "effective" intrinsic rate constant that takes account of the orientational requirement for reaction when the rotational diffusion of the ligand is fast compared to the translational diffusion of its center of mass. If the encounter complex L···R is in a steady state, then one can show (DeLisi, 1980) that this two-stage process can be replaced by a single-step process with effective forward and reverse rate constants k_f and k_r given by

$$k_f = k_+k_1^*/(k_1^* + k_-) \qquad (2)$$

$$k_r = k_-k_{-1}/(k_1^* + k_-) \qquad (3)$$

Equations 2 and 3 for the observed rate constants can also be derived by using an entirely different physical picture, viz., partially absorbing boundary conditions (Shoup & Szabo, 1982). The dependence of k_f on the receptor number and distribution arises through k_+ and k_-. In particular, for disk-like receptors of radius s, uniformly distributed over a spherical cell of radius a (Berg & Purcell, 1978)

$$k_+ = 4\pi aDNs/(Ns + \pi a) \qquad (4)$$

and (DeLisi & Wiegel, 1981)

$$k_- = g\frac{3D}{s^2}\frac{\pi a}{Ns + \pi a} \qquad (5)$$

where D is the diffusion coefficient of the ligand, and N is the number of unoccupied receptors per cell at any given time after mixing of the ligand and cells. g is a geometric factor of order unity that depends upon the geometry of the bound complex (DeLisi, 1982).

If one uses these relations the equation that describes the course of reaction

$$dN/dt = -k_fL \qquad (6)$$

where the bulk concentration L is the number of molecules of unbound ligand per milliliter, can be solved. The solution is given in the Appendix. Equation 6 neglects dissociation, which is negligible during the time of an association experiment.

(A) Diffusion-Limited Regime. If the rate at which bonds are formed between the ligand and the receptor is fast relative to the rate at which the encounter complex dissociates, i.e., $k_1^* \gg k_-$ in eq 1, then the reaction is said to be "diffusion limited". That is, $k_f \simeq k_+$ (eq 2). In the system such as the one we have examined, the cell radius (a) is $\sim 5 \times 10^{-4}$ cm (Isersky et al., 1975), the diffusion constant for the ligand (D) is $\simeq 3.5 \times 10^{-7}$ cm^2/s (Bennich & Johansson, 1971; Newman et al., 1977), and the radius of that portion of the receptor that binds IgE is $\simeq 3 \times 10^{-7}$ cm (Metzger et al., 1982).[2] Then, it can be calculated (Appendix) that when k_1^* is $\geq 10^8$ s^{-1}, it will be much greater than k_- and the reaction will be diffusion limited. In that case, the equation that describes the course of the reaction simplifies to a form such that the number ofempty receptors (N) will change according to the relation

$$N = \bar{N} - \bar{N}r[1 - \exp(-4\pi aDct)] \qquad (7)$$

where $\bar{N}$ is the number of vacant receptors per cell at the start of the reaction and r is the ratio of total ligand to the initial concentration of empty receptors, i.e., $r \equiv L_0/\bar{N}c$), c being the concentration of cells and L_0 the total ligand concentration. Note that the rate of binding averaged overall cells, $4\pi aDc$, is proportional to the cell concentration but *independent* of the number of receptors per cell. An example of receptor density independence of the forward rate is given in Table IV. The rate of binding shown in Table IV is about 100 times faster than the experimentally determined rate. However, one cannot conclude from this difference that the rate is not diffusion limited since the rate constant $4\pi aD$ neglects orientational factors. One can draw qualitative but not quantitative conclusions from the table. Finally, it can also be shown that the rate of dissociation is *dependent* on the number of vacant sites (DeLisi & Wiegle, 1981).

Table IV: Bound Ligand as a Function of Time Calculated for a Hypothetical Diffusion-Limited System[a]

time (s)	$4\pi aDct$	molecules bound/cell $\bar{N} = 10^5$	molecules bound/cell $\bar{N} = 4 \times 10^5$
0.40	2×10^{-3}	4 846	4 958
1.6	8×10^{-3}	19 280	19 769
6.5	3.2×10^{-2}	74 666	78 092

[a] Calculated using eq A2 (Appendix) with $\lambda_1 + \lambda_2 = 3 \times 10^3$ and $L_0/c = 2.5 \times 10^6$ molecules/cell.

(B) Reaction Is Not Diffusion Limited. The predictions are simplest when (for the system examined here) $k_1^* \ll 3 \times 10^5$ s^{-1}. In that case, it can be shown (Appendix) that k_f is linearly proportional to N and that eq 6 becomes the standard differential equation for describing a bimolecular reaction: the rate of change of vacant sites is proportional to the product of free ligand and free sites. The initial rate of binding will therefore be proportional to $\bar{N}$, the number of vacant receptors at time zero. Since k_1^* is $\ll k_-$, $k_r \simeq k_{-1}$ (eq 3); i.e., dissociation is expected to be occupancy *independent*.

Interpretation of Results. The data in Figure 2 clearly show a linear relationship between the initial velocity of the reaction of IgE with the receptors on cells and the concentration of vacant receptors per cell. On the other hand, the rate of dissociation was occupancy independent (Figure 4). Both results are consistent with the regime predicted for a reaction that is not diffusion limited. It was previously shown that when the course of the binding reaction was followed, the data were consistent with a bimolecular reaction (Figure 3 in Kulczycki & Metzger, 1974), which also apparently is inconsistent with a diffusion-controlled reaction. However, it can be calculated that the precision required to rule out the diffusion-limited mechanism by the latter type of experiment is too great to be

[2] The portion of the receptor that binds IgE—the α chain—has a molecular weight of $\simeq$50 000 and a partial specific volume of $\simeq$0.72 (Kanellopoulos et al., 1980). If we assume a disklike shape with the height being equal to half the radius, the latter would be equal to 34 Å; if it were spherical, r would equal 24 Å. These differences are too small to affect the calculations significantly.

practical and the approach used in the present studies is much more reliable.

Another approach to discern whether the reaction of a ligand with a cell is diffusion limited is to compare the rate constants for the binding to cell-bound vs. dispersed (i.e., solubilized) receptors. Theory predicts (Tanford, 1961) that the ratio of the respective k_f's should change with the viscosity of the medium. We tested the feasibility of this approach by using metrizamide to vary the solvent viscosity. This compound appears to be harmless to these types of cells at high concentrations (Holgate et al., 1980; Coutts et al., 1980). However, its effect even on solubilized receptors was much greater than could be accounted for by changes in the viscosity of the medium (Figure 3). The metrizamide may be changing the solvent structure such that changes in rates due to viscosity are overshadowed by perturbations that are effecting the reaction mechanism itself.

Differences between Cell-Bound and Solubilized Receptors. Table III compares the estimated rate constants (k_f and k_r) for the cell-bound and solubilized receptors. While the estimates for the k_r's are likely to be somwehat inaccurate [cf. Isersky et al. (1979)], it is apparent that the k_r's are similar and that the dramatic (30-fold) difference is in the k_f's. Because of previous uncertainties about whether the rate of binding to the cell was diffusion limited or not, it had been unclear whether the difference in k_f for cell-bound and solubilized receptors (cf. Rossi et al., 1977) was real or an artifact of calculation. We now have proven that the latter is not the case.

How can one account for this difference? It is possible that solubilization of the receptors with Triton X-100 induces a change in conformation of the receptor. A priori, one would predict that this would more likely be reflected in k_r rather than in k_f.

Alternatively, the nonreceptor glycoproteins and glycolipids on the cell surface may be a factor. While their presence might limit the access of the ligand to the receptor, e.g., by restricting the possible angles of approach, such an effect should influence k_f and k_f symmetrically. However, if the glycolipids or glycoproteins or both that surround the receptors are flexible, they might act as a "gate"—alternately closing and opening and thus effecting the forward rate constant. If the IgE when bound restricts the motion of these membrane components to the "open gate" configuration, the free energy change thereby induced will effect the observed equilibrium constant for the binding of IgE. The latter speculation should be testable by using cells depleted of nonreceptor proteins by proteases [the receptor for IgE appears to be quite resistant to the latter (Metzger et al., 1976)] or by examining the binding of ligand to receptors reincorporated into liposomes.

A "gating effect" might explain the observation that the rate but not the amount of IgE binding is almost doubled when cells are treated with neuraminidase (Pecoud et al., 1981). However, whether the enzyme effected the receptor itself or other membrane glycoproteins was not analyzed.

Appendix

Solution to Eq 6. If one uses the conservation condition that the concentration of bound ligand equals the concentration of bound receptors, eq 6 (text) can be written as

$$dN/dt = k_f[L_0 - c(N - \bar{N})] \quad \text{(A1)}$$

where c is the number of cells per milliliter, $\bar{N}$ is the initial number of free receptors per cell, and L_0 is the total number of labeled IgE molecules per milliliter. Then with eq 2–5, eq A1 can be solved as a function of time. The result, with all labeled ligand initially free, is

$$\alpha(N)\left[\frac{Nc - \bar{N}c(1-r)}{L_0}\right] = \exp(-4\pi aDct) \quad \text{(A2)}$$

where

$$\alpha(N) \equiv \left[\frac{N}{\bar{N}}\frac{L_0}{Nc - \bar{N}c(1-r)}\right]^{-(\lambda_1+\lambda_2)/[\bar{N}(1-r)]} \quad \text{(A3)}$$

and

$$r \equiv L_0/(\bar{N}c) \quad \text{(A4a)}$$

$$\lambda_1 \equiv 3\pi aD/(k_1^* s^3) \quad \text{(A4b)}$$

and

$$\lambda_2 \equiv \pi a/s \quad \text{(A4c)}$$

Diffusion-Limited Regime. When

$$\lambda_1 \ll \lambda_2 \quad \text{(A5)}$$

$k_1^* \gg k_-$ and the reaction will be diffusion limited.

If one uses $a = 5 \times 10^{-4}$ cm, $D = 3.5 \times 10^{-7}$ cm^2/s, and $s = 30 \times 10^{-8}$ cm, condition A5 implies that

$$\lambda_1 \simeq 6.1 \times 10^{10}/k_1^* \ll \lambda_2 \simeq 5 \times 10^3$$

Consequently, k_1^* must be of the order 10^8 s^{-1} or larger for condition A5 to be fulfilled. If one assumes this requirement is met, then under a wide range of conditions and throughout most of the reaction, relationship A2 simplifies such that the free receptor number will change according to

$$N = \bar{N} - \bar{N}r[1 - \exp(-4\pi aDct)] \quad \text{(A6)}$$

If $r < 1$, then $N \to \bar{N}(1-r)$ as $t \to \infty$. If $r > 1$, then all sites become saturated at some finite time, t^*, where

$$t^* = -\frac{1}{4\pi aDc}\ln\frac{r-1}{r} \quad \text{(A7)}$$

Equation A6 can be obtained intuitively by noting that if the reaction is diffusion limited and N is not too small ($N \gtrsim \pi a/s$), k_f, given by $k_+k_1^*/(k_1^* + k_-)$, will be approximately independent of N, and under that condition eq A6 is the solution to eq 6. Thus when the reaction is diffusion limited, eq A6 holds, and the binding rate (averaged over all cells), $4\pi aDc$, is proportional to the cell concentration and independent of the number of receptors per cell. If the reaction were reversible (i.e., if the observation time were long enough), the dissociation rate would be occupancy dependent [eq 3 and 5 (text) and A5 with $Ns > \pi a$].

It is evident that since the cells used in the experiments reported here have $\gtrsim 3 \times 10^5$ Fc receptors, we expect eq A6 to be approximately valid under a wide range of values for L_0 and $\bar{N}$, *provided the reaction is diffusion limited.*

Reaction Is Not Diffusion Limited ($\lambda_1 > \lambda_2$). The predictions are simplest when $\lambda_1 \gg \bar{N}$. In that case, k_f is linearly proportional to N. In particular

$$k_f \simeq (4/3)k_1^* s^3 N \quad \text{(A8)}$$

and eq 6 (text) becomes the standard differential equation for describing a bimolecular reaction: the rate of change of free sites is proportional to the product of free ligands and free sites. Substituting eq A8 into eq 6 (text) and using the condition that the bound IgE concentration equals the bound receptor concentration

$$dN/dt = -(4/3)k_1^* s^3 N(L_0 - \bar{N}c + Nc) \quad \text{(A9)}$$

If s is expressed in centimeters, then L_0, $\bar{N}c$, and Nc are in

molecules per cubic centimeter ≃ molecules per milliliter. The initial rate of loss of free receptors is now linearly proportional to $\bar{N}$, and the time course of N is obtained by solving eq A9. The solution is

$$N = \bar{N}(1 - r)/(1 - re^{-\alpha t}) \qquad r \neq 1 \qquad \text{(A10)}$$

where

$$\alpha \equiv (4/3)k_1 s^3 \bar{N} c(1 - r) \qquad \text{(A11)}$$

Shortly after mixing, the rate of ligand binding is

$$\frac{dN}{dt} \simeq -(4/3)k_1 s^3 L_0 \bar{N}(1 - \alpha t)/\left(1 + \frac{r\alpha t}{1 - r}\right)^2 \qquad \text{(A12)}$$

The initial rate of binding ($t \rightarrow 0$) is therefore proportional to $\bar{N}$ (see, also, eq A9). This is in distinct contrast to behavior in the diffusion-limited regime in which the initial rate of binding is independent of $\bar{N}$. Therefore, if the reaction is not diffusion limited, we expect the reaction velocity shortly after mixing to satisfy eq A12, and the limiting velocity as $t \rightarrow 0$ should vary linearly with the initial number of free receptors per cell.

Registry No. Metrizamide, 31112-62-6; Triton X-100, 9002-93-1.

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A Novel Neutral Oligosaccharide Chain Found in Polysialoglycoproteins Isolated from Pacific Salmon Eggs. Structural Studies by Secondary Ion Mass Spectrometry, Proton Nuclear Magnetic Resonance Spectroscopy, and Chemical Methods†

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ABSTRACT: A novel carbohydrate chain possessing a hitherto unknown disaccharide unit, α-L-Fuc→D-GalNAc, has been isolated from salmon egg polysialoglycoproteins on alkali–borohydride treatment. Salmon egg polysialoglycoproteins contain O-glycosidically linked neutral pentasaccharide chains in addition to a number of oligosialosyl group containing sugar chains. Composition analysis of the neutral pentasaccharide gave fucose, galactose, 2-acetamido-2-deoxygalactose, and 2-acetamido-2-deoxygalactitol in a molar ratio of 1:2:1:1. The structure was determined to be α-L-Fuc(1→3)-β-D-GalNAc-(1→3)-β-D-Gal(1→4)-β-D-Gal(1→3)-D-GalNAcol by the following three major procedures: First, the sequential order of the constitutional monosaccharides was determined by secondary ion mass spectrometry before and after permethylation. Second, linkages were established by methylation analysis and Smith degradation and hydrazinolysis–nitrous deamination studies. Third, anomeric configuration of the glycosidic linkages involved was deduced from 270-MHz proton nuclear magnetic resonance spectroscopy.

Polysialoglycoproteins, first discovered in rainbow trout eggs by Inoue & Iwasaki (1978), are a novel type of glycoprotein molecules, characterized by the presence of as much as about 50% *N*-glycolylneuraminic acid by weight. Similar acidic glycoproteins are also found in the eggs of Pacific salmon, *Oncorhynchus keta* (Walbaum). They are primarily localized in the cytoplasm of the eggs and can be involved in the interaction with basic proteins although the biological role of the carbohydrate in these polysialoglycoproteins still remains veiled.

Salmon egg polysialoglycoproteins differ from the corresponding trout egg glycoproteins in the existence of neutral oligosaccharide chains conjugated to the protein through

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