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Mathematical Modeling the Kinetics of Cell Distribution in the Process of Ligand–Receptor Binding

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A statistical approach is presented to model the kinetics of cell distribution in the process of ligand–receptor binding on cell surfaces. The approach takes into account the variation of the amount of receptors on cells assuming the homogeneity of monovalent binding sites and ligand molecules. The analytical expressions for the kinetics of cell distribution have been derived in the reaction-limited approximation. In order to demonstrate the applicability of the mathematical model, the kinetics of binding the rabbit, anti-mouse IgG with Ig-receptors of the murine hybridoma cells has been measured. Anti-mouse IgG was labeled with fluorescein isothiocyanate (FITC). The kinetics of cell distribution on ligand–receptor complexes was observed during the reaction process by real-time measuring of the fluorescence and light-scattering traces of individual cells with the scanning flow cytometer. The experimental data were fitted by the mathematical model in order to obtain the binding rate constant and the initial cell distribution on the amount of receptors.

Introduction

The kinetics of binding ligands with receptors on a cell surface has received considerable theoretical attention. An analysis based on a steady-state calculation of the diffusive flux of ligands to receptors was presented by Berg & Purcell (1977), Shoup & Szabo (1982), and Zwanzig (1990). That approach was extended by Zwanzig and Szabo (1991) to provide a full time dependence of the reactive flux for infinitely capacious sinks. Another analysis is based on the hypothesis of Adam & Delbruck (1968) that the reaction rate between solutes and membrane receptors can be enhanced by a non-specific adsorption and subsequent two-dimensional diffusion to the receptors (Cukier, 1983; Berg, 1985; Wang *et al*., 1992). Axelrod & Wang (1994) presented a reduction-dimensionality rate enhancement for reaction-limited receptors. A model of binding multivalent ligands with multivalent receptors on a cell surface is also developed (Macken & Perelson, 1982;

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Sulzer & Perelson, 1996). Generally, all above-mentioned approaches consider a single cell, assuming that all cells in the system are equal. If a cell population has wide distribution on the amount of receptors (or other cell parameters), the mathematical model of the system becomes more complex. In order to model such system, a statistical approach can be applied in terms of distribution functions and stoichiometric binding constants. In the case of the reaction-limited approximation and neglecting native cell processes, the problem may be considered like that of the ligand binding to a multisubunit protein, and the general solution for the kinetics of distribution of ligand–receptor complexes may be presented in the form of series (Perelson, 1985; Bentz *et al*., 1988). Working well for relatively small amount of binding sites (1–100), the form of series becomes rather huge for systems with 10^4 and more binding sites per cell. In order to model such systems, a proper approximation would be very useful. On the other hand, until recently, the development of the statistical approach to model the ligand–receptor binding was devoted mainly to multivalent receptors (Perelson, 1985; Bentz *et al*., 1988; Muller *et al*., 1997; Jose & Jose, 1998), and it does not consider the shape and native properties of individual cells. Therefore, such models have rather limited application for real cell systems, especially for intact cells. From the most general point of view, the problem is that the mathematical model of the ligand–cell interaction should unite the statistical approach with the comprehensive model of ligand–receptor binding on a single cell.

The development of the mathematical model depends on the information, which is obtained in experiments on ligand–receptor binding (Sklar *et al*., 1984; Murphy, 1991; Malmborg *et al*., 1992; Liebert & Prieve, 1995; Quesada *et al*., 1997; Zuber *et al*., 1997). Well-known flow cytometry allows measuring the amount of ligand–receptor complexes on a single cell and collecting much statistical information for a relatively short time. Therefore, processes in a cell system can be studied by real-time measurements with a flow cytometer in terms of cell distributions (Kraemer *et al*., 1972; Bohn, 1976; Steinkamp & Kraemer, 1979; Murphy, 1991). For example, Steinkamp & Kraemer (1974) described a method in which the kinetics of cell distribution on fluorescence intensity was recorded during the binding process for a sample continuously incubated with a labeled ligand. Applying flow cytometry, such experiments greatly support the development of the statistical approach to model the ligand–cell binding.

The aim of this paper is to develop a framework for the statistical characterization of a cell system reacting with homogeneous monovalent ligands by applying a distribution function approach for the amount of cell receptors. In the case of reaction-limited process, analytical expressions have been derived for the kinetics of the two-dimensional cell distribution on occupied and free receptors. In order to demonstrate the applicability of the model, the kinetic of binding of the rabbit polyclonal anti-mouse IgG antibody to surface receptors of intact murine hybridomas have been examined. For this purpose, we have combined the scanning flow cytometry (Soini *et al*., 1998) with fluorescence immunoassay.

The cell distribution on free receptors cannot be measured directly because the free receptors are unlabeled. This paper presents the method to calculate the cell distribution on free receptors from the kinetics of the cell distribution on occupied receptors measured by flow cytometry.

Mathematical Model

The purpose of this section is to formulate the statistical approach to describe the kinetics of cell distribution during the process of ligand–receptor binding. The monovalent ligand is dissolved in the medium. The cell receptor is considered as a monovalent binding site. We assume that all receptors act independently, binding is reaction limited, no other events (e.g. internalization) occurring, and the total amount of cell receptors (occupied and free) is a constant (Lauffenburger & Linderman, 1993).

REACTION SCHEME

Let a cell have initially n free receptors. The first ligand molecule bound with the cell creates one ligand–receptor complex and reduces the amount of free receptors from n to $n-1$. The next ligand molecule bound with the cell creates one

more complex and reduce the amount of free receptors by one. Therefore, the consecutive reactions of ligand molecules with a single cell can be presented by the following scheme:

$$\begin{aligned} &A + C_{n,0} \underset{k^{diss}_{n-1\,1}}{\overset{k^{ass}_{n0}}{\rightleftarrows}} C_{n-1,1}, \\ &\cdots \\ &A + C_{x,y} \underset{k^{diss}_{x-1\,y+1}}{\overset{k^{ass}_{xy}}{\rightleftarrows}} C_{x-1,y+1}, \\ &\cdots \\ &A + C_{1,n-1} \underset{k^{diss}_{0n}}{\overset{k^{ass}_{1n-1}}{\rightleftarrows}} C_{0,n}, \end{aligned} \tag{1}$$

where A is the dissolved ligand molecule, k^{ass}_{xy} is the forward (association) rate constant for binding of the ligand to a receptor on the cell with x free and y occupied receptors on the surface, k^{diss}_{xy} is the corresponding reverse (dissociation) rate constant for the cell, and $C_{x,y}$ denotes a single cell with x free and y occupied receptors. For a single cell, we have the following conservation law:

$$x + y = n = const. \tag{2}$$

Generally, in the system there are many cells with different number of receptors, n. Then, the concentration of cells may be represented as a function of n, i.e. $C(n)$. The distribution function $C(n)$ is the statistical characteristic of the cell population and is not changed during ligand–receptor binding, if other native cell processes (internalization etc.) are neglected. During the reaction of ligand–receptor binding, the cells become partially covered by ligands, and the cell concentration may be represented by the function $C(x, y, t)$, where x is the amount of free receptors, y is the amount of occupied receptors, and t is time. It should be noted that x and y are considered as independent variables for that distribution function. As soon as we describe a cell population, where a cell with any combination (x, y) is available, the distribution function $C(x, y, t)$ of independent variables is used. Instead of the conservation law for a single cell, eqn (2), the following conservation law for a cell population holds:

$$I(t) + B(t) = I(0) = I_0 = const, \tag{3}$$

where $I(t)$ is the total amount of free sites $I(t)$, $B(t)$ is the total amount of binding sites, and I_0 is the total amount of all cell receptors per unit volume in the system. We will take into account eqn (3).

CONTINUOUS CELL DISTRIBUTION FUNCTION

If the width of the distribution $C(x, y, t)$ and mean values of x and y are big enough, it is possible to consider $C(x, y, t)$ as a continuous function. Then the system of master kinetic equations can be presented as follows:

$$\begin{aligned} \frac{\partial C(x,y,t)}{\partial t} = &\left(\frac{\partial}{\partial x} - \frac{\partial}{\partial y}\right) k^{ass}(x,y) C(x,y,t) A(t) \\ &- \left(\frac{\partial}{\partial x} - \frac{\partial}{\partial y}\right) k^{diss}(x,y) C(x,y,t), \end{aligned} \tag{4}$$

$$\begin{aligned} \frac{\mathrm{d}A(t)}{\mathrm{d}t} = \int_0^\infty \int_0^\infty &(-k^{ass}(x,y) A(t) C(x,y,t) \\ &+ k^{diss}(x,y) C(x,y,t)) \,\mathrm{d}x \,\mathrm{d}y \end{aligned} \tag{5}$$

and

$$I(t) = \int_0^\infty \int_0^\infty x C(x,y,t) \,\mathrm{d}x \,\mathrm{d}y, \tag{6}$$

$$B(t) = \int_0^\infty \int_0^\infty y C(x,y,t) \,\mathrm{d}x \,\mathrm{d}y. \tag{7}$$

REACTION-LIMITED KINETICS

The reaction-limited process is generally assumed for ligand–receptor binding (Lauffenburger & Linderman, 1993). Taking into account that all receptors act independently, and binding is reaction-limited, the forward rate constant $k^{ass}(x, y)$ and the dissociation rate constant $k^{diss}(x, y)$ can be presented as follows:

$$k^{ass}(x,y) = k^{ass}(x) = x k_f, \tag{8}$$

$$k^{diss}(x,y) = k^{diss}(y) = y k_d, \tag{9}$$

where k_f and k_d are association and dissociation rate constants, respectively, for a single ligand–receptor complex. Using eqns (8) and (9), the system of kinetics equation (4) and (5) can be solved analytically (see the appendix), and the solution is

$$A(t) = \frac{A_1 - A_2((A_0 - A_1)/(A_0 - A_2))\mathrm{e}^{k_f(A_2 - A_1)t}}{1 - ((A_0 - A_1)/(A_0 - A_2))\mathrm{e}^{k_f(A_2 - A_1)t}}, \tag{10}$$

$$C(x, y, t) = f(t)C(x', y', 0), \tag{11}$$

where

$$x' = xf(t) - (x + y)k_d \int_0^t f(w)\,\mathrm{d}w, \tag{12}$$

$$y'(t) = (x + y)\left(1 + k_d \int_0^t f(w)\,\mathrm{d}w\right) - xf(t), \tag{13}$$

$$f(t) = \exp\left(\int_0^t (k_f A(w) + k_d)\,\mathrm{d}w\right). \tag{14}$$

A_1 and $A_2 (A_1 > A_2)$ are the square roots of the equation

$$A^2 + A\left(I_0 - A_0 + \frac{k_d}{k_f}\right) - \frac{k_d}{k_f} A_0 = 0 \tag{15}$$

and A_0 is the initial concentration of ligand molecules. Expression (11) describes the dynamics of the two-dimensional cell distribution on free and occupied receptors.

CELL DISTRIBUTION FUNCTION ON OCCUPIED RECEPTORS

Generally, in the experiments on ligand–receptor binding, the cell distribution on occupied (labeled) receptors $\tilde{\tilde{C}}(y, t)$ is measured by a flow cytometer:

$$\tilde{\tilde{C}}(y, t) = \int_0^\infty C(x, y, t)\,\mathrm{d}x. \tag{16}$$

The expression for $\tilde{\tilde{C}}(y, t)$ can be derived analytically from eqns (10)–(15). Assuming that cells have no occupied receptors at the beginning of the reaction, the following initial condition is used:

$$C(x, y, 0) = \begin{cases} 0 & y > 0, \\ \tilde{C}(x, 0) & y = 0, \end{cases} \tag{17}$$

where $\tilde{C}(x, 0)$ is the initial cell distribution on free receptors $\tilde{C}(x, t)$:

$$\tilde{C}(x, t) = \int_0^\infty C(x, y, t)\,\mathrm{d}y. \tag{18}$$

Using the initial condition (17), after rearrangements, the following expression is derived:

$$\tilde{\tilde{C}}(y, t) = \frac{1}{1 - z(t)/f(t)}\,\tilde{C}(y'', 0), \tag{19}$$

where

$$y'' = \frac{y}{1 - z(t)/f(t)}, \tag{20}$$

$$z(t) = 1 + k_d \int_0^t f(w)\,\mathrm{d}w. \tag{21}$$

Equations (19)–(21) describe the kinetics of cell distribution in occupied receptors in the general case of reversible binding, when the dissociation reaction cannot be neglected.

IRREVERSIBLE BINDING

Although the reaction of ligand–receptor binding is reversible, in particular cases of specific binding the dissociation reaction can be neglected. Then the analytical solution is simplified, and it can be represented as follows:

$$A(t) = A_0 \frac{1 - I_0/A_0}{1 - (I_0/A_0)\mathrm{e}^{-k_f(A_0 - I_0)t}}, \tag{22}$$

$$\tilde{\tilde{C}}(y, t) = a(t)\tilde{C}(ya(t), 0), \tag{23}$$

$$a(t) = \frac{\mathrm{e}^{k_f(A_0 - I_0)t} - (I_0/A_0)}{\mathrm{e}^{k_f(A_0 - I_0)t} - 1}. \tag{24}$$

According to eqn (23), the distribution on occupied receptors $\tilde{\tilde{C}}(y, t)$ at given time t is expressed by scaling the initial distribution on free receptors $\tilde{C}(x, t)$, where the scaling parameter $a(t)$ is

a function of time. The well-known expression for the kinetics of mean cell fluorescence $\bar{y}(t)$ can be derived from eqn (23):

$$\bar{y}(t) = \int_0^\infty y\tilde{\tilde{C}}(y,t)\,\mathrm{d}y = \frac{I_0}{a(t)}$$

$$= I_0 \frac{\mathrm{e}^{k_f(A_0-I_0)t}-1}{\mathrm{e}^{k_f(A_0-I_0)t}-I_0/A_0}. \qquad (25)$$

Equation (23) was used to fit the experimental data on the kinetics of the cell distribution $\tilde{\tilde{C}}(y,t)$ on occupied receptors measured with the SFC scanning flow cytometer. The experimental data were considered as a two-dimensional histogram $\tilde{\tilde{C}}(y_i,t_j)$, which was evaluated at certain points (y_i,t_j), and the two-dimensional nonlinear Levenberg–Marquart method was applied for the fitting. The following independent parameters were varied and obtained in the fitting: the rate parameter $k_f(A_0-I_0)$, the ratio I_0/A_0, and the initial distribution $\tilde{C}(x,0)$ on free receptors. In order to vary and to obtain $\tilde{C}(x,0)$, the distribution was represented as a histogram $\tilde{C}(x_i,0)$, where $i=1,\ldots,10$. Therefore, the distribution was represented by ten independent parameters $\tilde{C}(x_i,0)$, which were obtained in the fitting. An optimal distance between the equidistant points x_i was found and fixed in order to minimize the fitting error.

Experimental

In order to demonstrate the applicability of the presented approach, fluorescence experiments on ligand–receptor binding were carried out, and the kinetics of the cell distribution on labeled receptors was measured with the scanning flow cytometer Anti-mouse IgG labeled by FITC and murine hybridoma cells were used in these experiments.

LIGAND AND CELLS

Murine hybridomas 7H10 and 9G6 (Razumov *et al*., 1998) were used as receptor-bearing cells. The cultivation of murine hybridomas was done according to the description given by Razumov *et al*. (1991). The cells were washed 3 times and resuspended in the buffered saline (0.01 M phosphate buffer, pH 7.2 with 0.15 M NaCl). Then the cells were stored at 4°C and used within a working day. All kinetic experiments were carried out at room temperature 20°C. Affinity-purified rabbit anti-mouse IgG antibody (Sigma) was used as a ligand for the IgG-like receptors on the cells. Anti-mouse IgG immunoglobulins were labeled by FITC. FITC labeled immunoglobulins were freeze-dried and stored at 4°C. The FITC-conjugates were dissolved in buffered saline immediately before experiments. The number of FITC molecules conjugated per single IgG molecule was determined by a spectrophotometer employing the following equation:

$$\frac{[\mathrm{FITC}]}{[\mathrm{Protein}]} = \frac{2.87\times OD_{495}}{(OD_{280}-0.35\times OD_{495})}, \qquad (26)$$

where OD_{495} and OD_{280} are the optical density of the IgG solution at 495 and 280 nm, respectively (Storz, 1984). FITC-conjugates dissolved in buffered saline were mixed with the cells in a plastic tube that was attached to the inlet capillary of the scanning flow cytometer. The kinetics of the cell distribution on fluorescence intensity was recorded by the flow cytometer during the binding process in the sample incubated continuously with the labeled ligand.

SCANNING FLOW CYTOMETER

The scanning flow cytometer (SFC) was developed in our group and was described in details elsewhere (Chernyshev *et al*., 1995; Soini *et al*., 1998; Shvalov *et al*., 1999). In contradistinction to the ordinary flow cytometer the SFC measures the entire angular dependency of the intensity of light scattered by a single cell (light-scattering indicatrix), and that provides additional information to distinguish individual cells. The fluorescence of the FITC-labeled IgG on a cell surface was measured simultaneously with the light-scattering signal from a single cell. A 15-mW air-cooled 488 nm argon-ion laser (Spectra Physics) was applied for fluorescence excitation.

Results and Discussion

We used a commercially available fluorescent microspheres (Polyscience, Inc., mean size

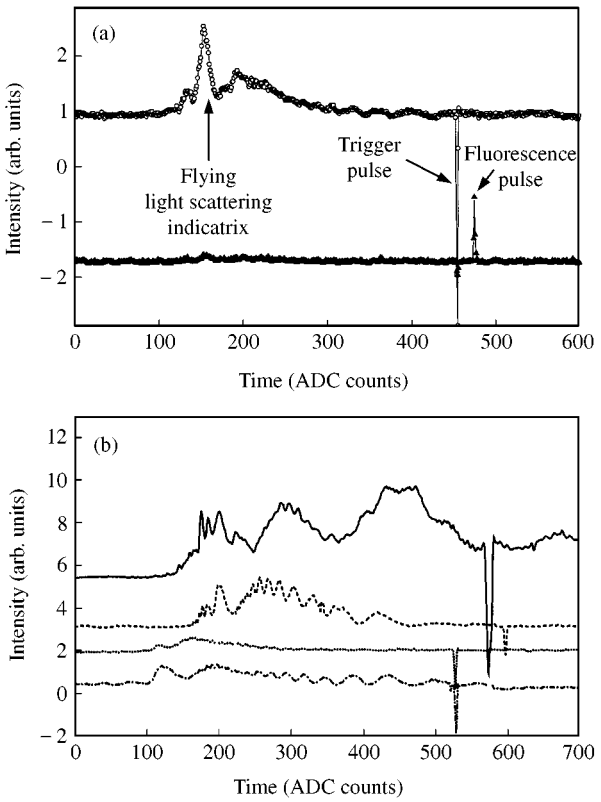

FIG. 1. Experimental signals for a single fluorescent latex particle (Polyscience, Inc.) of size 1.8 μm (a): ( ) light scattering channel; ( ) fluorescence channel and native SFC traces for light-scattering patterns from different single cells (b): ( ), mouse hibridoma (7h10); ( ), human sarcoma (H-143); ( ), E.coli (TG-1); ( ), E.coil (JS-5318).

— 1.8 μm, standard size deviation—3%) to calibrate the cytometer. Figure 1(a) shows the control measurement of the light-scattering trace (up) and the fluorescence pulse (down) of the calibrated fluorescent microsphere measured by the SFC. The SFC light-scattering traces (Shvalov *et al.*, 1999) were used to distinguish the cells among other particles in the sample: cell clumps, damaged or dead cells, protein aggregates, etc. Typical SFC traces of cells are shown in Fig. 1(b). For the presentation every curve was shifted in vertical direction. The fluorescence signal is proportional to the amount of FITC labeled ligand–receptor complexes on the cell surface. Figure 2 shows the distribution of fluorescence intensity of the calibrated fluorescent microspheres obtained from the control measurement.

The kinetics of distribution of cells 7H10 is presented in Fig. 3. The fluorescence signals of

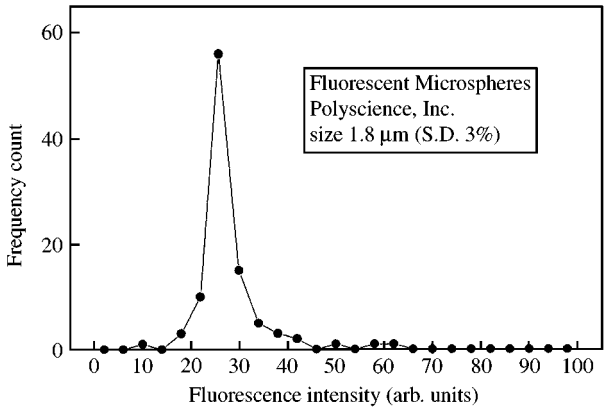

FIG. 2. The fluorescence intensity histogram of calibrated fluorescent latex particles (Polyscience, Inc., size —1.8 μm, S.D. of size —3%).

500 cells were collected to build every cell distribution on fluorescent intensity. It follows from experimental data that the whole reaction takes approximately 1 h. The experimental data on the kinetics of cell distribution were fitted with eqn (23). The solid lines shown in Fig. 3 are the best fit to the experimental data. The obtained parameters and known experimental conditions are presented in Table 1 and Fig. 4. The known conditions are initial concentrations of cells (C) and ligand molecules ($A(0)$). The parameters obtained from the fitting are the ratio $I(0)/A(0)$, the parameter $(k_f(A(0) - I(0)))^{-1}$, and the initial cell distribution (histogram) $\tilde{C}(x_i, 0)$ on free receptors (Fig. 4). The association rate constant k_f of a single binding site was calculated from the obtained parameters $I(0)/A(0)$ and $(k_f(A(0) - I(0)))^{-1}$. The average amount $\bar{n}$ of binding sites on a cell was estimated from the obtained histogram $\tilde{C}(x_i, 0)$.

In order to demonstrate the advantage of the presented method of cell distributions, the kinetics of mean fluorescence intensity (Fig. 5) was treated with eqn (25) to find the binding parameters. As a result, the only parameter, which was obtained by such treatment, is $(k_f(A(0) - I(0)))^{-1}$. The parameter $I(0)/A(0)$ could not be obtained from the mean fluorescence kinetics.

Using the values from Table 1, it is possible to verify the assumption that the ligand–cell binding was the reaction-limited process. In order to estimate the diffusion-limited rate constant, the

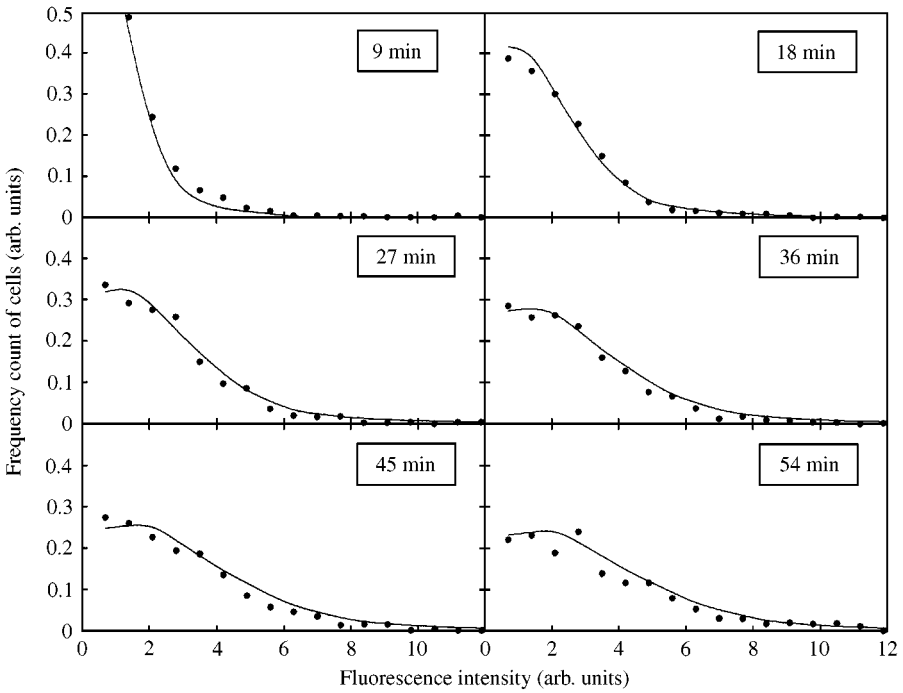

FIG. 3. The kinetics of fluorescence intensity histogram for 7H10 cells incubated with FITC-labeled ligand. The points and solid lines correspond to experimental data and theoretical calculation, respectively.

TABLE 1
Conditions and fitting results for ligand–receptor binding

Parameter	Sample 9G6_b	Sample 9G6_c	Sample 7H10_a
$A(0)$, cm^{-3}	3.1×10^{13}	6.3×10^{13}	1.3×10^{14}
C, cm^{-3}	1.0×10^{6}	1.0×10^{6}	2.0×10^{7}
$I(0)/A(0)$	0.031 ± 0.009	0.016 ± 0.006	0.49 ± 0.13
$(k_f(A(0) - I(0)))^{-1}$, s	502 ± 124	290 ± 76	607 ± 84
k_f, $cm^3\,s^{-1}$	$(6.3 \pm 1.6) \times 10^{-17}$	$(5.4 \pm 1.4) \times 10^{-17}$	$(2.5 \pm 0.4) \times 10^{-17}$
$\bar{n}$	9.6×10^{5}	10.1×10^{5}	3.2×10^{6}

following approximated values for the translational diffusion coefficient D_{IgG} and the radius a_{IgG} of immunoglobulin G molecules are used:

$$a_{IgG} = 3.6 \times 10^{-7}\ \text{cm},$$

$$D_{IgG} = 2 \times 10^{-7}\ \text{cm}^2\,\text{s}^{-1}.$$

The radius r of a cell is about 5×10^{-4} cm. According to the expression for the association rate constant of diffusion-limited binding on a cell surface (Zwanzig & Szabo, 1991), the diffusion-limited time τ_D of the reaction is

$$\tau_D = \frac{1}{k_D A(0)} = \frac{1-p}{4nbD_A A(0)} + \frac{1}{4\pi r D_A A(0)}, \quad (27)$$

where k_D is the diffusion-limited rate constant, D_A is the diffusion coefficient of ligand molecules, n is the amount of cell receptors, b is the radius of a circular receptor site, p is the fraction of the cell

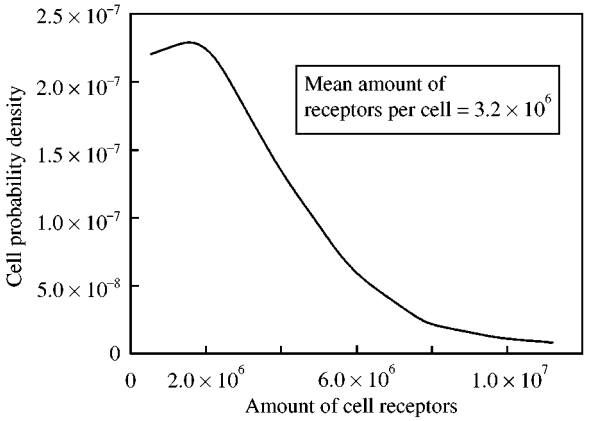

FIG. 4. Initial distribution on free binding sites for 7H10 cells obtained by fitting the experimental data.

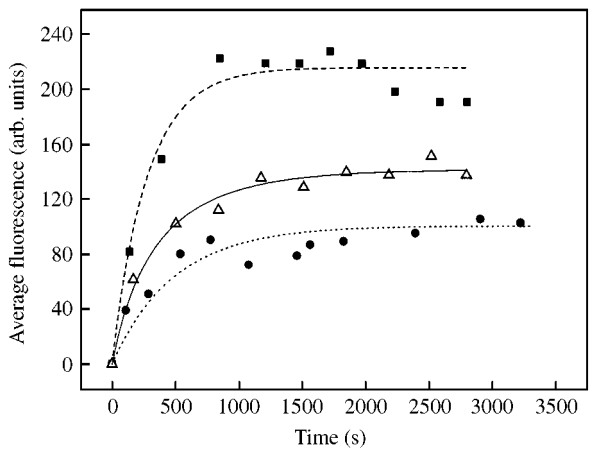

FIG. 5. Kinetics of the mean fluorescence intensity for hybridoma cells 9G6 and 7H10: A1–9G6, $A(0) = 3.1 \times 10^{13}$ cm^{-3}, $C = 10^6$ cm^{-3}; A2–9G6, $A(0) = 6.3 \times 10^{13}$ cm^{-3}, $C = 10^6$ cm^{-3}; B-7H10, $A(0) = 1.3 \times 10^{14}$ cm^{-3}, $C = 2 \times 10^7$ cm^{-3}: A1, ●; A2, ■; B1, △.

surface covered by receptors

$$p = \frac{nb^2}{4r^2}. \tag{28}$$

From the experiments we obtained the time of the reaction $\tau_r = (k_f(A(0) - I(0)))^{-1} \approx 500$ s (Table 1). Therefore, applying the condition of the reaction-limited process ($\tau_r \gg \tau_D$) to eqn (27), the following restriction on the amount of binding sites on a single cell takes place:

$$n \gg \frac{4\pi r^2}{4br(4\pi r D_A A(0)\tau_r - 1) + \pi b^2}. \tag{29}$$

Evaluating this estimation, one can see that condition (29) is realized in our system. Therefore, our assumption about the reaction-controlled process is supported.

From the fitting it was found that the initial cell distribution of free receptors coincides with the final cell distribution on occupied receptors. Such coincidence indicates that there is the high-affinity constant of the binding, and, therefore, nearly all cell receptors is occupied by ligand molecules at the end of the reaction. In the case of low-binding affinity there would be no such coincidence, and then, instead of eqn (23), expression (19) should be used to find the cell distribution on free receptors.

From the obtained parameters we can estimate the probability of ligand–receptor binding per one collision. According to the collision theory (Bamford & Tipper, 1969), the rate constant k_r of the reaction-controlled process can be represented by the following expression:

$$k_r = \frac{4\pi}{6} r^2 \bar{v}_A \varepsilon_0 p, \tag{30}$$

where $\bar{v}_A$ is the average velocity of molecules A in the medium, and ε_0 is the probability of binding per collision. From eqns (8), (28) and (30) it is possible to estimate the probability ε_0:

$$\varepsilon_0 = \frac{6k_f}{\bar{v}_A \pi b^2} = \frac{6k_f}{\pi b^2 \sqrt{2RT/M}}, \tag{31}$$

where M is the molar mass of IgG molecule, $R = 8.31$ is the gas constant, T is the temperature. Using known values of $M = 1.5 \times 10^5$ g mol^{-1}, k_f, a, and $T = 300$ K, the binding probability is estimated: $\varepsilon_0 \approx 0.005$. Therefore, the IgG molecule–receptor complex is formed after approximately 200 collisions.

The experimental data (Fig. 3) and the obtained result (Fig. 4) present rather wide cell distribution on number of receptors. According to our consideration such wide spread is the inherent property of the cell population, which was measured. The wide spread is not due to an apparatus effect, because the control measurements of calibrated fluorescence microspheres by the SFC demonstrate rather narrow distribution (Fig. 2).

Following the concept of distribution function approach, we have presented a mathematical model of the ligand–receptor binding, considering the receptor number to vary from cell to cell. Analytical expressions have been derived in order to find the initial cell distribution on free binding sites from the kinetics of cell distribution on occupied receptors. This method may be of particular interest for the statistical characterization of cell populations, and for detailed quantitative description of immune reactions in cell systems. The presented statistical approach can be expanded also on diffusion-limited and partially diffusion-limited processes, as well as a native cell processes (i.e. two-dimensional diffusion of ligand molecules on cell surface, internalization, etc.) can be included into the consideration.

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APPENDIX

We are going to find the solution for the system of equations:

$$\frac{\partial C(x,y,t)}{\partial t} = A(t)\left(\frac{\partial}{\partial x} - \frac{\partial}{\partial y}\right) x k_f C(x,y,t) - \left(\frac{\partial}{\partial x} - \frac{\partial}{\partial y}\right) y k_d C(x,y,t), \tag{A.1}$$

$$\frac{\mathrm{d}A(t)}{\mathrm{d}t} = \int_0^\infty \int_0^\infty (-x k_f A(t) C(x,y,t) + y k_d C(x,y,t))\,\mathrm{d}x\,\mathrm{d}y. \tag{A.2}$$

It follows from eqn (A.2) that

$$\frac{\mathrm{d}A(t)}{\mathrm{d}t} = -k_f A(t) I(t) + k_d B(t), \tag{A.3}$$

where $I(t)$ and $B(t)$ are concentrations of free and occupied binding sites, as these defined by eqns (6)–(7). Then the following conservation laws take place:

$$B(t) + I(t) = I(0) = I_0, \tag{A.4}$$

$$A(t) + B(t) = A(0) = A_0 \tag{A.5}$$

and the solution of eqn (A.3) is

$$A(t) = \frac{A_1 - A_2(A_0 - A_1)/(A_0 - A_2)\mathrm{e}^{k_f(A_2 - A_1)t}}{1 - (A_0 - A_1)/(A_0 - A_2)\mathrm{e}^{k_f(A_2 - A_1)t}}, \tag{A.6}$$

where A_1 and $A_2 (A_1 > A_2)$ are square roots of the equation

$$A^2 + A\left(I_0 - A_0 + \frac{k_d}{k_f}\right) - \frac{k_d}{k_f} A_0 = 0. \tag{A.7}$$

In order to solve the partial differential equation (A.1), the well-known method of characteristics is applied. Using eqn (A.3), after rearrangements, the following equation is derived from eqn (A.1):

$$\frac{\partial C}{\partial t} - \frac{\partial C}{\partial x}(A x k_f - y k_d) + \frac{\partial C}{\partial y}(A x k_f - y k_d) = (A k_f + k_d) C. \tag{A.8}$$

The following set of differential equations on corresponding characteristics arise from eqn (A.8):

$$\begin{aligned} \frac{\mathrm{d}t}{\mathrm{d}\tau} &= 1, \\ \frac{\mathrm{d}x}{\mathrm{d}\tau} &= y k_d - A x k_f, \\ \frac{\mathrm{d}y}{\mathrm{d}\tau} &= -y k_d + A x k_f, \\ \frac{\mathrm{d}C}{\mathrm{d}\tau} &= (A k_f + k_d) C. \end{aligned} \tag{A.9}$$

The solution of system (A.9) is

$$t(\tau) = \tau, \tag{A.10}$$

$$y(\tau) = a_1 - x(\tau), \tag{A.11}$$

$$x(\tau) = \frac{1}{f(\tau)}\left(a_2 + a_1 k_d \int_0^\tau f(w)\,\mathrm{d}w\right), \tag{A.12}$$

$$C(\tau) = a_3 f(\tau), \tag{A.13}$$

$$f(\tau) = \exp\left(\int_0^\tau (A(w)k_f + k_d)\,\mathrm{d}w\right), \tag{A.14}$$

where a_1, a_2, and a_3 are constants. According to eqn (A.13), the ratio $C(\tau)/f(\tau)$ is a constant along the characteristic curve $(x(\tau), y(\tau))$. Therefore, the solution of eqn (A.1) can be represented by the following expression:

$$C(x, y, t) = f(t)C(x', y', 0), \tag{A.15}$$

where x' and y' are derived from eqns (A.10)–(A.14):

$$x' = xf(t) - (x + y)k_d \int_0^t f(w)\,\mathrm{d}w, \tag{A.16}$$

$$y' = (x + y)\left(1 + k_d \int_0^t f(w)\,\mathrm{d}w\right) - xf(t) \tag{A.17}$$

and

$$f(t) = \exp\left(\int_0^t (A(w)k_f + k_d)\,\mathrm{d}w\right). \tag{A.18}$$