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THE INFLUENCE OF DIFFUSION ON THE RATE OF A REVERSIBLE QUASI-UNIMOLECULAR REACTION IN ONE, TWO OR THREE DIMENSIONS

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The role of diffusion in the kinetics of a reversible quasi-unimolecular reaction is considered. Equations that couple diffusion and reversible reaction are defined. From these equations are derived expressions for the concentrations of the reacting species, as a function of time, after a perturbation from their equilibrium concentrations. These expressions demonstrate how the time-dependent approach by a concentration to its equilibrium value is determined by the binding rate of adjacent molecules, the dissociation rate, the diffusion coefficients, the distance of closest approach of the reactants, the concentrations of the reactants, and the dimensionality. The expressions are applicable to perturbation-relaxation experiments in one, two, and three dimensions. The formalism is compared with previously existing theories.

1. Introduction

Molecular recognition, or specific binding, is of fundamental importance in biological processes. Although the specific binding of two biological molecules is frequently an irreversible event, recognition involving specific but reversible interactions is also of biological importance. For example, the two nonconducting gramicidin A monomers in a membrane that associate to form a conducting dimer have an average association time of approx. 1 s [1] and many antibody-hapten pairs have average binding times of only a few milliseconds [2]. Many ligand-receptor or enzyme-substrate pairs may also have relatively low average binding times [3]. In addition, cell-cell adhesion that is mediated by specific interactions between proteins anchored in adjacent cell membranes may depend on many weak, reversible interactions.

Because reversible interactions in biology may involve diffusion of the reacting molecules in solution or in a cell membrane, or in other geometries such as along a linear structure, it is important to know how diffusion affects the reaction rate. The role of diffusion in irreversible reactions has been more thoroughly investigated [4–9] than that in reversible reactions [10–20].

One type of process that may be important in the function of biological cell membranes is molecular adsorption from solution to a surface, followed by two-dimensional diffusion and reaction with specific sites on the surface [21,22]. Results of experiments designed to measure biomolecular adsorption with surface diffusion have been presented [20,23,24]. However, nonspecific and specific sites on the surface are not differentiated in these works. In this paper, expressions are derived for the concentrations as a function of time for a reversible bimolecular reaction occurring on a surface (in two dimensions). The theory is generalized to reactions occurring in one and three dimensions.

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The usual method of describing the kinetics of an irreversible bimolecular reaction is to solve for the number of bindings per time, either until all conjugates are formed (given a finite number of molecules at time zero), or until a steady state is reached (given a constant supply of molecules). To describe the kinetics of a reversible reaction, the appropriate method is to begin at time zero with nonequilibrium concentrations of reacting molecules and to solve for the concentrations, as a function of time, as equilibrium is approached. The temporal rate and shape of decay of the concentrations to their equilibrium values depends in a complex manner on the intrinsic binding and dissociation probabilities, on the diffusion coefficients, and on the geometry. Thus, the concentration as a function of time observed in a relaxation-type experiment (such as a concentration jump) also depends on these kinetic and diffusional parameters. Fluctuation-type experiments [1,24,25] measure the same parameters as relaxation-type experiments. In this paper it is demonstrated how to obtain and under what conditions it is possible to obtain intrinsic binding and dissociation rates and/or diffusion coefficients from kinetic data.

2. General solution

Our approach is similar to that of Smoluchowski [4] and Collins and Kimball [5]. In Smoluchowski's description of diffusion-control in irreversible, bimolecular reactions, the reaction rate is proportional to the flux of molecules of one type at the surface of a molecule of the other type. Collins and Kimball generalized Smoluchowski's problem to one of partial diffusion control by setting the reaction rate proportional to only a fraction of this flux. The problem can be further generalized by assigning a finite lifetime to the bound state of the molecules, as demonstrated below.

A reversible bimolecular reaction is described schematically by

$$\mathrm{A} + \mathrm{C} \underset{k_2}{\overset{\phi_n k_1}{\rightleftharpoons}} \mathrm{P}, \tag{1}$$

where $\phi_n k_1 \langle \mathrm{A} \rangle \langle \mathrm{C} \rangle$ is the concentration of bindings per time and $k_2 \langle \mathrm{P} \rangle$ is the concentration of dissociations per time, at equilibrium. $\langle \mathrm{A} \rangle$, $\langle \mathrm{C} \rangle$ and $\langle \mathrm{P} \rangle$ are equilibrium concentrations and k_1 and k_2 are rate constants. The concentrations are in one, two or three dimensions, denoted by $n = 1$, 2 or 3. Rate constant k_1 in eq. 1 is scaled by the parameter ϕ_n, where

$$\begin{aligned} \phi_1 &= 1 \\ \phi_2 &= 2\pi a \\ \phi_3 &= 4\pi a^2, \end{aligned} \tag{2}$$

and a is the distance of closest approach of the two types of molecules (the 'reaction radius'). Constant k_1 thus has units of length time^{-1} molecule^{-1} in all dimensionalities. Constant k_2 has units of time^{-1}. The sum of the concentrations of reactant $A(t)$ and product $P(t)$ is a constant N; these molecules are called 'A molecules'. The sum of the concentrations of reactant $C(t)$ and product $P(t)$ is a constant M; these are called 'C molecules'.

A single A molecule at the origin of a coordinate system in n dimensions is shown in fig. 1. C molecules freely diffuse and bind reversibly to the A molecule. The probability that the A molecule is occupied at time t is $G(t)$. The concentration of C molecules at a distance r from the center of the site at time t is $\hat{C}(r,t)$, where ^ denotes that the concentration is a function of the spatial coordinate r. Given an initial value of G and an initial uniform concentration of C molecules, the functions $\hat{C}(r,t)$ and $G(t)$ approach the equilibrium values $\langle \mathrm{C} \rangle$ and $\langle \mathrm{G} \rangle$ as time proceeds. $G(t)$ and $\hat{C}(r,t)$ represent ensemble averages over many A molecules and are thus continuous functions of their arguments.

The average distance between two A molecules is $2b$. The relationships between the ensemble averages G and $\hat{C}$ around a single A molecule and the macroscopic concentrations A, C and P are thus:

$$\begin{aligned} P(t) &= NG(t) \\ A(t) &= N[1 - G(t)] \\ C(t) &= a^{-n} d_n \int_a^b \hat{C}(r,t) r^{n-1} \mathrm{d}r, \end{aligned} \tag{3}$$

where

$$d_n = a\phi_n/v_n = n/(\gamma^n - 1)$$
$$v_n = V_n(b) - V_n(a) \qquad (4)$$
$$\gamma = b/a;$$

and volume elements $V_n(r)$ are

$$V_1(r) = r$$
$$V_2(r) = \pi r^2 \qquad (5)$$
$$V_3(r) = 4\pi r^3/3.$$

Concentration $\hat{C}(r,t)$ obeys the diffusion equation for n dimensions:

$$\frac{\partial}{\partial t}\hat{C}(r,t) = Dr^{1-n}\frac{\partial}{\partial r}\left[r^{n-1}\frac{\partial}{\partial r}\hat{C}(r,t)\right], \qquad (6)$$

where the diffusion coefficient of the C molecules is D. If the A molecules also diffuse, then the coordinate system in fig. 1 is a moving frame, and D is approximately equal to the sum of the diffusion coefficients of the C molecules and the A molecules. The approximation becomes exact as $b \to \infty$ [17,26].

Function $G(t)$ obeys the following nonlinear equation describing reaction at the sites:

$$\mathrm{d}G(t)/\mathrm{d}t = \phi_n k_1 \hat{C}(a, t)[1 - G(t)] - k_2 G(t). \qquad (7)$$

The first term on the right-hand side of eq. 7 follows from the assumption that the association reaction proceeds with proportionality constant $\phi_n k_1$ to the product of the local concentration C at $r = a$ and the probability that the A molecule is unoccupied. Diffusion effects are introduced by this term because the binding rate is not proportional to the overall concentration of C molecules but only to the concentration immediately adjacent to an A molecule; thus, a C molecule must first diffuse to an A molecule and then bind. The second term on the right-hand side of eq. 7 defines the dissociation rate k_2. The equilibrium constant K is

$$K = \phi_n k_1/k_2 = \langle \mathrm{G}\rangle/[(1 - \langle \mathrm{G}\rangle)\langle \mathrm{C}\rangle]. \qquad (8)$$

At time zero the values of $\hat{C}(r,t)$ and $G(t)$ are

$$\hat{C}(r,0) = \langle \mathrm{C}\rangle + \Delta C$$
$$G(0) = \langle \mathrm{G}\rangle + \Delta G, \qquad (9)$$

where Δ denotes the amount by which a starting value is different from an equilibrium value. (Conservation of molecules implies that $v_n \Delta C = -\Delta G$.) Normalized functions $\mathscr{G}$ and $\hat{\mathscr{C}}$ can be defined, where

$$\mathscr{G}(t) = [G(t) - \langle \mathrm{G}\rangle]/\Delta G$$
$$\hat{\mathscr{C}}(r,t) = [\hat{C}(r,t) - \langle \mathrm{C}\rangle]/\Delta C, \qquad (10)$$

so that $\mathscr{G}$ and $\hat{\mathscr{C}}$ decrease from 1 to 0 with increasing time.

Eq. 7 can be linearized with either of the following assumptions. First, if the experiment involves a

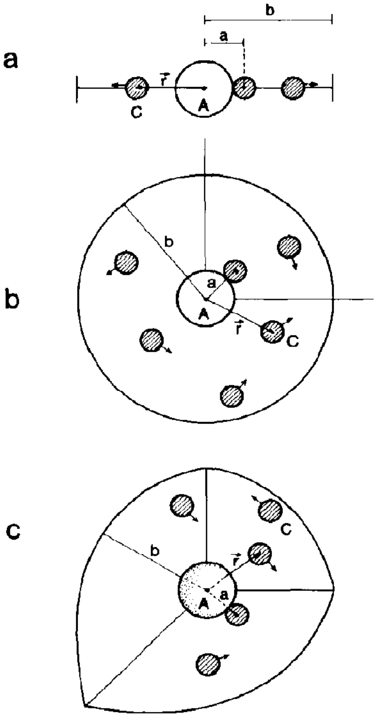

Fig. 1. Reaction geometry in one, two and three dimensions. An A molecule of occupancy $G(t)$ is at the origin of a (a) one, (b) two- or (c) three-dimensional coordinate system. C molecules of concentration $\hat{C}(r,t)$ diffuse and react with the site. Coordinate r is the distance from the site, distance a the closest approach of the two reactants, and distance $2b$ the average distance between two sites.

small perturbation, so that $\Delta G \ll G(t)$ and $\Delta C \ll \hat{C}(a,t)$, then eq. 7 can be written as (dropping a nonlinear cross-term proportional to $\Delta G \Delta C$):

$$\mathrm{d}\mathscr{G}(t)/\mathrm{d}t = -d_n R_{\mathrm{on}} \hat{\mathscr{C}}(a,\, t) - R_{\mathrm{off}} \mathscr{G}(t), \tag{11}$$

where

$$R_{\mathrm{on}} = (k_1/a)(1 - \langle \mathrm{G} \rangle)$$
$$R_{\mathrm{off}} = k_2/(1 - \langle \mathrm{G} \rangle) \quad \text{(small perturbation)}. \tag{12}$$

Second, if the A molecules remain at all times far from saturated, then $P(t) \ll A(t)$, $G(t) \ll 1$, and eq. 7 can be written as eq. 11 with

$$R_{\mathrm{on}} = k_1/a$$
$$R_{\mathrm{off}} = k_2 \quad \text{(far from saturation)}. \tag{13}$$

Eq. 6 can in all cases be rewritten with $\hat{C}(r,t) \rightarrow \hat{\mathscr{C}}(r,t)$.

The flux of C molecules between A molecules is zero by symmetry, so that

$$(\partial \mathscr{C}/\partial r)_{r=b} = 0. \tag{14}$$

This condition has been described in more detail by Berg and Blomberg [22]. The flux of C molecules at the A molecule surface is equal to the rate of reaction (see eq. 11):

$$D(\phi_n/v_n)\left[\frac{\partial}{\partial r}\hat{\mathscr{C}}(r,t)\right]_{r=a} = -\mathrm{d}\mathscr{G}/\mathrm{d}t. \tag{15}$$

The general solution to eqs. 6 and 11 is:

$$\hat{\mathscr{C}}(r,\, t) = \int_0^\infty c(\beta)\, \exp(-\beta^2 R_a t) U_n(\beta r/a) \mathrm{d}\beta \tag{16}$$

$$\mathscr{G}(t) = \int_0^\infty g(\beta)\, \exp(-\beta^2 R_a t) \mathrm{d}\beta \tag{17}$$

where

$$R_a = D/a^2 \tag{18}$$

and

$$U_1(\beta x) = \cos(\beta x) + h_1(\beta)\sin(\beta x)$$
$$U_2(\beta x) = J_0(\beta x) + h_2(\beta) N_0(\beta x) \tag{19}$$
$$U_3(\beta x) = j_0(\beta x) + h_3(\beta) n_0(\beta x).$$

R_a is the characteristic rate of diffusion through the area a^2, J_ν and N_ν Bessel functions of order ν, j_ν and n_ν spherical Bessel functions of order ν, and $x = r/a$ a dimensionless variable corresponding to the distance from the center of an A molecule to the point of observation. Parameter β, a separation constant, function $h_n(\beta)$, and weighting functions $c(\beta)$ and $g(\beta)$ are determined by the boundary and initial conditions.

3. Evaluation of boundary conditions

The boundary condition in eq. 14 determines functions $h_n(\beta)$ defined in eqs. 19, so that:

$$h_1(\beta) = \tan(\beta\gamma)$$
$$h_2(\beta) = -J_1(\beta\gamma)/N_1(\beta\gamma) \tag{20}$$
$$h_3(\beta) = -j_1(\beta\gamma)/n_1(\beta\gamma).$$

Evaluating eq. 16 and its first spatial derivative at $r = a$, and using this with eq. 17 in eqs. 11 and 15, implies that β is constrained by the condition:

$$R_{\mathrm{on}} U_n(\beta) = (R_{\mathrm{off}} - \beta^2 R_a) T_n(\beta) \tag{21}$$

where

$$\beta^2 T_n(\beta) = -\left[\frac{\mathrm{d}}{\mathrm{d}x} U_n(\beta x)\right]_{x=1}. \tag{22}$$

Functions $T_n(\beta)$ are sinusoidal functions ($n = 1$), Bessel functions of order 1 ($n = 2$), or spherical Bessel functions of order 1 ($n = 3$).

In general one would like to know how many values of β satisfy eq. 21. When $n = 1$, eq. 21 assumes the form of $\tan[\beta(\gamma - 1)]$ equaling a known function of β, which is a transcendental equation having an infinite number of discrete positive roots. When $n = 3$, the spherical Bessel functions $j_0(z)$, $n_0(z)$, $j_1(z)$ and $n_1(z)$ can be represented as ratios of sinusoidal functions and z or z^2; in this case, eq. 21 implies that $\tan[\beta(\gamma - 1)]$ equals a known function of β and thus that there are an infinite number of discrete positive roots of eq. 21. When $n = 2$ and β is large, $J_\nu(\beta)$, $J_\nu(\beta\gamma)$, $N_\nu(\beta)$ and $N_\nu(\beta\gamma)$ can be approximated by sinusoidal functions divided by the square root of their argument (ref. 29, p. 364). This leads in eq. 21 to a situation analogous to $n = 1,3$. For $n = 2$ and β upper-bounded, it has been demonstrated numerically (see below) that only a finite number of roots exist for many combinations of R_a, R_{on}, R_{off}, and γ, and thus that eq. 21 has an infinite number of discrete positive roots. The allowed values of β in the n-th dimension are called β_{ni}, where i is an integer greater than zero.

Eqs. 11, 15–17 also imply that:

$$g_i/c_i = -d_n T_n(\beta_{ni}), \tag{23}$$

where $g_i = g(\beta_{ni})$, $c_i = c(\beta_{ni})$. Thus, the general solution to eqs. 6 and 11 is

$$\mathscr{C}(r,t) = \sum_{i=1}^{\infty} c_i U_n(\beta_{ni} r/a) \exp(-\beta_{ni}^2 R_a t) \tag{24}$$

$$\mathscr{G}(t) = -\sum_{i=1}^{\infty} c_i d_n T_n(\beta_{ni}) \exp(-\beta_{ni}^2 R_a t). \tag{25}$$

4. Evaluation of initial condition

The amplitudes c_i in eqs. 24 and 25 indicate the relative importance of the exponential terms with rates $\beta_{ni}^2 R_a$. The amplitudes are determined with the initial condition $\mathscr{C}(r,0) = 1$ by inverting eq. 24 at $t = 0$. Inversion of eq. 24 would be straightforward if the functions $U_n(\beta_{ni} r/a)$ were orthogonal. However, they are not orthogonal under the appropriate inner product, $\int_a^b \mathrm{d}r\, r^{n-1}$. This is because the spatial differential operator in the separated form of eq. 6 is not self-adjoint under the boundary condition in eq. 15 [27]. In fact, the functions $U_n(\beta_{ni} r/a)$ are orthogonal for the β_{ni} specified by eq. 21 only when $R_{\mathrm{off}} = 0$. In this and the following derivations, we used ref. 28, pp. 350–352, for $n = 1$; ref. 29, pp. 484–485, for $n = 2$; and simply derived analogies to the $n = 2$ case for $n = 3$.

An orthogonality relation between the set of functions $U_n(\beta_{ni} x)$ and another set of functions $F_n(\beta_{ni} x)$ does exist. Noting that for $n = 1,2$ or 3 and for any value of R_{off},

$$\begin{aligned}\int_1^\gamma x^{n-1} U_n(\beta_{ni} x) U_n(\beta_{nj} x) \mathrm{d}x &\\ = I_{ni} & \qquad (i=j)\\ = -(R_{\mathrm{off}}/R_{\mathrm{on}}) T_n(\beta_{ni}) T_n(\beta_{nj}) & \qquad (i \neq j),\end{aligned} \tag{26}$$

where

$$I_{1i} = \left[(\gamma - 1) U_1^2(\beta_{1i}\gamma) - U_1(\beta_{1i}) T_1(\beta_{1i})\right]/2$$

$$I_{2i} = \left[\gamma^2 U_2^2(\beta_{2i}\gamma) - U_2^2(\beta_{2i}) - \beta_{2i}^2 T_2^2(\beta_{2i})\right]/2 \tag{27}$$

$$\begin{aligned}I_{3i} = \Big[& x^3 \{ j_0^2(\beta_{3i} x) + n_0(\beta_{3i} x) j_1(\beta_{3i} x) \}\\ & + h_3^2(\beta_{3i}) x^3 \{ n_0^2(\beta_{3i} x) - j_0(\beta_{3i} x) n_1(\beta_{3i} x) \}\\ & + 2 h_3(\beta_{3i}) x^2 n_0^2(\beta_{3i} x)/\beta_{3i} \Big]_1^\gamma /2,\end{aligned}$$

and that

$$\int_1^\gamma x^{n-1} U_n(\beta_{ni} x) \mathrm{d}x = -T_n(\beta_{ni}), \tag{28}$$

one can show that

$$\int_1^\gamma x^{n-1} U_n(\beta_{ni} x) F(\beta_{nj} x) \mathrm{d}x = H_{ni} \delta_{ij} \tag{29}$$

where δ_{ij} is the Kronecker delta,

$$F_n(\beta_{nj} x) = U_n(\beta_{nj} x) - (R_{\mathrm{off}}/R_{\mathrm{on}}) T_n(\beta_{nj}), \tag{30}$$

and

$$H_{ni} = I_{ni} + (R_{\mathrm{off}}/R_{\mathrm{on}}) T_n^2(\beta_{ni}). \tag{31}$$

Evaluating eq. 24 at $t = 0$, multiplying by $x^{n-1} F_n(\beta_{ni}) \mathrm{d}x$, using $x = r/a$, and integrating from 1 to γ, it is found that

$$\begin{aligned}c_i = & -T_n(\beta_{nj})[1 + (R_{\mathrm{off}}/d_n R_{\mathrm{on}})]/\\ & [I_{ni} + (R_{\mathrm{off}}/R_{\mathrm{on}}) T_n^2(\beta_{ni})].\end{aligned} \tag{32}$$

Eqs. 24, 25, 27 and 32 provide a complete solution to eqs. 6 and 11 that satisfies all boundary and initial conditions.

5. Characteristic rates

The reaction-diffusion process involves three characteristic rates (R_a, R_{off}, R_{on}), the dimensionality, and the parameter γ. The general solution has limiting cases in which the relative values of these parameters vary such that only one or two are important.

Rate R_{off} (eqs. 12 and 13), the dissociation rate, is inversely proportional to the average lifetime of a conjugate, $1/k_2$. Rate R_{on} (eqs. 12 and 13) is proportional to the forward rate constant k_1. The equilibrium constant K (eq. 8), related to R_{on} and R_{off}, depends only on the molecular details of binding between two adjacent molecules and does not depend on the diffusion coefficient. Rate R_a (eq. 18) is the inverse of the time for a C molecule to diffuse through the area of a site, and is not directly related to or limited by R_{on}. One expects, however, at least for irreversible reactions ($R_{off} = 0$), that the reaction rate is limited by the smallest of R_a and R_{on}.

In many reversible reactions, typical molecules remain bound longer than it would take a C molecule to diffuse across the area of an A molecule, so that $R_{off} < R_a$. (For small molecules, $1/R_a = (10\ \text{Å})^2/10^{-6}\ \text{cm}^2\ \text{s}^{-1} = 10$ ns.) Eq. 11 is isotropic in that C molecules are released from A molecules in all directions with equal probability. Thus, it has been implicitly assumed that R_{off} is smaller than the rate of A molecule rotational diffusion R_r (for $n = 2$, 3). Because for many molecules $R_a \sim R_r$ [30] the largest R_{off} considered is thus $R_{off} \approx R_a$.

6. Relation of observed concentration(s) to $\mathscr{G}$

As shown in eq. 24, concentration $\hat{C}(r,t)$ varies with position r from a typical A molecule. These variations can in principle be experimentally detected. However, in most experiments, the spatial average of $\hat{C}(r,t)$, denoted by $C(t)$, is measured. The relevant concentration value, in its normalized form, is (referring to eqs. 3 and 24):

$$\begin{aligned}\mathscr{C}(t) &= [C(t) - \langle \text{C} \rangle]/\Delta C \\ &= d_n \int_1^{\gamma} x^{n-1} \hat{\mathscr{C}}(ax,t)\,\mathrm{d}x \\ &= -\mathscr{G}(t).\end{aligned} \tag{33}$$

The third equation is derived by using eqs. 24 and 28 in the second equation, and comparing the result with eq. 25.

At time zero, the macroscopic concentrations are A_0, C_0 and P_0. The magnitudes of the initial deviations from equilibrium are equal ($\Delta = \Delta A = \Delta C = \Delta P$), and are determined by the initial concentration values and the equilibrium constant K:

$$\begin{aligned}&\langle \text{A} \rangle \langle \text{C} \rangle K = \langle \text{P} \rangle \\ &(A_0 - \Delta)(C_0 - \Delta)K = (P_0 + \Delta).\end{aligned} \tag{34}$$

The observed concentrations in time are thus given by

$$\begin{aligned}A(t) &= A_0 - \Delta[1 - \mathscr{G}(t)] \\ C(t) &= C_0 - \Delta[1 - \mathscr{G}(t)] \\ P(t) &= P_0 + \Delta[1 - \mathscr{G}(t)].\end{aligned} \tag{35}$$

Eqs. 35 mean that, in most experimental cases, the information necessary for data interpretation is contained in the function $\mathscr{G}(t)$.

7. Limiting case: simple bimolecular reaction

In the simplest scheme, a bimolecular reaction proceeds according to the equation:

$$\mathrm{d}P/\mathrm{d}t = \phi_n k_1 A C - k_2 P, \tag{36}$$

where C is not a function of position.

Assuming either that $\Delta \ll \langle \text{A} \rangle$, $\langle \text{C} \rangle$, $\langle \text{P} \rangle$ or that $G(t) \ll 1$, eq. 36 can be written as

$$\mathrm{d}\mathscr{G}/\mathrm{d}t = -(d_n R_{on} + R_{off})\mathscr{G}(t), \tag{37}$$

where R_{on} and R_{off} are shown in eq. 12 and eq. 13. Eq. 37 has the solution

$$\mathscr{G}(t) = \exp[-(R_{off} + d_n R_{on})t]. \tag{38}$$

Thus, diffusion (R_a) does not affect the reaction rate. Under some conditions, the general solution will reduce to eq. 38.

The approach to equilibrium after a perturbation is in general the sum of an infinite number of exponential decays, as shown in eqs. 24 and 25. The slowest decay rate is $R_a \beta_{n1}^2$, and its relative importance is determined by c_1. For some systems, the smallest root of eq. 21 might be $\ll 1$. Thus, for small β such that β, $\beta\gamma \ll 1$, the functions $U_n(\beta)$ and $T_n(\beta)$ can be written with small argument approximation for sinusoidal and Bessel functions so that $U_n(\beta) \approx U_n(\beta\gamma) \approx 1$ and $T_n(\beta) \approx -1/d_n$. This means that eq. 21 reduces to

$$R_a \beta_{n1}^2 = d_n R_{on} + R_{off}, \tag{39}$$

where β_{n1} is the smallest solution for β. Thus, if $(d_n R_{on} + R_{off}) \ll R_a$, then β_{n1} will be given by eq. 39. Similar approximations in eqs. 23 and 32 indicate that g_i and c_i for this β_{n1} will be ≈ 1. In addition, $\beta_{n1}x \ll 1$ (since $\beta\gamma \ll 1$ and $1 < x < \gamma$), so that $U_n(\beta_{n1}x) \approx 1$, and $\hat{\mathscr{C}}(r,t)$ and $\mathscr{G}(t)$ are given by eq. 38.

Eqs. 38 and 39 have been numerically verified for several parameter combinations. In two dimensions, for (1) $R_{on}/R_a = 10^{-3}$ and $R_{off}/R_a = 10^{-3}$, 10^{-4}, ..., 10^{-8}, $\gamma = 20.0$ and for (2) $R_{on}/R_a = R_{off}/R_a = 10^{-3}$, and $\gamma = 1.1$, 1.5, 2.0, 5.0, 10.0, the first root of eq. 21 is found to be given by eq. 39, and its coefficient to equal 1, within 0.2%.

8. Limiting case: irreversible diffusion-controlled reactions

The time course of an irreversible reaction is given by the solution with $R_{off} = 0$. In this case, the set of functions $U_n(\beta_{ni})$ specified by the β_{ni} satisfying eq. 21 with $R_{off} = 0$ are orthogonal under the usual inner product. All explicit dependence on R_{on} is removed from the coefficients c_i, but not from the root equation. The rate of binding is limited by the slowest rate R_{on} or R_a. When $R_{on} \gg R_a$, and $R_{off} = 0$, eq. 21 reduces to $U_n(\beta_{ni}) = 0$; thus, neither the roots β_{ni} nor the coefficients g_i and c_i depend on R_{on}, and the relaxation process depends only on R_a. This is mathematically equivalent to the diffusion-limited theory of Smoluchowski [4] in which the boundary condition eq. 15 is replaced by the condition $\hat{C}(a,t) = 0$. When $R_{on} \approx R_a$ and $R_{off} = 0$, eq. 21 reduces to $U_n(\beta_{ni})/T(\beta_{ni}) = -\beta_{ni}^2 R_a/R_{on}$. This is mathematically consistent (with $\gamma \to \infty$) to the partially diffusion-controlled theory of Collins and Kimball [5]. The relaxation is slower in this case and depends both on R_{on} and R_a. When $R_{on} \ll R_a$, relaxation depends only on R_{on}. This R_{on} vs. R_a behavior has been numerically verified for $n = 2$, $\gamma = 10$, and $R_{off} \to 0$.

9. General form of $\mathscr{G}(t)$ for different values of R_{on}, R_{off}, R_a, n and γ

Given values of the three characteristic rates, n and γ, the function $\mathscr{G}(t)$ is entirely determined by the roots of eq. 21 and their coefficients as determined by eqs. 32 and 23. The roots and coefficients have been numerically calculated for a range of parameter values. The results of these calculations are shown in table 1 for $n = 2$ and $\gamma = 10$ and in table 2 for $n = 3$ and $\gamma = 100$. The time is scaled by R_a, so that the shape of $\mathscr{G}(t)$ is determined only by the ratios of R_{on} and R_{off} to R_a. The bottom number in tables 1 and 2 for each pair of ratios gives the number of roots of β_{ni} with coefficient g_i greater than 0.01. The other terms in eq. 25 contribute only a small part to the function $\mathscr{G}(t)$ and are thus neglected. The top row in tables 1 and 2 shows a weighted average of the important rates:

$$\sum_i g_i \beta_{ni}^2 / \sum_i g_i. \tag{40}$$

The numerator of eq. 40 is also proportional to the reaction rate at $t = 0$.

As shown, when $R_{off} + d_n R_{on} \ll R_a$ (simple reaction limit), the average rate is equal to $R_{off} + d_n R_{on}$. When $R_{off} \to 0$ (irreversible reaction), the rate is limited by the smallest of R_{on} and R_a.

If $R_{on} \ll R_a$, and R_{off} is increased from zero, the rate becomes R_{off}, and the decay is nonexponential. Physically, this occurs because C molecules, once released from A molecules, are more likely to be transported away from the A molecules than to rebind, and the rate thus depends only on R_{off}. It is not the slowest of R_{off} and R_a that dominates (as for R_{on} and R_a), but simply R_{off} (given that $R_{on} < R_a$).

If $R_{on} \gg R_a$, and R_{off} is increased from zero, the rate is not very sensitive to changes in R_{off} or R_{on} and depends mostly on R_a (and on n and γ). When $R_{off} > R_a$, the rate weakly depends on R_{on}/R_{off}.

When both kinetic rates are much smaller than the diffusional rate (calculations not shown), the shape and rate of $\hat{\mathscr{C}}(r,t)$ at $r > a$ equal those of $\mathscr{G}(t)$. However, when either of the kinetic rates is larger than or equal to the diffusional rate, $\hat{\mathscr{C}}(r,t)$ can follow a different course than $\mathscr{G}(t)$. We have verified this behavior for $\gamma = 10$, $n = 2$, $x = \gamma$, and $R_{off}/R_a = 0.0001$, 100, with $R_{on}/R_a = 10\,000$, 0.01.

Table 1

Average relaxation rate and degree of nonexponentiality for two dimensions and $\gamma = 10$

Different values of R_{on}/R_a and R_{off}/R_a are shown. Each block shows the initial rate given by eq. 40 and the number of terms in eq. 25 that have coefficients greater than 0.01. When $R_{\mathrm{on}}/R_a = 10$ and $R_{\mathrm{off}}/R_a = 100$, the number of terms is > 30.

R_{on}/R_a		10000	1000	100	10	1	0.1	0.01
R_{off}/R_a	100	0.0333 3	0.181 5	3.00 14		98.7 14	100 5	100 3
	10	0.0183 2	0.0332 3	0.179 5	2.69 13	8.43 14	9.88 5	9.99 2
	1	0.0176 2	0.0183 2	0.0330 3	0.167 5	0.719 6	0.958 5	0.998 2
	0.1	0.0175 2	0.0176 2	0.0182 2	0.0249 2	0.0680 3	0.0926 2	0.0975 1
	0.01	0.0175 2	0.0175 2	0.0175 2	0.0172 2	0.0148 2	0.0103 1	0.0100 1
	0.001	0.0175 2	0.0175 2	0.0174 2	0.0164 2	0.0104 2	0.00260 1	0.00116 1
	0.0001	0.0175 2	0.0175 2	0.0174 2	0.0163 2	0.00998 2	0.0183 1	0.000297 1

10. Relation of general solution to 'encounter complex' theory

One method of treating reversible bimolecular reactions [2,11,17] is to propose a scheme:

$$A + C \underset{\kappa_{-1}}{\overset{\kappa_1}{\rightleftharpoons}} AC \underset{\kappa_{-2}}{\overset{\kappa_2}{\rightleftharpoons}} P$$

where A and C are reactant concentrations, AC is an 'encounter complex' concentration, and P is the bound state concentration. Rates κ_1 and κ_{-1} correspond to inward and outward diffusive fluxes of the reactants and can be estimated from known values of the diffusion coefficients and the reaction radius. Rate κ_1 has units of concentration^{-1} time^{-1} and rate κ_{-1} has units of time^{-1}. In this

Table 2

Average relaxation rate and degree of nonexponentiality for three dimensions and $\gamma = 100$

The initial rate and the number of significant exponential terms are given as in table 1. When $R_{\mathrm{on}}/R_a = 10$ and $R_{\mathrm{off}}/R_a = 100$, more than 35 terms have coefficient $g_i \approx 0.01$.

R_{on}/R_a:		10000	1000	100	10	1	0.1	0.01
R_{off}/R_a:	100	0.0108 5	0.122 15	1.28 32		98.4 33	99.9 14	100 6
	10	0.00103 2	0.0108 5	0.121 15	1.27 34	8.58 35	9.90 14	9.99 5
	1	0.000105 1	0.000990 1	0.0107 5	0.110 14	0.619 21	0.949 10	0.995 4
	0.1	0.0000132 1	0.0000105 1	0.00102 2	0.00984 5	0.0532 8	0.0917 4	0.0991 3
	0.01	0.00000407 1	0.0000132 1	0.000104 1	0.000936 2	0.00514 4	0.00915 2	0.00989 1
	0.001	0.00000316 1	0.00000407 1	0.0000131 1	0.0000950 1	0.000503 1	0.000909 1	0.000990 1
	0.0001	0.00000307 1	0.00000315 1	0.00000433 1	0.0000120 1	0.0000519 1	0.0000913 1	0.0000991 1

formalism, the association/dissociation process and the isomerization process are treated in separate formalisms and then pieced together. (For a thorough explanation, see the references cited above). It is the purpose of this paper to solve both processes in a coupled manner.

The process shown in eq. 41 is related to the process described in this paper, in the following manner. The concentration AC is related to $\hat{C}(a,t)$. Rate κ_{-2} equals k_2. The concentration of forward isomerizations per unit time at equilibrium, according to eq. 41 equals $\kappa_2\langle \mathrm{AC}\rangle$. Because $\kappa_1\langle \mathrm{A}\rangle\langle \mathrm{C}\rangle = \kappa_{-1}\langle \mathrm{AC}\rangle$, then $\kappa_2\langle \mathrm{AC}\rangle = \kappa_2\kappa_1\langle \mathrm{A}\rangle\langle \mathrm{C}\rangle/\kappa_{-1}$. The concentration of forward isomerizations per time at equilibrium, according to eq. 7, is $\phi_n k_1\langle \mathrm{C}\rangle\langle \mathrm{A}\rangle$. Thus, the 'on' rate constants of the two formalisms are related as $k_1\phi_n \approx \kappa_2\kappa_1/\kappa_{-1}$.

Our formalism can be used to determine if the steady-state assumption, $\mathrm{d}[AC(t)]/\mathrm{d}t = 0$, often employed [2,17] is justified. To do this, one would calculate $\mathrm{d}[\hat{C}(a,t)]/\mathrm{d}t$ for a given set of parameters and insure that it is nearly equal to zero at all times.

11. Application to experimental data

Thus far, the manner in which the relative magnitudes of the diffusional and kinetic rates interact to determine the time dependence of concentrations in different dimensionalities has been investigated. In practice, one may wish to determine experimentally which rates are important in a given system and what the values of these rates are. The following procedure could be followed as a method of data analysis:

(i) Function $\mathscr{G}(t)$ is calculated from experimental data according to eqs. 35.

(ii) Rate R_a is calculated from known values of D and a.

(iii) The observed rates R_i^{obs} and their coefficients g_i^{obs} are calculated from the data, where

$$\mathscr{G}(t) = \sum_i g_i^{\mathrm{obs}} \exp(-R_i^{\mathrm{obs}} t). \qquad (42)$$

(iv) The observed rates and coefficients are related to the theoretical rates and coefficients as

$$\beta_{ni}^2 = R_i^{\mathrm{obs}}/R_a$$
$$g_i = g_i^{\mathrm{obs}} \qquad (43)$$

(v) Parameter γ (which can be experimentally varied) and the dimensionality n are known independently of the experiment. They specify a table of the form shown in tables 1 and 2. In practice, only the diagonal in the table that is consistent with the (usually known) equilibrium constant K needs to be calculated.

(vi) The appropriate diagonal is scanned for those areas that are consistent with the number and magnitude of pairs β_{ni}^2, g_i. They may or may not specify a unique position in the table.

(vii) The region in the table where the system lies determines which rates determine the kinetics. For example, if the data are consistent with a place in the lower right-hand part of the table, then the process is reaction-limited and depends only on the rate $d_n R_{\mathrm{on}} + R_{\mathrm{off}}$. If the data are consistent with a place in the lower left-hand region, the process is diffusion-limited and depends only on the rate R_a.

(viii) The ratios of R_{on} and R_{off} to R_a can be directly read from the column and row headings; with R_a, the absolute values of R_{on} and R_{off} (and thus k_1 and k_2) can be obtained.

12. Example: antibody-hapten interactions in solution

An example is the time dependence of the concentration of antibody-hapten conjugates as would be measured in a concentration jump experiment. In particular, we can assume that the concentration of hapten is sufficiently small so that the fraction of haptenated antibody fragments is small [$G(t) \ll 1$]. One example of such a system would be an anti-dinitrophenyl antibody, which has reduced tryptophan fluorescence in the haptenated state. If the antibody were monoclonal, it could be expected to have single rates k_1 and k_2.

Typically, the equilibrium coefficient of this reaction is 10^6 M^{-1} and the dissociation rate is 10^3 s^{-1} [2]. If we assume that the reaction radius a

is ≈ 20 Å, then $R_{\text{off}} \approx 10^3$ s^{-1} and $R_{\text{on}} = Kk_2/\phi_3 = (10^6\ \text{M}^{-1})(10^3\ \text{s}^{-1})/[4\pi(20\ \text{Å})^3] = 1.7 \times 10^7$ s^{-1}. Given a hapten diffusion coefficient of 2.5×10^{-6} cm^2/s, and an antibody diffusion coefficient of 5×10^{-7} cm^2/s, then $R_a \approx 7.5 \times 10^7$ s^{-1}. In this application, the total antibody concentration is approximately equal to $[4\pi(\gamma a)^3/3]^{-1}$. Thus, for a concentration of 2.5 μg/ml and a molecular weight of 55000, $\gamma = 100$.

For these parameters, we find (numerically) a single observed rate that is slightly larger than R_{off} (precisely, it equals 1.02×10^3 s^{-1}). This means that concentration jump experiments in this range of antibody concentrations, and for these kinetics rates k_1 and k_2, do not depend on k_1 or D (as is usually assumed). However, when $\gamma = 500$, the reaction rate is smaller than R_{off} (822 s^{-1}). This means that the kinetics are slowed by diffusion, and that the experimenter could easily misinterpret the data to imply an off-rate of 822 s^{-1} instead of 1000 s^{-1}.

13. Example: protein-protein interactions in or on a cell membrane

Reversible reactions between protein molecules either embedded in or adsorbed to a cell membrane are important in biological systems. For typical protein concentrations and diffusion coefficients, it is of interest to know when the rate of an association reaction depends on the diffusion coefficients. Typical concentrations are 1000 molecules/μm^2, so that, if the reaction radius is 30 Å, then the value of γ is 10. For a (typical) diffusion coefficient of 10^{-10} cm^2/s, rate $R_a \approx 10^3$ s^{-1}. A typical reversible association might be relatively long-lived ($R_{\text{off}} \approx 10^3$ s^{-1} to 0.1 s^{-1}). If we (arbitrarily) assume that the reactants are present in equal concentrations and that 1% of each is present in the bound form (at equilibrium), then the ratio of R_{on} to R_{off} would equal $k_1/k_2 a = \langle G\rangle/\phi_2\langle C\rangle a = (0.01)/[2\pi(30\ \text{Å})^2(10^3\ \text{molecules}/\mu\text{m}^2)] \approx 0.2$. Referring to table 1, with all of the rates about equal, it is seen that the kinetics are highly nonmonoexponential and dependent on the value of all of these rates.

15. Summary

A solution has been presented for the kinetics of a reversible quasi-unimolecular reaction in one, two or three dimensions. Unlike previous approaches to this problem, diffusion and reaction are coupled at the outset. The solution demonstrates that the reaction rate is determined by an intrinsic off-rate, an intrinsic on-rate, and a diffusional rate. Exactly which of these three rates determines the reaction rate depends on their relative magnitudes. The expressions presented can be directly applied to kinetic data obtained through concentration perturbations.

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