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Basic kinetics of binding and incorporation with supramolecular aggregates

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Supramolecular particles of spherical shape (such as micelles, vesicles, etc.) are imagined to interact with small (bio)molecules so that either binding or incorporation occurs. This is generally described as an association process whose rate is determined by appropriate diffusion and actual transformation steps. Along these lines pertinent expressions for the phenomenological rate constants are given. In addition, an upper bound for the rate of incorporation has been derived. The results are discussed with respect to their usefulness in practice.

1. Introduction

In a liquid system of chemical or biological interest one may deal with supramolecular entities of spherical shape, e.g., microdroplets, micelles or phospholipid vesicles, which are composed of a large number of individual molecules. Such aggregate particles possibly interact with another molecular species added to the system, resulting in some kind of association. More specifically, one can distinguish between (i) binding or adsorption to the surface of an aggregate and (ii) incorporation into its interior.

An example of the latter type is encountered in the case of unilamellar lipid vesicles whcich are exposed to the antibiotic peptide alamethicin. This peptide is known to penetrate the bilayer, giving rise to the formation of voltage-dependent pores [1]. We have recently investigated the thermodynamics of the partitioning of alamethicin between the lipid and aqueous phases, theoretically as well as experimentally [2,3]. In order to explore the underlying kinetics appropriate stopped-flow measurements have subsequently been carried out and interpreted as reported elsewhere (G. Schwarz, V. Rizzo and H. Gerke, unpublished results). The fundamental approach used to describe the rate process is also applicable to many similar colloidal and biological systems. Therefore, we shall give here a quite general treatment of the argumentations and results.

From the phenomenological point of view a most simple kinetic scheme is envisaged. It comprises the transition of a comparatively small (bio)molecule from the 'free' state B_2 in the given solution to an 'associated' state B_2^* formed with spherically shaped supramolecular aggregates made up by molecules B_1. The apparent rate constants of the forward and reverse reactions (being of the second and first order, respectively) will be expressed in terms of diffusion of B_2 towards and off the aggregate particles while the actual conversion $B_2 \rightleftharpoons B_2^*$ occurs on close contact with these particles. In addition, we shall investigate the rate of an incorporation process which is solely controlled by diffusion within to the aggregates.

Dedicated to Professor Manfred Eigen on the occasion of his 60th birthday.

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2. The system

In a given solvent So (e.g., water) the molecules B_1 (concentration: c_1) are supposed to conglomerate as large supramolecular particles Ag (ν_1 molecules of B_1 per aggregate). Furthermore, small molecules B_2 (concentration: c_2) of another agent (the substrate) may exist freely dissolved in So. They can associate with Ag, thus being transformed into a somehow distinguishable state B_2^* (concentration: c_2^*). This general picture applies to binding (or adsorption), incorporation and permeation processes.

For the sake of simplicity, a sufficient excess of B_1 over B_2 is assumed so that the aggregate-substrate interaction is not appreciably affected in the course of reaction. Under these circumstances one should expect the forward rate, $(dc_2^*/dt)_{\text{for}}$, to be proportional to the concentrations of Ag and B_2. Therefore, a second-order rate constant k_{as} is introduced according to

$$(dc_2^*/dt)_{\text{for}} = k_{\text{as}} c_1 c_2$$

Instead of c_a (the concentration of aggregates) this involves c_1 which will be more directly available in practice. Actually, the step $B_2 \rightarrow B_2^*$ is a pseudo first-order reaction in the ordinary case of a closed system (where c_1 remains constant). On the whole, a reversible scheme can be formulated as

$$B_2(+B_1) \underset{k_{\text{dis}}}{\overset{k_{\text{as}}}{\rightleftharpoons}} B_2^* \tag{1}$$

involving a first-order rate constant k_{dis} for the reverse step, $B_2^* \rightarrow B_2$. It appears suitable to relate the amount of associated substrate to the amount of given B_1 by introducing the quantity

$$r = c_2^*/c_1$$

which measures the concentration of B_2^* in the aggregate domain. The total phenomenological rate equation then reads

$$dr/dt = k_{\text{as}} c_2 - k_{\text{dis}} r \tag{2}$$

(t: time). At equilibrium ($r \rightarrow \bar{r}$, $c_2 \rightarrow \bar{c}_2$) one obtains

$$\bar{r} = \Gamma \bar{c}_2 \tag{3a}$$

$$\Gamma = k_{\text{as}}/k_{\text{dis}} \tag{3b}$$

involving the thermodynamic parameter Γ which is interpreted as the appropriately defined partition coefficient for the partitioning of substrate between the Ag and So phases [2].

Mass conservation of substrate in the system implies the relation

$$c_2 + r c_1 = c_2^0$$

where c_2^0 is the total concentration of substrate. Taking this into account, eq. 2 can be readily converted to

$$dr/dt = -(1/\tau)(r - \bar{r}) \tag{4}$$

$$1/\tau = k_{\text{as}} c_1 + k_{\text{dis}} \tag{5a}$$

$$\bar{r} = [\Gamma/(1 + \Gamma c_1)]\, c_2^0 \tag{5b}$$

Accordingly, r will approach $\bar{r}$ following a single exponential time function with the relaxation time τ, even at large perturbations of equilibrium.

3. Anatomy of the rate constants

In order that the conversion of substrate to the bound or incorporated state can actually take place, the B_2 molecules must come sufficiently near to the aggregate. This is accomplished spontaneously by random translocation steps owing to thermal motion. In addition, there may be interaction forces which promote or hamper the encounter of substrate and aggregate. On average, the

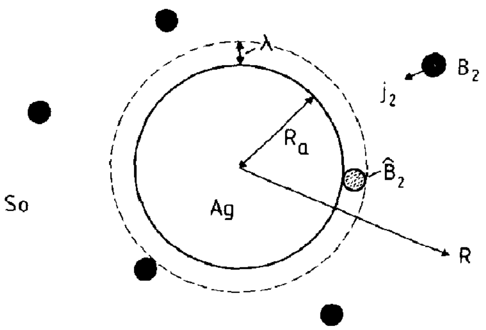

Fig. 1. Schematic representation of the radial flux (net density: j_2) of substrate molecules (B_2) towards a spherical aggregate (radius: R_a). Molecules $\hat{B}_2$ inside the 'reaction layer' of depth λ can undergo an 'association' reaction (see text).

effective migration is described as a (possibly directed) diffusion process in combination with a turnover of substance when the molecules have arrived at appropriate positions. Such an approach has been employed in the analysis of simple diffusion-controlled bimolecular reactions [4,5]. An advanced version of this method will be used here to express k_{as}, k_{dis} in a more detailed way which reveals to what extent the overall rate is determined by diffusion and structural transition, respectively.

The starting point is sketched in fig. 1. We consider a spherically shaped aggregate of radius R_a. This particle is surrounded by a 'reaction layer' up to a distance λ from the surface. The B_2 located in that space will be denoted $\hat{B}_2$. Only such $\hat{B}_2$ can actually turn into B_2^*. We shall ascribe the first-order rate constants k_r and k_{-r} to the forward and reverse reaction steps $\hat{B}_2 \rightarrow B_2^*$ and $B_2^* \rightarrow \hat{B}_2$, respectively. In other words, we set

$$\mathrm{d}n_2^*/\mathrm{d}t = k_r \hat{n}_2 - k_{-r} n_2^* \qquad (6)$$

(n_2^* and $\hat{n}_2$ being the instantaneous mole numbers of B_2^* and $\hat{B}_2$). The relevant value of $\hat{n}_2$ can be related to c_1, c_2 and c_2^* by solving a pertinent radial diffusion problem.

The net flux density of B_2 directed towards the center of a given aggregate may be formulated as

$$j_2 = D(\partial c/\partial R) + (1/f)(\partial U/\partial R)c \qquad (7)$$

where c is the local concentration of free substrate at a distance R. We note that in the vicinity of Ag this c is expected to deviate from its overall value c_2. The first term of j_2 refers to ordinary diffusion with $D = D_a + D_2$ (D_a and D_2 are the diffusion coefficients of the aggregate and the free substrate in the medium So). The second term considers the effect of a possible interaction force, $-\partial U/\partial R$ (with U representing the appropriate potential energy and f denoting the frictional coefficient of B_2 when moving relative to the aggregate). The total net flux, $I_2 = 4\pi R^2 j_2$, flowing towards a single aggregate, accordingly becomes

$$I_2 = 4\pi R^2 D \exp(-U/kT) \times \{\partial[c \exp(U/kT)]/\partial R\} \qquad (8)$$

as is easily verified on the basis of eq. 7 when the relation $f = kT/D$ is taken into account (k, Boltzmann's constant; T, absolute temperature).

Once the system has reached a stationary state, I_2 must be independent of R and t. After some rearranging, eq. 8 can then be integrated between $R_\lambda = R_a + \lambda$ and $R \rightarrow \infty$ resulting in

$$I_2 \int_{R_\lambda}^{\infty} R^{-2} \exp(U/kT)\,\mathrm{d}R = 4\pi D[c_2 - c_\lambda \exp(U_\lambda/kT)] \qquad (9)$$

This implies $c \rightarrow c_2$, $U \rightarrow 0$ at $R \rightarrow \infty$ whereas c_λ, U_λ are the values of c, U taken locally at $R = R_\lambda$. For the sake of convenience we define

$$1/\phi_U = R_\lambda \int_{R_\lambda}^{\infty} R^{-2} \exp(U/kT)\,\mathrm{d}R \qquad (10)$$

(which simply yields $\phi_U = 1$ if there is no interaction energy). Furthermore, with v_λ being the volume of the reaction layer of one aggregate and N denoting the total number of aggregates we may set $c_\lambda = \hat{n}_2/(N v_\lambda)$. Eq. 9 can now be rewritten to yield

$$I_2 = 4\pi D R_\lambda \phi_U \{c_2 - [\exp(U_\lambda/kT)/N v_\lambda]\hat{n}_2\} \qquad (11)$$

Under the condition of stationary state, the law of continuity requires that I_2 is equal to the value of $\mathrm{d}n_2^*/\mathrm{d}t$ for one aggregate. With respect to the whole system one therefore arrives at

$$N I_2 = k_r \hat{n}_2 - k_{-r} n_2^* \qquad (12)$$

when eq. 6 is recalled. Inserting the I_2 of eq. 11 and solving for $\hat{n}_2$ eventually yields

$$\hat{n}_2 = [k_d/(k_{-d} + k_r)] n_1 c_2 + [k_{-r}/(k_{-d} + k_r)] n_2^* \qquad (13)$$

where

$$k_d = 4\pi N_A D(R_\lambda/\nu_1)\phi_U \qquad (14a)$$

$$k_{-d} = 4\pi D(R_\lambda/v_\lambda)\phi_U \exp(U_\lambda/kT) \qquad (14b)$$

(N_A: Avogadro's number). Finally, let us use eq. 6 with the expression for $\hat{n}_2$ from eq. 13 and divide it by n_1. This results in the phenomenological rate equation, introduced before as eq. 2, provided we put

$$k_{as} = [k_r/(k_{-d} + k_r)] k_d \tag{15a}$$

$$k_{dis} = [k_{-d}/(k_{-d} + k_r)] k_{-r} \tag{15b}$$

In other words, the apparent rate constants of the reaction between free substrate and the aggregated material can be discussed in terms of some appropriate rate constants which are associated with the occurrence and disappearance of an intermediate encounter state. We may express this by formulating a more extended version of the phenomenological scheme (eq. 1), namely

$$B_2(+B_1) \underset{k_{-d}}{\overset{k_d}{\rightleftharpoons}} \hat{B}_2 \underset{k_{-r}}{\overset{k_r}{\rightleftharpoons}} B_2^* \tag{16}$$

Ordinarily, the amount of $\hat{B}_2$ remains quite small so that a steady-state condition applies to it. Then eqs. 15a and b are easily verified to describe the overall process. The second-order rate constant k_d apparently refers to the formation of $\hat{B}_2$ as a result of diffusion of free substrate up to the immediate vicinity of an aggregate. On the other hand, k_{-d} represents the first-order rate constant for the decay of B_2 as caused by diffusion out of the reaction layer.

As must be borne in mind, the above relations for k_{as} and k_{dis} have been obtained on the understanding that diffusion has reached a stationary state. Strictly speaking, this will not apply immediately after the reaction process has been initiated (at $t = 0$). According to a rigorous solution of the general problem [6] one must wait until the condition

$$\phi_U \sqrt{\pi D t} \gg R_\lambda \exp(-U_\lambda/kT)$$

has become effective. Such will ordinarily hold good in practice, except for rather large concentrations of the B_1 molecules (G. Schwarz, V. Rizzo and H. Gerke, unpublished results).

4. The case of incorporation

In certain cases $\hat{B}_2 \rightarrow B_2^*$ may actually be a translocation step from a position on the surface of the aggregate Ag $(R = R_\lambda)$ to another somewhere in the interior $(R = R_*)$. The rate constants k_r and k_{-r} can then be derived by means of a diffusion model approach similar to that employed above (see fig 2).

We consider I_2^*, i.e., the net flux of substrate inside the spherical aggregate. It would be formally the same as that given in eq. 8 except for replacing D by D_2^*, the diffusion coefficient of substrate within Ag. Under stationary-state conditions we find in strict analogy to eqs. 9–11

$$I_2^* = 4\pi D_2^* R_\lambda \phi_U^* \{ c_\lambda \exp(U_\lambda/kT) - c_* \exp(U_*/kT) \} \tag{17a}$$

where c_*, U_* refer to $R = R_*$ and ϕ_U^* is defined by

$$1/\phi_U^* = R_\lambda \int_{R_*}^{R_\lambda} R^{-2} \exp(U/kT)\, dR \tag{17b}$$

Let us first envisage the final equilibrium which naturally implies that $I_2^* = 0$. Accordingly

$$\exp(-\Delta U_*/kT) = (c_*/c_\lambda)_{eq} = \gamma_\lambda \tag{18a}$$

where $\Delta U_* = U_* - U_\lambda$. The quantity γ_λ will be equal to the ordinary partition coefficient of substrate between the aggregate and reaction layer phases. This γ_λ is determined by the difference in free energy between the two relevant states (strictly speaking, ΔU_* turns out to be equal to the difference of the appropriate standard chemical potentials for a single B_2 molecule). In practice, the partition coefficient between the incorporated and free substrate states

$$\gamma = \exp(-U_*/kT) = \gamma_\lambda \exp(-U_\lambda/kT) \tag{18b}$$

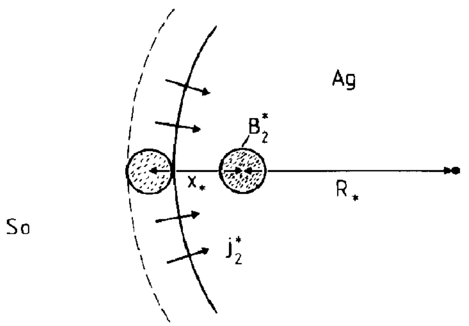

Fig. 2. Schematic representation of incorporation flux (net density: j_2^*) of $\hat{B}_2$ molecules from the reaction layer into an aggregate. The position of stable incorporation is assumed to be at $R = R_*$ (see text).

would be more directly accessible. It can be evaluated from measured values of the quantity Γ as has been introduced in eq. 3.

Under nonequilibrium conditions we have generally $I_2^* = \mathrm{d}n_2^*/\mathrm{d}t$ so that because of eq. 6

$$I_2^* = k_r \hat{n}_2 - k_{-r} n_2^*$$

Since this must hold true for any $\hat{n}_2$, n_2^* values, application of eq. 17a readily leads to

$$k_r = 4\pi D_2^* (R_\lambda / v_\lambda) \phi_U^* \exp(U_\lambda / kT) \qquad (19a)$$

$$k_{-r} = 4\pi D_2^* (R_\lambda / v_*) \phi_U^* \exp(U_* / kT) \qquad (19b)$$

v_* is that volume inside the aggregate which is accessible to the substrate in its stable state of incorporation.

5. Discussion

The various relations derived so far will now be examined in a little more detail with a view to their practical use. Let us start with the phenomenological forward rate constant. According to eq. 15a we can quite generally write

$$k_{as} = \kappa k_d, \text{ with } \kappa = k_r/(k_r + k_{-d}) \leqslant 1 \qquad (20)$$

There is an apparent 'transmission coefficient' κ approaching an optimum value of unity if k_r becomes sufficiently large ($k_r \gg k_{-d}$). Then, every B_2 molecule which has entered the reaction layer is rapidly converted to B_2^* without having a chance to diffuse back into the bulk solvent phase. Under these circumstances k_{as} assumes an upper bound k_d, the rate constant of the diffusion-controlled formation of encounter states $\hat{B}_2$. The expression for k_d is presented in eq. 14a. For most practical applications it can, however, be approximated using more appropriate terms. With comparatively large aggregates and small substrate molecules the existence of long-range interaction forces may well be disregarded so that $\phi_U = 1$. This actually leads to the well-known equation of Von Smoluchowski [4] which is only modified here by the explicit appearance of ν_1 (owing to the fact that our k_d is defined relative to c_1 instead of c_a). Furthermore, we can neglect D_a as compared to D_2 and set $R_\lambda \approx R_a$. It remains for us to find a serviceable way to express ν_1. We could, for instance, divide v_a, the volume of an aggregate, by v_1, the volume taken up by a single molecule. Approximating v_1 as $\bar{V}_1/N_A$ ($\bar{V}_1$ partial molar volume of B_1 in the system) then results in

$$\nu_1 = \varphi_1 (4\pi/3) N_A R_a^3 / \bar{V}_1$$

where φ_1 is the actual volume fraction of B_1 in the sphere of radius R_a. Note that in the case of unilamellar phospholipid vesicles

$$\varphi_1 = 1 - \left[1 - (d/R_a)\right]^3 < 1$$

(d: thickness of the bilayer) in contrast to the situation of micelles where $\varphi_1 = 1$. Along this line one obtains

$$k_d = 3(D_2/R_a^2)(\bar{V}_1/\varphi_1) \qquad (21)$$

Under certain conditions, k_d may thus turn out somewhat too small since possibly $\bar{V}_1 < N_A v_1$ (due to an increase in density in the solvation shell, particularly if the solvent is water). Let us therefore propose an alternative approach which does not make use of $\bar{V}_1$. It involves the quantities A_1, i.e., the area covered by one B_1 molecule on the surface of the aggregate, and β_1, which denotes the fraction of all B_1 molecules actually bordering on the surface. As is easily seen we obtain

$$\nu_1 = 4\pi R_a^2 / (\beta_1 A_1)$$

For a phospholipid vesicle one could put

$$\beta_1 = R_a^2 / \left[R_a^2 + (R_a - d)^2\right]$$
$$= 1/\left[1 + (1 - (d/R_a))^2\right]$$

(whereas $\beta_1 = 1$ in the case of micelles). Employing this relation for ν_1 one arrives at

$$k_d = \beta_1 N_A D_2 (A_1/R_a) \qquad (22)$$

In order to form an idea of the order of magnitude of such k_d, eqs. 21 and 22 may be evaluated for a typical case, namely, small biomolecules in water ($D_2 \sim 0.5 \times 10^{-5}$ cm^2/s at a radius of about 4 Å) reacting with unilamellar vesicles of, for instance, dioleoylphosphatidylcholine ($R_a \sim 150$ Å, $d \sim 30$ Å, $\bar{V}_1 \sim 0.8$ dm^3/mol, $A_1 \sim 70$ Å^2) [3]. One finds $k_d \sim 10^7$ M^{-1} s^{-1}. Generally, it can be concluded that k_d decreases with an increasing size of the aggregate.

Turning to k_{-d}, the same simplifying assumptions are to be made. With $v_\lambda = 4\pi R_a^2\lambda$ eq. 14b then changes into

$$k_{-d} = (D_2/\lambda R_a)\exp(U_\lambda/kT) \tag{23}$$

This includes the effect of a possible short-range interaction force described by the potential energy U_λ. As a physically reasonable value of λ, the span of the reaction layer, we adopt the (average) diameter of the substrate molecule. For the above numerical example we therefore have $\lambda \sim 8$ Å and accordingly obtain a $k_{-d} \sim 4 \times 10^7\ s^{-1}$ if $U_\lambda = 0$. The diffusion-controlled average lifetime of free substrate in the reaction layer (which is generally equal to $1/k_{-d}$) them amounts to about 2.5×10^{-8} s. Whether or not this is long enough to allow the actual transformation to B_2^* will be crucial for the transmission coefficient in eq. 20.

Upper bounds of k_r relative to k_{-d} can be derived depending on the specific nature of the reaction step $B_2 \rightarrow B_2^*$. Let us first examine the situation in the case of a binding process. Provided there is no activation barrier when the substrate molecule has arrived at a binding site on the surface of an aggregate, the average lifetime of $\hat{B}_2$ with respect to binding would be determined by the relevant (two-dimensional) diffusion coefficient D_λ in the reaction layer. This results in an estimated time of $s^2/4D_\lambda = 1/k_r$, where s denotes the square mean of the distance up to the nearest binding site. Considering a simple case with $D_\lambda = D_2$ and $U_\lambda = 0$ we thus find for a completely diffusion-controlled binding reaction

$$k_r/k_{-d} = 4\lambda R_a/s^2 \tag{24}$$

If there is only a single binding site on the aggregate we calculate $s^2 \sim 3R_a^2$. Hence, k_r is expected to be substantially smaller than k_{-d} under these circumstances. According to eq. 20 it then follows that k_{as} falls much below the value of k_d (by about a factor of 15 in our above example with the lipid vesicles). Naturally, the rate increases in the event of more binding sites per aggregate. Once a greater number of sites is distributed over the whole surface one could set $s \approx (s_0/2)$, with s_0 being the average distance between neighbouring sites. In this case the condition

$$s_0^2 \ll 16\lambda R_a$$

is adequate to have $k_r \gg k_{-d}$ so that k_{as} will actually approach k_d (in our numerical example distances $s_0 \lesssim 40$ Å would be sufficient).

Finally, we switch over to pure incorporation steps as described by eqs. 19a and b. Under the given simplifying assumptions we obtain

$$k_r/k_{-d} = (D_2^*/D_2)\phi_U^* \tag{25}$$

The principal problem consists in the evaluation of the term ϕ_U^* as defined by eq. 17b. Apparently, it can be rather well approximated according to

$$1/\phi_U^* = (1/R_a)\int_0^{x_*}\exp(U/kT)\,dx \tag{26}$$

($x = R - R_*$) provided the penetration depth, $x_* = R_\lambda - R_*$, is small compared to R_a. This holds true, for instance, for incorporation into the bilayer of a vesicle. Assuming a linear change of U in the course of the incorporation step we set

$$U = U_* - (\Delta U_*/x_*)x$$

Then integration is readily performed, resulting in

$$\phi_U^* = (R_a/x_*)\gamma\{\ln\gamma + (U_\lambda/kT)\}/\{\gamma\exp(U_\lambda/kT) - 1\} \tag{27}$$

where eqs. 18 have been taken into account. Considering negligible interaction in the reaction layer ($U_\lambda = 0$) and strong tendency of incorporation ($\gamma \gg 1$) eq. 27 becomes much simpler so that eq. 25 can be rewritten as

$$k_r/k_{-d} = (D_2^*/D_2)(R_a/x_*)\ln\gamma \tag{28}$$

Usually the aggregate phase can be assumed to allow only comparatively very slow diffusion as compared to the ordinary solvent. Thus, we must expect $D_2^* \ll D_2$ (two or three orders of magnitude, for instance, in lipid bilayers relative to an aqueous medium [7]). This most unfavorable situation regarding k_r is somewhat relieved owing to the short distance of migration as reflected in the factor R_a/x_*. Another favorable effect is exerted by a large partition coefficient, indicating a substantial driving force due to a strong negative gradient of free energy. However, even for quite large partition coefficients, e.g., $\gamma \sim 10^5$, the k_r/k_{-d} may still fall well below unity (G. Schwarz, V. Rizzo and H. Gerke, unpublished results). In other words, even a completely diffusion-controlled incorporation process may be described by a k_{as} which is substantially smaller than k_d.

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