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MICROSCOPIC DIFFUSION-REACTION COUPLING IN STEADY-STATE ENZYME KINETICS

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The theory of diffusion-controlled processes is applied to describe the steady state of a reversible enzymatic reaction with special emphasis on the effects of enzyme saturation. A standard macroscopic steady-state treatment requires only that the average diffusional influx of substrate equals the net reaction flux as well as the average diffusional efflux of product. In contrast, the microscopic diffusion-reaction coupling used here takes properly into account the conditional concentration distributions of substrate and product: Only when the enzyme is unoccupied will there be a diffusional association flux; when the enzyme is occupied, the concentration distributions will relax towards their homogeneous bulk values. In this way the relaxation effects of the non-steady state will be constantly reoccurring as the enzyme shifts between unoccupied and occupied states. Thus, one is forced to describe the steady state as the weighted sum of properly time-averaged non-stationary conditional distributions. The consequences of the theory for an appropriate assessment of the parameters obtained in Lineweaver-Burk plots are discussed. In general, our results serve to justify the simpler macroscopic coupling scheme. However, considerable deviations between the standard treatment and our analysis can occur for fast enzymes with an essentially irreversible product release.

1. Introduction

In any enzymatic process, the diffusion of the reactants to and from the enzyme must play an integral part. It has been argued that the extent of diffusion control for a reaction can be used as a measure of the evolutionary perfection of the enzyme involved [1]. However, in spite of its importance, a strict treatment of the microscopic couplings between the diffusion of reactants and the reaction appears to be lacking even for simple steady-state enzyme processes. It is our aim in this communication to fill this void with the aid of the theory of (partially) diffusion-controlled association [2] and its consequences for the corresponding dissociation reaction [3].

Most commonly, it is implicitly assumed that the rate constants for substrate and product association in a reaction scheme can simply be identified with the corresponding estimates from the theory of diffusion-controlled reactions. Since such estimates are based on stationary diffusion fluxes coupled to independent irreversible reactions, they are not directly applicable to the steady-state description of an enzyme reaction where substrate and product formations are intimately coupled. The physical characteristics of the enzyme as an absorbing sink – and thereby the boundary conditions for the diffusion fluxes – are ever-changing as the enzyme shifts between the occupied and unoccupied states. The traditional macroscopic treatment implies an instantaneous setting up of the steady-state diffusion fluxes when the enzyme becomes unoccupied as well as an instantaneous relaxation to homogeneous (bulk) concentration distributions when the enzyme is occupied. The

microscopic coupling, on the other hand, will take into account also how the diffusion fluxes change when the enzyme changes its state. Effects which in an irreversible association (or dissociation) reaction only are transient will be continuously reoccurring for each substrate-to-product cycle of the enzyme; each time a substrate (or product) associates the concentration distributions of all other molecules will relax towards their bulk values, and each time a substrate (or product) dissociates the distributions will again approach those that correspond to a steady-state influx.

Furthermore, the theory of diffusion-controlled processes implies that a macroscopic dissociation is preceded by a large number of very short-lived microscopic dissociations, each of which just return to the bound state. The microscopic coupling will take into account also the possibility that the association reaction of one kind of molecule (e.g., substrate) can interfere with these microscopic dissociations of another (e.g., product) by blocking reassociation and thereby increasing its effective dissociation rate from the enzyme. Thus, a proper microscopic flux balancing over all the steps from substrate diffusion via the reaction steps to product diffusion requires the usage of conditional distributions of substrate and product, conditional on the state of the enzyme being occupied or not. Although the ensemble average over these conditional distributions must be a steady-state distribution, they are not by themselves steady-state solutions of the diffusion equation.

Thus, it is important to account in detail for the time evolution of the conditional substrate and product distributions around the enzymes and take rigorous ensemble or time averages to derive the steady-state distributions. To make the problem tractable we shall confine the calculations to a simple two-step isomerization process

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_b \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P + E \qquad (1)$$

where E_b denotes the bound state of the enzyme with a substrate (product) molecule complexed with it.

In section 2 below we shall briefly review the steady-state properties of the macroscopic reaction, scheme 1, as well as the relevant parts of the theory of diffusion-controlled processes. In section 3 the microscopic diffusion coupling is introduced, explicitly taking into account the conditional distributions of substrate and product. These distributions and their influence on the overall steady-state flux are calculated, first in section 4 for a totally irreversible case ($k_{-1} = k_{-2} = 0$). To give a correct description of the reversible steady state, section 5 starts with a development of the formalism for the equilibrium state of a reversible partially diffusion-controlled process which is subsequently applied to the steady state of the reversible reaction scheme 1. In general, our results serve to justify a simple macroscopic diffusion coupling in accordance with the traditional approach; the differences are small under most circumstances of physical interest. However, effects of the explicit diffusion coupling may become pronounced for enzymes which are fast – i.e., with high turnover number – and also characterized by an essentially irreversible product release. Deviations from the simple macroscopic coupling scheme are limited by the ratio of the diffusional relaxation time to the occupation time of substrate (product) on the enzyme. In general, this ratio is small because the diffusional relaxation time (approx. R^2/D where R is the effective collision radius and D the relative diffusion constant) is a small quantity even for macromolecular objects, and the occupation time is related to the turnover time which is relatively large for most enzymes. As discussed in section 6, the prediction of a small effect from the proper microscopic diffusion coupling can be extended also to more complex reaction schemes involving several substrates and/or products. Thus, in most cases there is no need to go beyond an appropriate identification of the rate constants in the macroscopic reaction scheme with estimates from the theory of diffusion-controlled processes. This conclusion, however, is in sharp contrast to the results of some recent theoretical works [4,5]. In these treatments of diffusion coupling for reversible enzymatic reactions in the steady state, the bound states of the enzyme have been neglected and the law of mass action is assumed to hold locally on the enzyme surface.

2. Macroscopic diffusion coupling

2.1. Steady-state flux

Under the usual assumptions – i.e., fixed bulk concentrations S_0 and P_0 of substrate and product and a large excess of substrate over enzyme – the steady-state substrate-to-product flux per enzyme from the macroscopic reaction (scheme 1) is (e.g., ref. 6)

$$j_{mac} = \frac{k_1 k_2 S_0 - k_{-1} k_{-2} P_0}{k_{-1} + k_2 + k_1 S_0 + k_{-2} P_0} \tag{2}$$

This expression is based on the balancing of the overall substrate flux from the usual chemical kinetic equation

$$j_{mac} = k_1 S_0 (1-\theta) - k_{-1}\theta$$

and the overall product flux

$$j_{mac} = k_2 \theta - k_{-2} P_0 (1-\theta)$$

where

$$\theta \equiv [E_b]/([E]+[E_b])$$

is the probability that the enzyme is occupied.

The diffusion effects can be introduced [7] by identification of the association rate constants k_1 and k_{-2} with those given by the theory of (partially) diffusion-controlled reactions [2]

$$k_1 = \frac{4\pi D_S R_S}{1+4\pi D_S R_S/k_S} = \frac{4\pi DR}{1+4\pi DR/k_S} \tag{3}$$

$$k_{-2} = \frac{4\pi D_P R_P}{1+4\pi D_P R_P/k_P} = \frac{4\pi DR}{1+4\pi DR/k_P} \tag{4}$$

where R_S and R_P are the reaction radii, D_S and D_P the relative diffusion constants, and k_S and k_P the surface reactivities for the substrate-enzyme and product-enzyme association, respectively. For simplicity, we shall assume $R_S = R_P = R$ and $D_S = D_P = D$ throughout the following. Then the differences between the substrate and product association appear only in the surface reactivities k_S and k_P. When these are very large, k_S, $k_P \gg 4\pi DR$, the usual Smoluchowski limits for the association rates of an ideal sink are recovered. Similarly, one finds [3] that the corresponding dissociation rate constants can be expressed as

$$k_{-1} = \frac{\lambda_S}{1+k_S/4\pi DR} \tag{5}$$

$$k_2 = \frac{\lambda_P}{1+k_P/4\pi DR} \tag{6}$$

where λ_S and λ_P are microscopic dissociation rate constants related to the surface reactivities k_S and k_P through the equilibrium binding constants K_S and K_P

$$K_S = k_S/\lambda_S = k_1/k_{-1} \tag{7}$$

$$K_P = k_P/\lambda_P = k_{-2}/k_2 \tag{8}$$

In the scheme considered, $K_S/K_P = k_1 k_2/k_{-1}k_{-2}$ is also the equilibrium constant for the substrate-to-product conversion.

Thus, a macroscopic diffusion coupling would consist of using the rate constants, eqs. 3–6, in the expression, eq. 2, for the overall steady-state flux, and the nature of the molecular interactions would enter through the microscopic rate constants k_S, k_P, λ_S and λ_P. However, the macroscopic rate constants (eqs. 3–6) are calculated from steady-state solutions for irreversible association and dissociation reactions, respectively. Thus, they describe the fluxes between the surface of the enzyme and the bulk solution a long distance away from the enzyme without interference by the reaction and without regard to transient inhomogeneities in the distribution of molecules around the enzyme.

The surface reactivity k (k_S or k_P) is given by the boundary condition for the diffusion equation at the enzyme surface (of eq. 10 below). Recently, Razi-Naqvi et al. [8] discussed the relation between this parameter and the underlying microscopic diffusion characteristics like the mean free path, root-mean-square displacement and reaction probability per microscopic encounter. Since these parameters are largely inaccessible, the main conclusion to be drawn is that the surface reactivity well serves the purpose of a molecular characteristic which is independent of the concentrations; i.e., in this case it is independent of how far the enzymatic reaction is driven from equilibrium. Furthermore, the factor $(1+4\pi DR/k)^{-1}$ can be interpreted as the reaction probability per macroscopic encounter. More specifically, this factor expresses the probability that a molecule which has reached the enzyme surface will bind eventually – after an indeterminate number of microscopic collisions. The limit $k \gg 4\pi DR$ (or $k_1 =$

$4\pi DR$) consequently serves as a practical definition of diffusion control.

This interpretation of diffusion control also implies that a macroscopic dissociation (given by k_{-1} or k_2) is preceded by a large number of very short-lived microscopic dissociations (given by λ_S or λ_P) which just return to the bound state. Thus, when the coupled reaction is considered, the association reaction of one kind of molecule (e.g., the substrate) can interfere with the microscopic dissociations of the other (product) and in effect 'catch' the transient dissociation by blocking reassociation.

2.2. Diffusion flux

In section 3 below we shall investigate the validity of the identifications, eqs. 3–6, for the flux, eq. 2. For later use, let us first briefly review their derivation in a pure association process [2,3]. Consider a single spherical molecule (the enzyme) with an absorbing surface, reaction radius R. The distribution $c(r,t)$ of particles (substrate or product) at distance r from the center of the enzyme obeys the diffusion equation

$$\frac{\partial c}{\partial t} = Dr^{-2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c}{\partial r}\right) \tag{9}$$

with the boundary condition at the enzyme surface

$$kc = 4\pi DR^2\frac{\partial c}{\partial r};\ r = R \tag{10}$$

Here, D is the relative diffusion coefficient and k the surface reactivity ($k = k_S$ for substrate and $k = k_P$ for product) which takes care of the case when not every diffusional encounter will lead to binding; in the limit $k \to \infty$ the ideal absorbing boundary condition $c(R,t) = 0$ for the Smoluchowski limit is recovered. If the distribution is homogeneous at time $t = 0$

$$c(r,0) = c_0 \tag{11}$$

one finds the distribution at later times

$$c(r,t) = c_0 - \frac{kc_0}{4\pi rD + kr/R}\left\{\operatorname{erfc}\left[\frac{r-R}{(4Dt)^{\frac{1}{2}}}\right] - \exp\left[\frac{k}{k_a}\left(\frac{r}{R}-1\right)+\left(\frac{k}{k_a}\right)^2\frac{Dt}{R^2}\right]\times\operatorname{erfc}\left[\frac{r-R}{(4Dt)^{\frac{1}{2}}}+\frac{k}{k_a}\cdot\frac{(Dt)^{\frac{1}{2}}}{R}\right]\right\} \tag{12}$$

where we have defined

$$k_a \equiv \frac{4\pi DR}{1+4\pi DR/k} \tag{13}$$

(Actually, $c(r,t)$ is the conditional distribution at time t given that no association has taken place before this time.) The flux over the absorbing surface is from eq. 10

$$\phi^0(t) \equiv kc(r,t) = k_ac_0\left\{1+\frac{k}{2\pi DR}\exp\left[\left(\frac{k}{k_a}\right)^2\frac{Dt}{R^2}\right]\operatorname{erfc}\left[\frac{k}{k_a}\cdot\frac{(Dt)^{\frac{1}{2}}}{R}\right]\right\} \approx k_ac_0\left\{1+\frac{(R^2/\pi Dt)^{\frac{1}{2}}}{1+4\pi DR/k}\right\} \tag{14}$$

For times such that $(R^2/\pi Dt)^{\frac{1}{2}} \ll 1 + 4\pi DR/k$, the transient part contributes very little and the flux is constant

$$\phi^0(t) \approx k_ac_0 \tag{15}$$

and k_a from eq. 13 is the effective (macroscopic) association rate constant. In the case when the enzyme can only bind one particle, there will be a saturation factor and the real association flux per enzyme is

$$I(t) = \phi^0(t)\exp\left[-\int_0^t\phi^0(t')\mathrm{d}t'\right] \tag{16}$$

Thus, the mean time of association is

$$\tau \equiv \int_0^\infty tI(t)\mathrm{d}t = \int_0^\infty\exp\left[-\int_0^t\phi^0(t')\mathrm{d}t'\right]\mathrm{d}t = \frac{1}{k_ac_0}\left[1-(\pi)^{\frac{1}{2}}z\exp(z^2)\operatorname{erfc}(z)\right] \tag{17}$$

where the approximation is the same as in eq. 14 and

$$z \equiv \frac{(k_ac_0R^2/D)^{\frac{1}{2}}}{1+4\pi DR/k}$$

As long as $z \ll 1$, the mean time of association τ does not differ from that expected from the steady-state approximation, eq. 15. Thus, the association during the transient is negligible when $z \ll 1$. In the diffusion-controlled case, $k \gg 4\pi DR$

and $k_a = 4\pi DR$, this translates to

$$(4\pi R^3 c_o)^{\frac{1}{2}} \ll 1 \tag{18}$$

a condition which will be satisfied except at the very highest concentrations.

3. Microscopic diffusion coupling

3.1. The conditional distributions

In the steady state there will be inhomogeneous distributions of substrate and product molecules around the enzymes. The distributions that belong to the average steady-state flux j are

$$\bar{S}(r) = S_o - j/4\pi Dr \tag{19}$$

$$\bar{P}(r) = P_o + j/4\pi Dr \tag{20}$$

Obviously, these distributions are ensemble averages over all enzyme molecules. In the simple reaction scheme, eq. 1, the enzymes have two states, occupied E_b and unoccupied E. Assume that the occupied state occurs with the probability θ. Then by the general rules of probability theory, the average distributions, eqs. 19 and 20, can be subdivided

$$\bar{S}(r) = \bar{S}_0(r)(1-\theta) + \bar{S}_1(r)\theta \tag{21}$$

$$\bar{P}(r) = \bar{P}_0(r)(1-\theta) + \bar{P}_1(r)\theta \tag{22}$$

where $\bar{S}_0(r)$ $[\bar{P}_0(r)]$ is the conditional distribution of substrate [product] given that the enzyme is unoccupied, and $\bar{S}_1(r)$ $[\bar{P}_1(r)]$ the conditional distribution given that the enzyme is occupied. Of course, these conditional distributions are also averages over all occupied or unoccupied enzymes, respectively. This subdivision is extremely important, since the boundary conditions at the enzyme surface are strongly dependent on whether or not the enzyme is occupied.

Furthermore, the fluxes have to be balanced at the elementary reaction steps:

$$j = k_S \bar{S}_0(R)(1-\theta) - \lambda_S \theta \tag{23}$$

$$j = \lambda_P \theta - k_P \bar{P}_0(R)(1-\theta) \tag{24}$$

These relations have the same structure required from probability theory as eqs. 21 and 22; the average forward flux of the first step is the weighted sum of the conditional forward flux, $k_S \bar{S}_0(R)$, given that the enzyme is unoccupied – probability $(1-\theta)$ – and the conditional forward flux, $-\lambda_S$, given that the enzyme is occupied, and similarly for the second step. When the microscopic rate constants k_S, k_P, λ_S and λ_P are replaced by their corresponding macroscopic ones through eqs. 3–6, eqs. 19–24 can be rearranged to give for the flux $j = j_{mic}$

$$j_{mic} = \frac{k_1 k_2 S_o - k_{-1} k_{-2} P_o + k_1 k_{-2}\left[S_o \bar{P}_1(R) - P_o \bar{S}_1(R)\right]}{k_{-1} + k_2 + k_1 \bar{S}_1(R) + k_{-2} \bar{P}_1(R)} \tag{25}$$

Consequently, the macroscopic rate constants k_1, k_{-1}, k_2, k_{-2} – although defined for pure association or dissociation – turn up as useful phenomenological rate constants for the steady-state reversible enzyme reaction as well. However, eq. 25 gives the same flux as the macroscopic coupling of eq. 2 only when $\bar{P}_1(R) = P_o$ and $\bar{S}_1(R) = S_o$, i.e., when the average surface concentrations around the occupied enzyme are equal to the bulk concentrations; a requirement which can be strictly fulfilled only at equilibrium when there is no net flux. Consequently, we can expect the microscopic diffusion coupling to introduce deviations if the enzyme is fast and product release essentially irreversible. Under such conditions the surface concentration, which is very low at the moment of association, will not have time to relax to its bulk value during the processing time. We shall try to quantify this effect in the following sections.

Without the saturation effect, the coupling becomes much simpler since there is no need to distinguish the conditional distributions. Then, the overall averages, eqs. 19 and 20, can be used such that

$$j = k_S \bar{S}(R) - \lambda_S \theta$$

$$j = \lambda_P \theta - k_P \bar{P}(R) \tag{26}$$

where θ now has to be interpreted as the average number of bound molecules per enzyme. Then one finds

$$j_{mic} = j_{mac} = \frac{k_1 k_2 S_o - k_{-1} k_{-2} P_o}{k_{-1} + k_{-2}} \tag{27}$$

This result can be compared to the coupling proposed by Jönsson and Wennerström [4]

$$J = k\bar{S}(R) - \bar{k}\bar{P}(R) \tag{28}$$

which expressedly neglects the bound state but gives the same result as eq. 27 if the local reaction rates k and $\bar{k}$ are interpreted as

$$k = k_S/(1 + \lambda_S/\lambda_P) \tag{29}$$
$$\bar{k} = k_P/(1 + \lambda_P/\lambda_S)$$

in terms of our parameters. Thus, without saturation effects there is no fundamental difference between the various coupling schemes for the simple substrate-to-product reaction considered here. As discussed in section 6, this situation is drastically changed when several substrates and/or products are involved.

3.2. On-off nature of enzyme binding

As stressed above, the main problem with the microscopic diffusion coupling is the limited binding capacity of the enzyme. For simplicity it has been assumed that it can bind only one molecule at a time. Thus, each time a molecule binds, the boundary condition at the enzyme surface for the diffusion of the remaining molecules (substrate and product) suddenly changes from an absorbing one like that of eq. 10 to a reflecting one such as

$$\left.\frac{\partial c}{\partial r}\right|_{r=R} = 0 \tag{30}$$

While the enzyme is occupied the distributions of the remaining molecules will relax towards their bulk values. Similarly, when a molecule dissociates, there will be a sudden influx into the enzyme again. It should be noted that the changing state of the enzyme cannot be expressed as a simple boundary condition, e.g., using a weighted average of eqs. 10 and 30 such that $4\pi DR^2(\partial c/\partial r)_{r=R} = k(1-\theta)c(R) - \lambda\theta$ at the enzyme surface. If $c(r)$ is to be an overall average distribution ($c = \bar{S}$ or $\bar{P}$), no such simple boundary condition can be made to agree with the subdivision into conditional averages as required by eqs. 21–24. It is necessary to consider each state separately.

Before going into the detailed calculations, let us outline the methods and notations. All functions and parameters with subscript P for the product are the same as those with subscript S for the substrate if S and P are exchanged in the expressions. Ensemble averages – denoted by a bar over the symbol – will be calculated by the proper time integrals. Also, the conditional distributions can be calculated as ensemble averages, then taken over all states of either occupied or unoccupied enzymes. All quantities with subscript 0 refer to the unoccupied state, and those with subscript 1 refer to the occupied one. Because of the continued switching between the two states of the enzyme, it is convenient to describe the steady state as a weighted sum of the proper time integrals over the lifetime of each state. This is not necessary, however, as the same results can be derived by considering the differential changes.

The rationale for the calculations is as follows. The steady state implies that the probability θ for the occupied state is constant and that the subdivision into conditional distributions is well defined. Also, the average distributions at the time of a change of state – i.e., association or dissociation – must be well defined. Then, the time evolution until the next change of the state of the system is determined by the diffusion equation. This time evolution also determines the probability density per unit time for the change, and consequently the average conditional distributions during the lifetime of each state can be calculated as the integral over this probability density. Also, the average distributions at the time of a change of state are uniquely defined in this way.

4. Irreversible case

For simplicity and to demonstrate the principles, let us first consider a steady-state system in the totally irreversible case where $k_{-1} = \lambda_S = k_{-2} = k_P = 0$. At time $t = 0$ pick out the subpopulation of enzymes which have just bound a substrate. Assume that the substrate distribution around these enzymes is $S_0(r)$ at time $t = 0$. Then at later times (but before any dissociation takes place) this distribution is

$$s_1(r,t) = \int_R^\infty 4\pi r'^2 G^R(r,r';t) S_0(r') \mathrm{d}r' \tag{31}$$

where $G^R(r,r';t)$ is the Green's function solution of the diffusion equation satisfying the reflecting boundary condition, eq. 26. From these bound enzymes a product will dissociate with rate λ_P. Hence, the average substrate distribution at the time of dissociation is

$$\bar{S}_1(r) = \lambda_P \int_0^\infty dt \exp(-\lambda_P t) s_1(r,t)$$
$$= \lambda_P \int_R^\infty dr' 4\pi r'^2 \bar{G}^R(r,r';\lambda_P) S_0(r') \quad (32)$$

where $\bar{G}^R(R,r':\lambda)$ is the Laplace transform of $G^R(r,r';t)$. Since dissociation follows an exponential law, the average substrate distribution during the lifetime of the bound state is the same as the averge distribution at the moment of dissociation. Thus, $\bar{S}_1(r)$ from eq. 32 is the same as that used in the previous section (eq. 21).

Now, $\bar{S}_1(r)$ can be used as a starting distribution for the substrate association. If product dissociation takes place at time $t = 0$, the substrate distribution at later times of the unoccupied state is

$$s_0(r,t) = \int_R^\infty 4\pi r'^2 G_S^A(r;r';t) \bar{S}_1(r') dr' \quad (33)$$

Here $G_S^A(r,r';t)$ is the Green's function solution of the diffusion equation satisfying the absorbing boundary condition, eq. 10, with $k = k_S$ for the substrate. Similarly to eqs. 14–17, one finds the association flux $I_S(t)$ and the mean association time τ_S

$$I_S(t) = \phi_S(t) \exp\left[-\int_0^t \phi_S(t') dt' \right] \quad (34)$$

where

$$\phi_S(t) = k_S s_0(R,t) = 4\pi D R^2 (\partial s_0 / \partial r)_{r=R} \quad (35)$$

and

$$\tau_S = \int_0^\infty \exp\left[-\int_0^t \phi_S(t') dt' \right] dt \quad (36)$$

Consequently, the average substrate distribution $\bar{S}_0(r)$ during the time of the unoccupied state is

$$\bar{S}_0(r) = \frac{1}{\tau_S} \int_0^\infty \exp\left[-\int_0^t \phi_S(t') dt' \right] s_0(r,t) dt \quad (37)$$

and the average substrate distribution at the time of association is

$$S_0(r) = \int_0^\infty \phi_S(t) \exp\left[-\int_0^t \phi_S(t') dt' \right] s_0(r,t) dt \quad (38)$$

Since the association is not properly exponential, $\bar{S}_0(r)$ differs from $S_0(r)$. $\bar{S}_0$ is the same conditional average as used in eq. 21 while S_0 is the starting distribution used in eqs. 31 and 32. Thus, the mathematical formulation is complete and the required conditional distributions can in principle be determined.

We can also check that it is consistent. First, differentiation of eq. 32 gives

$$Dr^{-2} \frac{d}{dr} \left(r^2 \frac{d\bar{S}_1}{dr} \right) = \lambda_P [\bar{S}_1(r) - S_0(r)] \quad (39)$$

Similarly, from eq. 37

$$Dr^{-2} \frac{d}{dr} \left(r^2 \frac{d\bar{S}_0}{dr} \right) = -\frac{1}{\tau_S} [\bar{S}_1(r) - S_0(r)] \quad (40)$$

Note that the right-hand sides of these equations are different from zero; i.e., the conditional distributions are not steady-state solutions of the diffusion equation. From eq. 21, one obtains for the total average steady-state distribution

$$Dr^{-2} \frac{d}{dr} \left(r^2 \frac{d\bar{S}}{dr} \right) = \frac{1}{\tau_S} [(1 + \lambda_P \tau_S)\theta - 1][\bar{S}_1(r) - S_0(r)] \quad (41)$$

The right-hand side must be zero if $\bar{S}(r)$ is to have a steady-state – i.e., r-independent – flux. This requirement is fulfilled only if the probability for the bound state is

$$\theta = \frac{1}{1 + \lambda_P \tau_S} = \frac{1/\lambda_P}{\tau_S + 1/\lambda_P} \quad (42)$$

A result which is consistent with the further requirement that the probability for the bound state be the ratio of the average occupation time, $1/\lambda_P$, and the average time for a cycle, $\tau_S + 1/\lambda_P$. Furthermore, from eq. 24 the flux per enzyme in the irreversible case is

$$j = \lambda_P \theta = \frac{1}{\tau_S + 1/\lambda_P} \quad (43)$$

which is simply the inverse of the cycling time. The same result, eq. 42, can be derived from eq. 23 using eqs. 35 and 37.

The main problem with the solution of eqs.

31–38 is the awkward coupling from eq. 35 where the flux ϕ_S is defined from a boundary condition on $s_0(r,t)$ which subsequently has to be integrated (eqs. 37 and 38). In principle, a solution can be found iteratively: assume a distribution $\bar{S}_1(r)$, calculate ϕ_S and $S_0(r)$ from eqs. 33, 35 and 38; then from eq. 32 a new $\bar{S}_1(r)$ can be calculated and the procedure repeated until convergence. Fortunately, it appears that one iteration is sufficient in this case. These calculations have been carried out in appendix A.

It is customary to plot the inverse of the flux as a function of the inverse of the substrate concentration (Lineweaver-Burk plot). From the macroscopic scheme, eq. 2, we have in the irreversible case

$$j_{\mathrm{mac}}^{-1} = \frac{1}{k_1 S_0} + \frac{1}{k_2} \tag{44}$$

which should be compared with the result from the microscopic scheme, eq. 29

$$j_{\mathrm{mic}}^{-1} = \frac{1}{k_1 S_0} + \frac{1}{k_2}\frac{\bar{S}_1(R)}{S_0} \tag{45}$$

(Note that $k_2 = \lambda_P$ in this case where $k_P = 0$.) Thus, the microscopic diffusion coupling differs by the term

$$\Delta = \frac{1}{k_2}\left(1 - \bar{S}_1(R)/S_0\right) \tag{46}$$

from the macroscopic result. This is exactly what we calculated in appendix A (eq. A7). The difference goes to zero for large substrate concentrations. The interesting part, however, is the linear region for lower concentrations where the approximate integration of eq. A6 is entirely justified. Here the relative difference is

$$1 - \bar{S}_1(R)/S_0 \approx 1/\left[1 + \left(D/k_2 R^2\right)^{1/2}\right]/(1 + 4\pi DR/k_S)$$

Since the maximum flux – or turnover number – is normally calculated by a linear extrapolation of the line down to infinite concentration, the maximum flux will also be overestimated by this amount (cf. fig. 1). Instead of the real maximum flux $j^{\max} = k_2 = \lambda_P$ from the irreversible scheme, a linear extrapolation of the data to infinite concentration

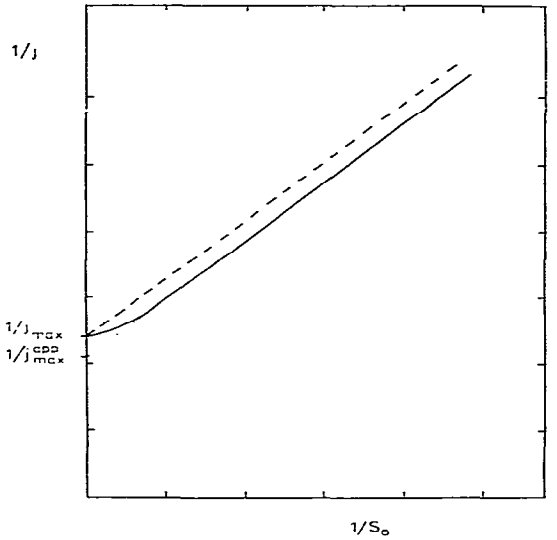

Fig. 1. Linewever-Burk plots. Full line is the microscopic result from eq. 45; dashed line is the macroscopic result from eq. 44. Units on the axes are arbitrary.

gives an apparent maximum flux

$$j_{\mathrm{app}}^{\max} = k_2 S_0/\bar{S}_1(R) = k_2\left[1 + \left(k_2 R^2/D\right)^{\frac{1}{2}}\right] \tag{47}$$

Consequently, we have shown that a proper microscopic diffusion coupling for the irreversible steady-state enzyme kinetics introduces deviations from the relations normally assumed. In particular, the maximum flux will be overestimated with the usual assumptions of the Lineweaver-Burk plot. Of course, the issue is not what the curve j^{-1} vs. S_0^{-1} really looks like when the concentration approaches infinity, since the diffusion equation will not remain valid in this limit. The point is that a linear extrapolation of the data to this limit does not give the true maximum flux.

5. Reversible reactions

5.1. Reversible binding at equilibrium

In order to give a proper treatment of the kinetic problem, we shall first check that the formalism to be used describes correctly the equi-

librium state of a reversible partially diffusion-controlled process. Consider again a single spherical enzyme which can bind one molecule of a species present at bulk concentration S_o. At equilibrium the average concentration $\bar{S}(r)$ at distance r from the center of the enzyme must be homogeneous. That is, with the same notations as in the previous sections, eq. 21 gives

$$\bar{S}(r) = S_o = \bar{S}_0(r)(1-\theta) + \bar{S}_1(r)\theta \tag{48}$$

The average flux into the enzyme is zero, and from eq. 23 one obtains

$$0 = k_S \bar{S}_0(R)(1-\theta) - \lambda_S \theta \tag{49}$$

At equilibrium, also the conditional distributions $\bar{S}_0(r)$ and $\bar{S}_1(r)$ must be homogeneous and equal to the bulk concentration S_o. We shall demonstrate that this is the case in our formalism. At $t = 0$, with an initial homogeneous concentration S_o around the enzyme, there is a dissociation. It was shown previously [3] that the probability $W(t)$ that no molecule has reassociated before time t is

$$W(t) = \left[1 - \int_0^t \varphi_S(t')\mathrm{d}t'\right] \exp\left[-\int_0^t \phi_S^o(t')\mathrm{d}t'\right] \tag{50}$$

The factor

$$1 - \int_0^t \varphi_S(t')\mathrm{d}t' \equiv \phi_S^o(t)/k_S S_o \tag{51}$$

is the probability that the dissociated molecule has not reassociated, and $\phi_S^o(t)$ is the flux from eq. 14 belonging to a homogeneous initial distribution. Thus, the factor $\exp[-\int_0^t \phi_S^o(t')\mathrm{d}t']$ is the probability that no other molecule has associated. Since these two events are independent and mutually exclusive, the total probability $W(t)$ must be given by the product of the respective probabilities as in eq. 50. The flux of molecules (a total of one) is given by

$$I(t) = -\frac{\mathrm{d}W}{\mathrm{d}t} \tag{52}$$

What is the average distribution $S_0(r)$ at the moment when a molecule has just been bound? Given that no molecule has associated before time t, the initial homogeneous distribution has relaxed to the one from eq. 12 or equivalently

$$s_0(r,t) = S_o \int_R^\infty 4\pi r'^2 G_S^A(r,r';t)\mathrm{d}r' \tag{53}$$

where G_S^A is the Green's function as in eq. 33. The dissociated molecule started just outside the boundary $r = R$ at $t = 0$. At later times its distribution is given by $G_S^A(r,R;t)$. However, under the condition that no molecule has associated at time t this distribution must be normalized such that the molecule remains in solution with probability one. Thus,

$$\sigma_S(r,t) = \frac{G_S^A(r,R;t)}{1 - \int_0^t \varphi_S(t')\mathrm{d}t'} = \frac{k_S S_o}{\phi_S^o(t)} G_S^A(r,R;t) \tag{54}$$

is the distribution of the dissociated molecule which satisfies

$$\int_R^\infty 4\pi r^2 \sigma_S(r,t)\mathrm{d}r = 1$$

If the dissociated molecule reassociates at time t, $\sigma_S(r,t)$ should not contribute at later times. The probability flux for such a reassociation is $\varphi_S(t)\exp[-\int_0^t \phi_S^o(t')\mathrm{d}t']$. However, if another molecule associates instead, $\sigma_S(r,t)$ will contribute fully at later times. The probability flux for the association of another molecule is $\phi_S^o(t)W(t)$. Thus, the average distribution right after a molecule has associated can be calculated as the weighted sum from the two possibilities:

$$\begin{aligned} S_0(r) &= \int_0^\infty \mathrm{d}t \varphi_S(t) \exp\left[-\int_0^t \phi_S^o(t')\mathrm{d}t'\right] s_0(r,t) \\ &\quad + \int_0^\infty \mathrm{d}t \phi_S^o(t) W(t)[s_0(r,t) + \sigma_S(r,t)] \\ &= \int_0^\infty \mathrm{d}t(-\mathrm{d}W/\mathrm{d}t) S_o \int_R^\infty \mathrm{d}r' 4\pi r'^2 G_S^A(r,r';t) \\ &\quad + \int_0^\infty \mathrm{d}t \phi_S^o(t) \exp\left[-\int_0^t \phi_S^o(t')\mathrm{d}t'\right] G_S^A(r,R;t) \end{aligned} \tag{55}$$

After partial integration over t and using the fundamental properties of the Green's function one finds

$$S_0(r) = S_o \tag{56}$$

as expected. Similarly, one can calculate the average distribution during the unoccupied state

$$\bar{S}_0(r) = \frac{1}{\tau}\int_0^\infty \mathrm{d}t W(t)[s_0(r,t) + \sigma_S(r,t)] \tag{57}$$

where the mean open time τ is defined as

$$\tau = \int_0^\infty t(-\mathrm{d}W/\mathrm{d}t)\,\mathrm{d}t = \int_0^\infty W(t)\mathrm{d}t = 1/k_S S_o \tag{58}$$

One finds $\bar{S}_0(r) = S_\infty$, as it should be. Also, the average distribution during the occupied state can be shown to be homogeneous, $\bar{S}_1(r) = S_\infty$. Thus, our formalism is consistent with respect to the equilibrium situation. The main conclusion to be drawn from this is the importance of giving the dissociated molecule a separate treatment. One does not get consistent results by simply using a starting distribution $S_0(r) = s_\infty + \delta(r-R)/4\pi R^2$ right after a dissociation.

5.2. Reversible steady-state enzyme kinetics

Now we are ready to treat in detail the full reaction scheme (eq. 1) with the microscopic diffusion coupling as described in section 3. Consider the subpopulation of enzymes which are occupied. According to the assumptions in section 3, these are surrounded by average distributions $\bar{S}_1(r)$ and $\bar{P}_1(r)$ of substrate and product, respectively. Assume that there is a dissociation at time $t = 0$. Let us call the total dissociation rate

$$\lambda_P + \lambda_S = \lambda$$

With probability λ_P/λ it is a product dissociation, and with probability λ_S/λ it is a substrate dissociation. Then the probability that no molecule – substrate or product – has associated after time t is

$$W(t) = \left\{ \frac{\lambda_P}{\lambda}\left[1 - \int_0^t \varphi_P(t')\mathrm{d}t'\right] + \frac{\lambda_S}{\lambda}\left[1 - \int_0^t \varphi_S(t')\mathrm{d}t'\right] \right\} \times \exp\left\{ -\int_0^t [\phi_S(t') + \phi_P(t')]\mathrm{d}t' \right\} \tag{59}$$

The flux $\phi_S(t)$ is that defined in eqs. 33 and 35. $\phi_P(t)$ is defined by analogous expressions for the product. The flux $\varphi_S(t)$ is that defined by eq. 51 and φ_P analogously for the product. Then in the same fashion as eqs. 55 and 57 we can calculate the average substrate distribution right after an association

$$S_0(r) = \int_0^\infty (-\mathrm{d}W/\mathrm{d}t)\, s_0(r,t)\mathrm{d}t + \frac{\lambda_S}{\lambda}\int_0^\infty [\phi_S(t) + \phi_P(t)] \exp\left\{ -\int_0^t [\phi_S(t') + \phi_P(t')]\mathrm{d}t' \right\} G_S^\wedge(r,R;t)\mathrm{d}t \tag{60}$$

and the average substrate distribution during the open time

$$\bar{S}_0(r) = \frac{1}{\tau}\int_0^\infty W(t)\, s_0(r,t)\mathrm{d}t + \frac{1}{\tau}\frac{\lambda_S}{\lambda}\int_0^\infty \exp\left\{ -\int_0^t [\phi_S(t') + \phi_P(t')]\mathrm{d}t' \right\} \times G_S^\wedge(r,R;t)\mathrm{d}t \tag{61}$$

$s_0(r,t)$ is defined in eq. 33 and the average lifetime τ of the unoccupied state is defined as in eq. 58,

$$\tau = \int_0^\infty W(t)\mathrm{d}t$$

with $W(t)$ from eq. 59. Analogous relations are found for $P_0(r)$ and $\bar{P}_0(r)$.

The average distributions at the time of association, $S_0(r)$ and $P_0(r)$, can now be used to calculate $\bar{S}_1(r)$ and $\bar{P}_1(r)$ as in eq. 33

$$\bar{S}_1(r) = \lambda\int_R^\infty 4\pi r'^2 \tilde{G}^R(r,r';\lambda) S_0(r')\mathrm{d}r'$$

$$\bar{P}_1(r) = \lambda\int_R^\infty 4\pi r'^2 \tilde{G}^R(r,r';\lambda) P_0(r')\mathrm{d}r' \tag{62}$$

Note that only the total dissociation rate $\lambda = \lambda_S + \lambda_P$ is relevant in these expressions. One can easily convince oneself that these average distributions are the same as the conditional ones given that a substrate – or product – dissociates.

Thus, the mathematical formulation is complete and the solutions can in principle be found iteratively (see appendix B). Similarly to the irreversible case we can check the consistency. One finds that the total average distributions $\bar{S}(r)$ and $\bar{P}(r)$ defined from eqs. 21 and 22 do indeed have a steady-state – i.e., r-independent – flux if the binding probability is

$$\theta = \frac{1}{1 + \lambda\tau} \tag{63}$$

This result can also be calculated from the boundary relations, eqs. 23 and 24, when it is realized from eq. 61 that the average lifetime τ of the unoccupied state satisfies $\tau = [k_P\bar{P}_0(R) + k_S\bar{S}_0(R)]^{-1}$.

5.3. Maximum flux

A common way of determining the maximum flux is to measure the 'initial' rate of reaction, i.e.,

the steady-state rate before the bulk concentration of product has reached any appreciable level. Thus, consider the limit $P_o = 0$. Then the assumed flux from the macroscopic scheme, eq. 2, is given by

$$j_{\mathrm{mac}}^{-1} = \frac{1}{k_2}\left[1 + \frac{k_2 + k_{-1}}{k_1 S_o}\right] \tag{64}$$

and the flux j_{mic} from the proper diffusion coupling is from eq. 25

$$j_{\mathrm{mic}}^{-1} = \frac{1}{k_2 + k_{-2}\bar{P}_1(R)}\left[\frac{\bar{S}_1(R)}{S_o} + \frac{k_2 + k_{-1} + k_{-2}\bar{P}_1(R)}{k_1 S_o}\right] \tag{65}$$

The difference between these two expressions is

$$\Delta = j_{\mathrm{mac}}^{-1} - j_{\mathrm{mic}}^{-1} = \frac{1}{k_2}\cdot\frac{1}{1 + K_P\bar{P}_1(R)}\left[1 - \frac{\bar{S}_1(R)}{S_o} + \frac{K_P\bar{P}_1(R)}{K_S S_o}(1 + K_S S_o)\right] \tag{66}$$

The calculations of the required conditional distributions have been carried out in appendix B. The relative error in the inverse flux, $k_2\Delta$ from eq. B11, is always smaller than $(1 + (D/\lambda R^2)^{\frac{1}{2}})^{-1}$ and is thereby similar to that for the totally irreversible process described in section 4. However, in the reversible case this factor is not sufficient as could be expected, and the other molecular parameters must also satisfy rather stringent conditions in order for the deviation to be significant. As discussed briefly in section 6, it is in fact difficult to assign physically reasonable values which still produce a significant deviation.

Let us also consider the Briggs-Haldane kinetics (cf. ref. 10) when the back-reaction of the second step is negligible, $k_{-2} = k_P = 0$. To better simulate a totally irreversible product formation, let us write this scheme as

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_b \overset{\lambda_P}{\rightarrow} E + P \tag{67}$$

where the second step is energy driven. Then from eqs. B7–B9, the deviation in the inverse flux will be

$$\Delta = \varepsilon_S/\lambda_P = \frac{1}{\lambda_P}\cdot\frac{1}{1 + (D/\lambda R^2)^{\frac{1}{2}}}\cdot\frac{1}{1 + \lambda_S/\lambda_P}\cdot\frac{1}{1 + 4\pi DR/k_S} \tag{68}$$

which may or may not be negligible depending on the unknown microscopic parameters. Like in the totally irreversible case, the dominant factor (i.e. the smallest one) is probably $1/[1 + (D/\lambda R^2)^{1/2}]$.

6. Discussion

We have carried out a detailed description of microscopic flux balancing in enzymatic reactions which is based strictly on the rules of probability theory and the theory of (partially) diffusion-controlled processes. Our main result is that a proper microscopic treatment leads to results differing from what is obtained when the rate constants in a macroscopic scheme simply are identified with the corresponding constants obtained from the theory of diffusion-controlled association. Such an identification is not a priori correct, since the macroscopic rate constants are defined for independent and irreversible reactions. Starting from molecular parameters, we have shown how these macroscopic rate constants nevertheless can be used to describe the steady-state flux for reversible enzymes. The deviations between the traditional approach and our results are in most cases small. However, when the enzyme is fast and has an essentially irreversible product release they become non-negligible.

In eq. 25 it is seen that the flux resulting from a proper microscopic flux balancing is somewhat larger than that normally assumed, eq. 2. The difference is determined only by the departure from the bulk values (S_o and P_o) of the average concentrations ($\bar{S}(R)$ and $\bar{P}_1(R)$) around the occupied enzyme. When the occupation time of the enzyme is very short, these concentrations will not have time to relax via diffusion to their bulk values. The diffusion-relaxation time is approx. R^2/D and the occupation time is defined as $1/\lambda$, which accounts for the requirement $D/\lambda R^2 < 1$ as the major determining factor for a non-negligible influence of the explicit diffusion coupling.

As an example, consider a case where in eqs. B10 and B11, $D/R^2 = 10^8\ \mathrm{s}^{-1}$, $\lambda_S = 8 \times 10^7\ \mathrm{s}^{-1}$, $\lambda_P = 2 \times 10^7\ \mathrm{s}^{-1}$, and $k/4\pi DR = 0.1$. Here one finds that the relative deviation between the two approaches is $k_2\Delta = \varepsilon = 0.48$. This difference is not negligible and would in a Lineweaver-Burk

plot lead to an overestimate of the turnover number by a factor of two. For this particular scheme, the real turnover number would be $k_2 = \lambda_P/1.1 = 2 \times 10^7\ s^{-1}$, while the estimated one would be $4 \times 10^7\ s^{-1}$ (cf. fig 1). The turnover number in this example was taken from catalase, one of the fastest enzymes known [10]. The effects of the microscopic diffusion coupling on the steady-state properties of enzymes will be confined by the moderate size as well as by the limited turnover of all known enzymes.

Our explicit calculations cannot easily be extended to more complex situations involving several substrates and/or products as well as several internal reaction steps. However, some general conclusions can be drawn also for such more complicated cases. If a more complex reaction scheme is analysed, one can easily derive the overall flux corresponding to eq. 25 expressed as a function of various conditional distributions. However, in analogy to the simple reaction, the average conditional distribution of a reactant in the neighbourhood of an occupied enzyme will be very close to its bulk value, and thereby the overall flux will be very close to that given by the corresponding macroscopic coupling. Thus, the saturation effects complicate the calculations in our approach, but they also provide a rationale for the conclusion that the traditional macroscopic coupling is sufficient in all but the most extreme cases (e.g., very fast and essentially irreversible reaction with a large diffusional cross-section R).

This general result differs from that obtained by another approach to derive an explicit diffusion-reaction coupling for reversible enzymes. In the scheme of Jönsson and Wennerström [4] which has been further developed by Chou and Forsén [5], the diffusion coupling is carried out with the stationary – i.e., overall averages corresponding to eqs. 19 and 20 – concentration distributions at the enzyme surface. Such a description can be correct only in the total absence of saturation effects. For the simple substrate-to-product reaction discussed above, such a coupling does not produce any significant deviations from the traditional macroscopic scheme, at least not in the limit of unsaturated enzymes. For several substrates and/or products, however, the differences become dramatic. As an example, let us consider a reaction with two products

$$E + A \rightleftharpoons E_b \rightleftharpoons E + B + C \tag{69}$$

which Jönsson and Wennerström [4] analysed in detail. Their crucial assumption is a diffusion-reaction coupling of the type

$$j = \bar{k} c_A(R) - \bar{k} c_B(R) c_C(R) \tag{70}$$

where the $c(R)$ terms are the local steady-state concentrations of the respective reactants at the enzyme surface. This boundary condition is of course motivated by the law of mass action, which in its original form says that the speed of a chemical reaction is proportional to the product of the bulk concentrations of the reactants. In spite of its simplicity, this motivation is deceptive. In fact, the calculations by Jönsson and Wennerström demonstrate that as a consequence of the local mass action assumption, the corresponding relation for the bulk concentrations is no longer valid. Although Chou and Forsén [5] in their generalization of the same theory ignore this result, it is equivalent to the nonexistence of macroscopic rate constants for the scheme, eq. 69.

This prediction differs drastically from the consequences of the traditional macroscopic treatment as well as from the results of the present one. It is therefore important to discuss the conditions under which the local mass action relation could be valid at the enzyme surface. First of all, such a relation is statistical and requires a sufficiently large number of molecules – i.e., significantly different from 0 or 1 – at each enzyme. Otherwise the averaging procedure implied by eq. 70 would in fact correspond to cross-reacting single B and C molecules bound to different enzymes. Thus, the enzyme surface must have such a large affinity for all reactants involved that their local concentrations correspond to sufficiently large numbers of non-specifically bound molecules. These numbers would then be proportional to the local concentrations just outside the enzyme which enter in the boundary condition, eq. 70. At the same time, however, these large numbers of bound ligands must not lead to saturation or exclusion effects either on the surface or – in particular – on the catalytic site. Thus, the local mass action assump-

tion, eq. 70, requires for its validity an enzyme with a strong non-specific affinity for all reactants while binding to its catalytic site is not favourable for any one of them. In fact, the situation which is best described by this boundary condition is a spherical surface with homogeneous catalytic activity; this would then represent a macroscopic surface rather than a molecular one. Thus, there would be serious problems both with overall efficiency and specificity if enzymes functioned in a way that satisfied the local mass action relation, and there are of course no indications that they do. On the contrary, the capacity of an enzyme to hold specifically one of the reactants at the catalytic site until the other has arrived is probably a major rate-enhancing mechanism.

In our approach, we have taken the other extreme and specifically considered the exclusion effects and their coupling to the diffusion field. The situation best described by our approach is when R is interpreted as a measure of the effective diffusional cross-section for binding to the catalytic site. In this interpretation, the influence from a non-spherical diffusion field around the enzyme is neglected, but the discussion of time scales above remains relevant. The cross-section R can approach molecular dimensions if substrates can bind non-specifically at the enzyme surface while remaining sufficiently free to diffuse along the surface into the catalytic site. Only for bulky substrates is one non-specifically bound molecule expected to exclude all others so that the saturation effects are fully expressed for the whole surface. If such non-specific binding is important, however, surface association and transport to the catalytic site must be included as separate steps in the reaction scheme; this is beyond the scope of the present theory.

The diffusional relaxation effects described above is a special example of how transients will be constantly reoccurring under steady-state conditions. In this case the influence on the interpretation of the phenomenological rate constants was small because the diffusional relaxation is fast on the time scale of the total reaction. The situation becomes different in other coupling schemes, e.g., if substrates and products can bind non-specifically to the enzyme and diffuse along the surface into the catalytic site. Then one has to consider the average numbers of surface-bound molecules conditional on whether or not the catalytic site is occupied. The conditional time averaging can be carried out in the same formalism as presented above; the steady-state result for the simple isomerization (S to P) reaction (Berg, unpublished calculations) is in some cases strongly dependent on the reoccurring transients in the numbers of surface-bound molecules. Such effects may be of particular importance in the living cell where both compartmentalization and couplings between several different reactions must be considered.

Appendix A

A.1. Iterative solution; irreversible case

To start with, assume a homogeneous distribution $\bar{S}_1(r) = S_0$ at the moment of product dissociation. Then one finds after partial integration of eq. 38

$$S_0(r) = S_0 - k_S S_0 \int_0^\infty \exp\left[-\int_0^t \phi_S^0(t')\mathrm{d}t' \right] G_S^{\hat{A}}(R,r;t)\mathrm{d}t \quad (A1)$$

where

$$\phi_S^0(t) = k_S S_0 \int_R^\infty 4\pi r^2 G_S^{\hat{A}}(R,r;t)\mathrm{d}r \quad (A2)$$

is the same flux as that given in eq. 14 belonging to the homogeneous initial distribution. The value at the boundary ($r' = R$) of the Green's function $G_S^{\hat{A}}(r',r;t)$ satisfying the partially absorbing boundary conditions, eq. 10, is [9]

$$G_S^{\hat{A}}(R,r;t) = (1/4\pi Rr)\left\{ (\pi Dt)^{-\frac{1}{2}} \exp\left[-(r-R)^2/4Dt \right] \right.$$
$$- \kappa_S \exp\left[\kappa_S^2 Dt + \kappa_S (r-R) \right]$$
$$\left. \times \operatorname{erfc}\left[(r-R)/(4Dt)^{\frac{1}{2}} + \kappa_S (Dt)^{\frac{1}{2}} \right] \right\} \quad (A3)$$

where

$$\kappa_S = (1 + k_S/4\pi DR)/R$$

Now, $\bar{S}_1(r)$ can be calculated anew from eq. 32. To determine the flux $j = j_{mic}$ from eq. 25, it is sufficient to calculate the boundary value $\bar{S}_1(R)$

which is

$$\bar{S}_1(R) = \lambda_P \int_R^\infty \bar{G}^R(R,r;\lambda_P) S_0(r) 4\pi r^2 dr \quad (A4)$$

where the Laplace-transformed Green's function is [9]

$$\bar{G}^R(R,r;\lambda_P) = \frac{1}{4\pi Dr} \cdot \frac{1}{qR+1} \exp[-q(r-R)] \quad (A5)$$

with

$$q = (\lambda_P/D)^{\frac{1}{2}}$$

Inserting eq. A1 into eq. A4 and changing the order of integrations one finds

$$1 - \frac{\bar{S}_1(R)}{S_o} = \frac{1}{qR+1} \cdot \frac{k_S \lambda_P}{4\pi DR} \int_0^\infty dt \exp\left[-\int_0^t \phi_S^o(t')dt' \right] \times \left\{ \frac{\kappa_S}{\kappa_S + q} \exp(\kappa_S^2 Dt) \operatorname{erfc}(\kappa_S^2 Dt)^{\frac{1}{2}} - \frac{q}{\kappa_S + q} \exp(q^2 Dt) \operatorname{erfc}(q^2 Dt)^{\frac{1}{2}} \right\} \quad (A6)$$

In the exponential function, the transient part of the flux ϕ_S^o contributes mainly for small t when the exponential is approximately equal to one. If this transient is neglected, i.e., $\phi_S^o \simeq k_1 S_o = k_S S_o/(1 + k_S/4\pi DR)$, the integration can easily be carried out giving

$$1 - \frac{\bar{S}_1(R)}{S_o} = \frac{1}{1 + (D/\lambda_P R^2)^{\frac{1}{2}}} \cdot \frac{1}{1 + (k_1 S_o/\lambda_P)^{\frac{1}{2}}} \cdot \frac{1}{1 + (4\pi DR/k_S)\left[1 + (k_1 S_o R^2/D)^{\frac{1}{2}}\right]} \quad (A7)$$

Note that the approximation does not imply a neglect of all transient effects; on the contrary, it is the transient substrate distribution which gives a non-zero integrand in eq. A6.

Appendix B

B.1. Iterative solution; reversible case

Like in the irreversible case above, we shall be satisfied with one iteration. Thus, assume homogeneous starting distributions $\bar{S}_1(r) = S_o$ and $\bar{P}_1(r) = P_o$. Then the expression, eq. 60, for $S_0(r)$ simplifies considerably, since the fluxes ϕ_S and ϕ_P are replaced by ϕ_S^o and ϕ_P^o belonging to the homogeneous initial distributions which are directly related to the fluxes φ_S and φ_P from eq. 51. Then one finds

$$S_0(r) = S_o - \frac{\lambda_P k_S S_o - \lambda_S k_P P_o}{\lambda} \int_0^\infty W_P^o(t) G_S^A(r,R;t) dt$$

$$P_0(r) = P_o + \frac{\lambda_P k_S S_o - \lambda_S k_P P_o}{\lambda} \int_0^\infty W_S^o(t) G_P^A(r,R;t) dt \quad (B1)$$

The Green's function G_P^A is of course the one belonging to the partially absorbing boundary condition for the product and is found from eq. A3 by replacing k_S with k_P. We have also defined the conditional reassociation probabilities

$$W_P^o(t) \equiv \frac{\phi_P^o}{k_P P_o} \exp\left\{ -\int_0^t [\phi_S^o(t') + \phi_P^o(t')] dt' \right\}$$

$$W_S^o(t) \equiv \frac{\phi_S^o}{k_S S_o} \exp\left\{ -\int_0^t [\phi_S^o(t') + \phi_P^o(t')] dt' \right\} \quad (B2)$$

conditional on whether it is a substrate or product that has dissociated. Using eq. 62 and the expressions, eqs. A3 and A5, for the respective Green's functions one finds

$$\bar{S}_1(R) = S_o - \frac{k_S \lambda_P S_o}{4\pi DR} \cdot \frac{1 - \delta_P}{qR+1} \int_0^\infty W_P^o(t) F_S(t) dt \quad (B3)$$

$$\bar{P}_1(R) = P_o + \frac{k_S \lambda_P S_o}{4\pi DR} \cdot \frac{1 - \delta_P}{qR+1} \int_0^\infty W_S^o(t) F_P(t) dt \quad (B4)$$

Here we have defined

$$F_S(t) \equiv \frac{\kappa_S}{\kappa_S - q} \exp(\kappa_S^2 Dt) \operatorname{erfc}(\kappa_S^2 Dt)^{\frac{1}{2}} - \frac{q}{\kappa_S - q} \exp(q^2 Dt) \operatorname{erfc}(q^2 Dt)^{\frac{1}{2}}$$

$$F_P(t) \equiv \frac{\kappa_P}{\kappa_P - q} \exp(\kappa_P^2 Dt) \operatorname{erfc}(\kappa_P^2 Dt)^{\frac{1}{2}} - \frac{q}{\kappa_P - q} \exp(q^2 Dt) \operatorname{erfc}(q^2 Dt)^{\frac{1}{2}} \quad (B5)$$

where

$$q \equiv (\lambda/D)^{\frac{1}{2}}$$

$$\kappa_S \equiv (1 + k_S/4\pi DR)/R = k_S/k_1 R$$

$$\kappa_P \equiv (1 + k_P/4\pi DR)/R = k_P/k_{-2} R$$

Furthermore

$$\delta_P \equiv \frac{P_o}{K_{eq} S_o} = \frac{k_P}{\lambda_P} \cdot \frac{\lambda_S}{k_S} \cdot \frac{P_o}{S_o} \quad (B6)$$

is the product displacement from equilibrium [11]. We see that the expressions, eqs. B3 and B4, are consistent in that at equilibrium – when $\delta_P = 1$ – there is no departure from the bulk concentrations at the enzyme surface.

Now, the expressions, eqs. B2–B5, can be used with eq. 26 to calculate the net flux j_{mic} per enzyme. In the special case when the initial rate of reaction is studied ($P_o = 0$), the difference between the macroscopic and microscopic flux couplings is given by eq. 66. Its magnitude is determined by the departures from the equilibrium distributions of substrate

$$\varepsilon_S \equiv 1 - \bar{S}_1(R)/S_o = \frac{1}{1+(\lambda R^2/D)^{\frac{1}{2}}} \cdot \frac{k_S \lambda_P}{4\pi DR} \cdot \frac{k_{-2}}{k_P} \times \int_0^\infty \left[1 + \frac{k_P}{4\pi DR} \exp(\kappa_P^2 Dt) \operatorname{erfc}(\kappa_P^2 Dt)^{\frac{1}{2}}\right] \times \exp\left[-\int_0^t \phi_S^o(t')\mathrm{d}t'\right] F_S(t)\mathrm{d}t \quad \text{(B7)}$$

and of product

$$\varepsilon_P \equiv K_P \bar{P}_1(R)/K_S S_o = \frac{1}{1+(\lambda R^2/D)^{\frac{1}{2}}} \cdot \frac{k_P \lambda_S}{4\pi DR} \cdot \frac{k_1}{k_S} \times \int_0^\infty \left[1 + \frac{k_S}{4\pi DR} \exp(\kappa_S^2 Dt) \operatorname{erfc}(\kappa_S^2 Dt)^{\frac{1}{2}}\right] \times \exp\left[-\int_0^t \phi_S^o(t')\mathrm{d}t'\right] F_P(t)\mathrm{d}t \quad \text{(B8)}$$

The required integrations can be carried out approximately if $4\pi R^3 S_o \ll 1$ (cf. eq. 18) and $k_1 S_o \ll \lambda$. These are not very restrictive conditions. The first one allows the approximation $\int_0^t \phi_S^o(t')\mathrm{d}t' = k_1 S_o t$ in the exponential functions (cf. eq. 14); the transient contributes significantly only when $t \ll R^2/D$ and then $\int_0^t \phi_S^o(t')\mathrm{d}t' \ll 4\pi R^3 S_o \ll 1$, in which case the exponential function is constant ($= 1$). Together, the two conditions ensure that the transient parts of $F_S(t)$ and $F_P(t)$ will have relaxed before the exponential function $\int_0^t \phi_S^o(t')\mathrm{d}t'$ differs appreciably from one. Thus, the main integral of eq. (B7) can be subdivided

$$\int_0^\infty \left[1 + \frac{k_P}{4\pi DR} \exp(\kappa_P^2 Dt) \operatorname{erfc}(\kappa_P^2 Dt)^{\frac{1}{2}}\right] \times \exp\left[-\int_0^t \phi_S^o(t')\mathrm{d}t'\right] F_S(t)\mathrm{d}t$$

$$= \int_0^\infty \exp(-k_1 S_o t) F_S(t)\mathrm{d}t + \frac{k_P}{4\pi DR} \times \int_0^\delta \exp(\kappa_P^2 Dt) \operatorname{erfc}(\kappa_P^2 Dt)^{\frac{1}{2}} F_S(t)\mathrm{d}t + \frac{k_P}{4\pi DR} \int_\delta^\infty (\kappa_P^2 \pi Dt)^{-\frac{1}{2}} \times \exp(-k_1 S_o t) \frac{\kappa_S + q}{\kappa_S^2 q^2} \left[4\pi (Dt)^3\right]^{-\frac{1}{2}} \mathrm{d}t \quad \text{(B9)}$$

where δ is some short time such that $k_1 S_o \delta \ll 1$ and $\kappa_P^2 D\delta$, $\kappa_S^2 D\delta$, $q^2 D\delta \gg 1$. In the last integral, the asymptotic expansion of $\exp(x^2)\operatorname{erfc}(x)$ has been introduced. All three integrals can be calculated and expressed in standard functions. One dominant contribution comes from the first integral which is $[(k_1 S_o)^{\frac{1}{2}} + \kappa_S (D)^{\frac{1}{2}}]^{-1} [(k_1 S_o)^{\frac{1}{2}} + q(D)^{\frac{1}{2}}]^{-1} = (q\kappa_S D)^{-1}$. The last integral is negligible to first order in $k_1 S_o \delta$, and the middle one gives approximately

$$\frac{k_P}{4\pi DR} \cdot \frac{1}{D} \cdot \frac{\kappa_P}{\kappa_S - q} \cdot \frac{1}{(\kappa_S^2 + \kappa_P^2)(\kappa_P^2 + q^2)} \left\{ \frac{2}{\pi} \left[\kappa_P^2 \ln(\kappa_S/q) + q^2 \ln(\kappa_S/\kappa_P) + \kappa_S^2 \ln(\kappa_P/q)\right] - (\kappa_S - q)(\kappa_P - q\kappa_S/\kappa_P)\right)$$

which to first order in $(\kappa_S \kappa_P D\delta)^{-1}$ and $(\kappa_P q D\delta)^{-1}$ is independent of the choice of δ. Similarly, the required integral for the product deviation (eq. B8) is recovered simply by exchange of subscripts S and P in the expressions above.

The deviations ε_S and ε_P are zero in the idealized limits of pure reaction control ($k_P, k_S, \lambda_S, \lambda_P \to 0$ while the binding constants $K_P = k_P/\lambda_P$ and $K_S = k_S/\lambda_S$ are kept fixed) as well as pure diffusion control ($k_P, k_S, \lambda_S, \lambda_P \to \infty$ while k_P/λ_P and k_S/λ_S are fixed). This is reasonable, since both limits correspond to local equilibrium at the enzyme surface. For a numerical example let us consider the simpler case when the extent of diffusion control is the same for substrate and product, i.e., $k_S = k_P = k$ and $\kappa_S = \kappa_P = \kappa$. Then the total deviation is

$$\varepsilon \equiv \varepsilon_S + \varepsilon_P(1 + K_S S_o) = (1 + kS_o/\lambda) \frac{qR}{qR+1} \cdot \frac{k}{4\pi DR} \cdot \frac{1}{(\kappa R)^2} \times \left\{1 + \frac{k}{4\pi DR} \cdot \frac{1}{1 - q/\kappa} \cdot \frac{q/\kappa}{1 + (q/\kappa)^2}\right.$$

$$\times\left[\frac{2}{\pi}\ln(\kappa/q)-\tfrac{1}{2}(1-q/\kappa)^2\right]\Bigg\} \qquad \text{(B10)}$$

which gives a relative error in the inverse flux from eq. 66

$$k_{2,3} = \frac{\varepsilon}{1+\varepsilon/(1+\lambda/kS_0)} \qquad \text{(B11)}$$

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