Biophysical Chemistry 7 (1978) 285–292

DIFFUSION CONTROL IN REVERSIBLE ENZYME REACTIONS. APPLICATIONS TO CARBONIC ANHYDRASE

Bo JÖNSSON and Håkan WENNERSTRÖM
Department of Physical Chemistry 2, Chemical Centre, S-220 07 Lund, Sweden

Received 18 May 1977

The limit to the possible rate of reversible enzymatic reactions set by the diffusional motion has been considered. It is found that not only the diffusion of the reactants to the enzyme but also the diffusion away of the products can be rate limiting. To avoid assumptions about the detailed nature of the enzyme only diffusion in the bulk aqueous medium is treated. By such an approach one obtains an upper limit to the possible rate. In the latter half of the paper the derived general equations are applied to the possible suggested reaction schemes for the enzyme carbonic anhydrase. It is found that a scheme involving HCO_3^- as substrate for the dehydration process and a direct reaction between buffer and enzyme is consistent with the limits set by the diffusional motion, while several other possibilities can be ruled out.

1. Introduction

For bi- and multimolecular reactions in solution it is necessary for the reactants to collide through brownian diffusional motion before a chemical transformation can take place. The diffusion thus puts a limit to the possible rate of the reaction. The diffusional behaviour of simple molecules in fluids has been thoroughly investigated both experimentally and theoretically. The accumulated knowledge about these processes makes it possible to determine rather accurate upper bounds for reaction rates. Although these bounds usually correspond to very high rates many diffusion controlled reactions has been observed. Well known examples are proton or electron transfer reactions but even some enzyme reactions are fast enough for the diffusion limit to apply [1,2].

The basic theoretical work on diffusion controlled reactions is due to Smulochowski [3] who considered the special case of aggregation of colloidal particles. He assumed spherical symmetry about the aggregation center and solved the diffusion equation

$$\frac{\partial}{\partial t} r\,c(r,t) = D\,\frac{\partial^2}{\partial r^2} r\,c(r,t) \qquad (1)$$

with the boundary conditions

$$c(\infty) = c_0; \quad c(R) = 0. \qquad (2)$$

Here c is the concentration, r the distance from the centre, D the translational diffusion constant and R the distance at which the particle is irreversibly captured. The solution of eq. (1) showed that, on a macroscopic timescale, a stationary state is rapidly reached with a concentration profile $c(r) = c_0(1-R/r)$ and a constant outgoing flux of particles $J(r) = -4\pi N_a D r^2 \times \partial c/\partial r = -4\pi N_a D R c_0$ where N_a is Avogadros number. In this model the diffusion limited second order rate constant is

$$k_2^{\lim} = 4\pi N_a D R. \qquad (4)$$

This expression for $k_2^{\lim}$ has been widely used on the merit of its simplicity.

In later work improvements of Smulochowskis theory has been mainly along three lines. The macroscopic diffusion approach is not valid on very short timescales, where the molecular nature of matter has to be accounted for [4]. In eq. (1) long range forces between the reactants are neglected and especially for Coulomb interactions sizeable correction terms may occur [1]. The boundary condition $c(R) = 0$ can be made more realistic both with respect to geometrical constraints on the relative orientation of the re-

actants for a reaction to occur and with respect to the probability of a reaction [5–10].

The present paper deals with the last of these aspects with special consideration of *reversible* enzyme reactions. This problem arouse during our study of the reaction between water and carbon dioxide to form carbonic acid [11] and during considerations of the possible enzymatic catalysis of this reaction by carbonic anhydrase.

2. Diffusion and reversible enzyme reactions

For a reversible enzymatic reaction not only the diffusion of the reactants to the active cleft can be a rate limiting step but also the diffusion away of the products. Since if the products are not transported away an equilibrium between reactants and products are established at the enzyme surface. One can thus obtain more stringent limits for the possible rate of the reaction by considering the diffusion of both reactants and products.

In the active cleft and at the enzyme surface in general the substrate, or product, interacts with the enzyme. Without a very detailed knowledge about the enzyme it is difficult to determine how these interactions affect the diffusional motion. One has, for example, the possibility of rapid surface diffusion. Usually very little is known about such specific effects. One method of avoiding arbitrary assumptions *is to consider the diffusional motion only in the bulk aqueous phase outside the enzyme.* In aqueous solution diffusion has been extensively studied and the isotropic translational diffusion constant has been determined for a large number of compounds. In the subsequent treatment we therefore assign to the enzyme a radius R (cf. fig. 1) outside which enzyme substrate interactions can be neglected and where the diffusion equation is directly applicable. In such an approach one can, at most, obtain an upper limit to the diffusion controlled reaction rate.

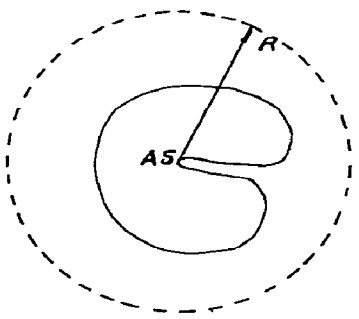

Fig. 1. Schematic picture of the enzyme with the active site (AS).

As a first example consider a simple enzymatic conversion between species A and B. The diffusion equation in a coordinate system fixed on the enzyme is at steady state [5].

$$\frac{1}{r}\frac{\partial^2}{\partial r^2} r c_{A,B} + \left(\frac{1}{r^2} + \frac{D_R}{D_{A,B}}\right)\Delta_\Omega c_{A,B} = 0, \tag{5}$$

where

$$\Delta_\Omega = \frac{1}{\sin\theta}\frac{\partial}{\partial\theta}\sin\theta\frac{\partial}{\partial\theta} + \frac{1}{\sin^2\theta}\frac{\partial^2}{\partial\phi^2}$$

is the angular part of the laplacian operator. The rotational diffusion constant is denoted D_R and $D_{A,B}$ is the sum of the translational diffusion constants of A(B) and of the enzyme. To find appropriate boundary conditions one has to consider the reaction in some detail. It is assumed that the reaction proceeds according to the scheme

$$A + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AE \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} BE \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + B, \qquad [I]$$

where E is the enzyme. If the binding of the substrate to the enzyme is negligible, that is if the Michaelis–Menten constant K_M is large relative to the substrate concentration, the rate is

$$\frac{dc_A}{dt} = -\frac{dc_B}{dt} = -\vec{k}c_A + \overleftarrow{k}c_B \tag{6}$$

per enzyme molecule. Here $\vec{k} = k_1k_2k_3/(k_{-1}k_3 + k_{-2}k_{-1} + k_2k_3)$ and $\overleftarrow{k} = k_{-1}k_{-2}k_{-3}/(k_{-1}k_3 + k_{-2}k_{-1} + k_2k_3)$ and $\vec{k}/\overleftarrow{k} = K$ is the equilibrium constant of the overall reaction. In eq. (6) the concentrations refer to those at the enzyme surface. The rate constants are orientation dependent since a molecule just outside the active cleft will have a higher probability of reaction than a molecule at the back of the enzyme.

At steady state mass balance requires that the flow of molecules equals the number produced by the reaction and an appropriate boundary condition would be

$$-J_A(R,\theta,\phi) = J_B(R,\theta,\phi)$$
$$= F(\theta,\phi)\{\vec{k}c_A(R,\theta,\phi) - \overleftarrow{k}c_B(R,\theta,\phi)\}. \quad (7a)$$

When applying eq. (7a) it is difficult to find a good expression for the distribution function $F(\theta,\phi)$ for the reaction probability. The function F might, for example, be finite over the entire surface due to the possibility of surface diffusion on the enzyme.

Eq. (5) has been solved by Solc and Stockmayer [6] with the boundary condition eq. (7a). The resulting limiting rate depends markedly on the choice of $F(\theta,\phi)$. To avoid the arbitrariness of a particular choice of $F(\theta,\phi)$ we have pursued the aim of obtaining an upper limit for the diffusion controlled rate. This is achieved by setting $F(\theta,\phi) = 1/4\pi$. With such a choice of $F(\theta,\phi)$, the boundary condition (7a) can be written as

$$-J_A(R) = J_B(R) = \vec{k}c_A(R) - \overleftarrow{k}c_B(R) \quad (7b)$$

and the diffusion problem reduces to a spherically symmetrical one and eq. (5) becomes

$$\frac{\partial^2}{\partial r^2} r\,c(r) = 0. \quad (8)$$

The boundary conditions are in addition to eq. (7b) that $c_A(\infty) = c_{A0}$ and $c_B(\infty) = c_{B0}$ where c_{A0} and c_{B0} are the bulk concentrations of A and B respectively.

The solution to the diffusion equation (8) is

$$c_A(r) = c_{A0} - n/r; \quad c_B(r) = c_{B0} + D_A n/(D_B r) \quad (9a,b)$$

$$n = \frac{Kc_{A0} - c_{B0}}{4\pi D_A N_a/\overleftarrow{k} + K/R + D_A/(D_B R)}. \quad (9c)$$

The effective forward rate constant is then

$$k_{eff} = 4\pi D_A N_a K R/(4\pi D_A N_a R/\overleftarrow{k} + K + D_A/D_B). \quad (10)$$

If $4\pi D_A N_a R/\overleftarrow{k} \gg K + D_A/D_B$ eq. (10) is equivalent to the kinetic expression eq. (6). If on the other hand $4\pi D_A N_a R/\overleftarrow{k} \ll K + D_A/D_B$ the process is diffusion controlled and

$$k_{eff} = k_2^{lim} K/(K + D_A/D_B). \quad (11)$$

The rate constant k_{eff} becomes equal to k_2^{lim} of eq. (4) if $K \gg D_A/D_B$ but in the opposite limit, $K \ll D_A/D_B$ it is the diffusion away of the products that is rate limiting and the maximum rate is reduced by a factor K. As will be shown later this extra factor can be of great importance.

For the reaction

$$A + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AE \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} BCE \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + B + C, \quad [II]$$

where in analogy with eq. (6) it is assumed that

$$-\frac{dc_A}{dt} = \frac{dc_B}{dt} = \frac{dc_C}{dt} = \vec{k}c_A - \overleftarrow{k}c_B c_C \quad (12)$$

per enzyme, the solution of the diffusion problem is somewhat more complex, but the same type of boundary conditions apply as in eq. (7b). However in this case the result cannot be expressed in terms of an effective rate constant, k_{eff}, since the reaction rate is not always proportional to the concentration. The flux of molecules A is

$$J_A = 4\pi N_a D_A \left\{ \frac{R}{2D_A}\left(\frac{4\pi N_a D_B D_C R}{\overleftarrow{k}} + \frac{D_B D_C}{D_A} + c_{B0}D_B + c_{C0}D_C \right) \right.$$
$$- \left[\frac{R^2}{4D_A^2}\left(\frac{4\pi N_a D_B D_C R}{\overleftarrow{k}} + \frac{D_B D_C K}{D_A} + c_{B0}D_B + c_{C0}D_C \right)^2 \right.$$
$$\left. \left. - \frac{R^2 D_B D_C}{D_A^2}(c_{B0}c_{C0} - K c_{A0}) \right]^{1/2} \right\}. \quad (13)$$

For small rate constants eq. (13) reduces to eq. (12), while in the diffusion limit with for example $c_{B0} = c_{C0} = 0$ the flux is

$$J_A = 4\pi N_a \frac{D_B D_C R K}{2D_A}\left\{ 1 - \left(1 + \frac{4D_A^2 c_{A0}}{D_B D_C K} \right)^{1/2} \right\}. \quad (14)$$

When the second term in the root expression is negligible eq. (14) is equivalent to eq. (4) while if the second term is the dominant one the flux is proportional to the square root of the concentration c_{A0}

$$J_A = -4\pi N_a (D_B D_C)^{1/2} R (K c_{A0})^{1/2}. \quad (15)$$

For future reference we also present the expression for the flux if the reaction proceeds in the opposite direction. When $c_{A0} = 0$ and in the limit of slow diffusion one obtains

$$J_A = \frac{4\pi N_a R}{2(D_B D_C K/D_A + c_{B0}D_B + c_{C0}D_C)}$$
$$\times \left\{ 1 - \left[1 - \frac{4D_B D_C c_{B0} c_{C0}}{(D_B D_C K/D_A + D_B c_{B0} + D_C c_{C0})^2} \right]^{1/2} \right\}. \quad (16)$$

A general conclusion of this section is that eq. (4) is a poor approximation of the diffusion limit for reversible enzymatic reactions for one of the directions of the reaction. It can also be noted that the expressions for J_A in the diffusion limit can be obtained by requiring equilibrium at the enzyme surface. This point of view is often essential for the conceptual understanding of the somewhat complex equations. For example the square root dependence of the flux on concentration in eq. (15) is readily understood in such a description.

3. Diffusion control and possible mechanisms for the enzyme carbonic anhydrase

The enzyme carbonic anhydrase acts to establish a rapid equilibrium between dissolved carbon dioxide and bicarbonate ions

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \qquad [III]$$

in a multitude of living systems. The enzyme is an extremely efficient catalyst and has one of the highest turnover numbers known. In spite of the fact that one has obtained extensive structural information about the enzyme [12] the mechanism for the catalytic activity has not yet been revealed. The substrate for the dehydration reaction has not even been firmly established. One usually assumes [13,14] that H^+ and HCO_3^- are the reactants but Koenig [15] has argued that H_2CO_3 must be the substrate because otherwise the supply of protons would exceed the diffusion limit. However, the same type of argument has been raised against H_2CO_3 as a possible substrate [13,14]. These arguments have been focussed on the dehydration reaction and the diffusion limit set by eq. (4) has been used.

The reaction in scheme [III] is under physiological conditions typically reversible and by analyzing the diffusion problem using the boundary conditions of eq. (7b) more consistent conclusions can be reached for the reaction proceeding in both directions.

Table 1
Values for the constants used in the numerical estimates

$D_{H_2O} = 9 \times 10^{-9}$ m^2 s^{-1}	$D_{CO_2} = 2 \times 10^{-9}$ m^2 s^{-1}
$D_{H_2CO_3} = 10^{-9}$ m^2 s^{-1}	$D_{HCO_3} = 10^{-9}$ m^2 s^{-1}
$R = 2.2 \times 10^{-9}$ m	
$K_1 = [HCO_3][H]/[H_2CO_3] = 2.5 \times 10^{-4}$ M	
$K_2 = [H_2CO_3]/[CO_2] = 1.7 \times 10^{-3}$	

3.1. Diffusion limits for the direct reactions

The case when the enzymatic reaction proceeds as

$$H_2O + CO_2 + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\cdot}H_2O{\cdot}CO_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E{\cdot}H_2CO_3 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + H_2CO_3 \qquad [IV]$$

is equivalent to scheme [I] since water is the solvent and thus present in excess. The equation (10) for the rate of the reaction should then be applicable and it is then possible to compare the calculated rates with actually measured rates.

To obtain numerical results actual values has to be assigned to the different parameters. The equilibrium constant K is 0.0017 [16] and the rate constants are either taken from the work of Steiner et al. [17] on the human C isoenzyme or they are assumed large to obtain the diffusion limited rate. Our choice of the radius R and the diffusion constants are summarized in table 1. Some of the parameters are estimated values but they could be wrong by at most 50%.

Interpreted within the kinetic scheme [IV] the experiments of Steiner et al. [17] give a forward rate constant of $\vec{k} \approx 1.4 \times 10^8$ s^{-1} M^{-1}. This value can now be compared with those calculated from eqs. (10) and (11). Using the constants of table 1 and the experimental value of $\vec{k}$ in eq. (10) one obtains $\vec{k}_{eff} = 2.27 \times 10^7$ s^{-1} M^{-1} and $\vec{k}_{eff} = 2.83 \times 10^7$ s^{-1} M^{-1} from eqs. (10) and (11), respectively. The rates for the dehydration reaction are obtained by dividing with the equilibrium constant K and thus they contain no additional information.

These estimates show that the experimental kinetic data are not consistent with the reaction scheme [IV]. It can be noted that the data of Steiner et al. has been reproduced [18,19] and the combined errors from experimental rates and the errors in the parameters is not large enough to eliminate the discrepancy.

For the other possible direct reaction scheme

$$H_2O + CO_2 + E \rightleftharpoons E{\cdot}H_2O{\cdot}CO_2 \rightleftharpoons E{\cdot}H^+{\cdot}HCO_3 \rightleftharpoons E + H^+ + HCO_3^- \qquad [V]$$

a similar analysis is possible. The difference is now

that it is the rate equation (13) that applies and that the result cannot be expressed in terms of rate constants. Instead one has to consider the measured flows. The value of the equilibrium constant K is now 4.3×10^{-7} M.

At, for example, pH = 7.5 and $c_{CO_2} = 1$ mM and $c_{HCO_3^-} = 0$ the measured rate is $J_{CO_2} = -8.8 \times 10^4$ s^{-1}. From eqs. (13) and (14) with $\vec{k} = 1.9 \times 10^{14}$ s^{-1} M^{-2} in eq. (13) one obtains the flows $J_{CO_2} = -8.1 \times 10^4$ s^{-1} and $J_{CO_2} = -1.0 \times 10^6$ s^{-1} respectively.

For the dehydration reaction also at pH = 7.5 and with $c_{CO_2} = 0$ and $c_{HCO_3^-} = 20$ mM the measured rate is $J_{CO_2} = 1.1 \times 10^5$ s^{-1}. In this case the eqs. (13) and (16) gives $J_{CO_2} = 4.5 \times 10^3$ s^{-1} and $J_{CO_2} = 4.7 \times 10^3$ s^{-1}. The measured rate for the dehydration reaction is thus faster than what can be accounted for in scheme [V] when the diffusion is taken into account.

It has thus been found that it is necessary to introduce additional steps in both reaction schemes [IV] and [V] to obtain consistency. It is clear that in both cases reactions occur in the bulk phase and this possibility will now be considered.

3.2. Effects of reactions in the bulk phase

Consider the reaction scheme [IV] where carbonic acid is produced or consumed at the enzyme surface. In the presence of a buffer system (HB, B^-) the reaction

$$HB + HCO_3^- \underset{k_-}{\overset{k_+}{\rightleftharpoons}} H_2CO_3 + B^- \qquad \text{[VI]}$$

will occur in the bulk phase. In the absence of buffer water may take the place of HB. In most experimental cases the concentrations of HCO_3^-, HB and B are large enough to be practically constant during the reaction. Denote their concentrations S_{HB}, S_B and S_{HCO_3}. In the diffusion equation for carbonic acid an extra term appears due to the reaction [VI] and at steady state

$$D\Delta c + (k_+ S_{HB} S_{HCO_3} - k_- S_B c) = 0 \qquad (17)$$

the solution to this equation is

$$c(r) = c_0 + n \exp(-\sqrt{b}\, r)/r, \qquad (18)$$

where $b = k_- S_B / D_{H_2CO_3}$. The constant n is determined by the boundary condition of eq. (7). The final expression for the reaction rate is

$$J_{CO_2} = -4\pi D_{CO_2} N_a (K c_{0,CO_2} - c_{0,H_2CO_3}) \times \left(\frac{4\pi D_{CO_2} N_a}{\vec{k}} + \frac{K}{R} + \frac{D_{CO_2}}{D_{H_2CO_3}(1 + \sqrt{b}R)R} \right)^{-1}. \qquad (19)$$

A comparison with eq. (10) reveals that in the diffusion limit and when K is small as in the present case, one achieves enhancement of the rate with a factor of $(1 + \sqrt{b}R)$. Using the values $S_B = 100$ mM, $k_- = 10^{10}$ M^{-1} s^{-1}, *a very fast proton transfer*, and other parameters as in table 1 one obtains $(1 + \sqrt{b}R) = 3.2$. This should give an upper limit to the contribution from reactions in the solution. The calculated effective rate constants are now

$$\vec{k}_{eff} = 5.0 \times 10^7 \text{ s}^{-1} \text{ M}^{-1}, \quad \bar{k}_{eff} = 9.1 \times 10^7 \text{ s}^{-1} \text{ M}^{-1},$$

using eqs. (10) and (11), respectively. The calculated values do still not reach the experimentally observed rate constant. If the uncertainty in the diffusion coefficients is taken into account the value 9.1×10^7 s^{-1} M^{-1} could, however, reach the experimental one. On the other hand the calculations have been designed to give an upper limit to the rates and approximations made in this process should by far outweight the possibility of a small error in the diffusion coefficients.

When the enzymatic reaction proceeds directly through HCO_3^- and H^+, as in scheme [V], the possible effect of a buffer is to act as a reservoir for protons through the reaction

$$HB \underset{k_{-B}}{\overset{k_B}{\rightleftharpoons}} H^+ + B^-. \qquad \text{[VII]}$$

Assuming the concentrations of the buffer species constant at S_{HB} and S_B, respectively, the solution for the proton concentration is the same as in eq. (18). The expression for the rate is rather complex. For the dehydration process, where consistency between theory and experiments was not obtained in the direct scheme, one has the relations $c_{0,CO_2} = 0$ and

$$c_{0,HCO_3} \gg \left(\frac{K D_H}{D_{CO_2}} + c_{0,H} \frac{D_H}{D_{HCO_3}} \right) \cdot (1 + R\sqrt{b})$$

and obtains in the diffusion limit the flow

$$J_{CO_2} = 4\pi N_a D_{HCO_3} R\, c_{0,H} (1 + R\sqrt{b}), \qquad (20)$$

which is analogous to eq. (16). With the numerical values as above $(1 + Rb) = 1.7$, and the enhancement is

not sufficient to account for the observed rate.

3.3. Direct participation by buffer in the enzymatic mechanism

As has been shown in the previous sections the high efficiency of carbonic anhydrase is hardly in agreement with theoretical calculations on the diffusion of the different species. This is true regardless of whether H_2CO_3 or $HCO_3^- + H^+$ are the products for the hydration reaction. The inclusion of reactions proceeding in the bulk solution does not change the results significantly.

It has been suggested by several authors [13,14] that buffer molecules present under in vitro experimental conditions and also in vivo may react directly with the enzyme and thereby transport the protons needed. In a recent paper by Jonsson et al. [20] it has been shown that the hydration rate of carbonic anhydrase is in fact dependent on the buffer concentration and that it reaches a limiting value at increasing buffer concentration. A possible scheme for the buffer dependent reaction would be the following one

$$E^+ + H_2O + B^- \underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} EOH + HB,$$

$$EOH + CO_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EOHCO_2 \underset{k_{-c}}{\overset{k_c}{\rightleftharpoons}} EHCO_3 \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} E^+ + HCO_3^-,$$

$$E^+ + H_2O \underset{k_{-e}}{\overset{k_e}{\rightleftharpoons}} EOH + H^+. \qquad \text{[VIII]}$$

Denoting $CO_2 = 1$, $HCO_3^- = 2$, $HB = 3$ and $B^- = 4$ one may write the reaction on the enzyme as

$$\frac{dCO_2}{dt}\Big/E_0 = \frac{1}{y}\{k_{-b}c_3(R)x - k_b c_4(R)(y-x)\},$$

$$\begin{aligned} y = {} & [N + k_2(k_{-1}+k_c+k_{-c})c_2(R)]k_{-b}c_3(R) \\ & + [N + k_1(k_{-2}+k_{-c}+k_c)c_1(R)]k_b c_4(R) \\ & + k_1 k_c k_{-2} c_1(R) + k_{-1}k_{-c}k_2 c_2(R) \\ & \times c_1(R)c_2(R)k_1 k_2[k_c k_{-2}(k_{-1}+k_c+k_{-c}) \\ & + k_{-c}k_{-1}(k_{-2}+k_c+k_{-c})]/N, \end{aligned} \qquad (23)$$

It is straightforward although somewhat tedious to solve the diffusion equations for all four of the reacting molecules. The use of boundary conditions (7b) leads to the following expression for the flow and the concentrations, assuming for simplicity that all diffusion coefficients are equal,

$$J_1(R) = \frac{1}{y}\left\{k_{-b}c_3(R)x - \frac{k_b c_4(R)[yN - x(N + k_1(k_{-2}+k_{-c}+k_c)c_1(R))]}{N + k_2(k_{-1}+k_c+k_{-c})c_2(R)}\right\}, \qquad (24$$

$$N = k_{-1}k_{-c} + k_{-2}k_{-1} + k_c k_{-2},$$

$$c_1(R) = c_{10} - \frac{J_1}{4\pi D N_a R}, \qquad c_2(R) = c_{20} + \frac{J_1}{4\pi D N_a R},$$

$$c_3(R) = c_{30} + \frac{J_1}{4\pi D N_a R}, \qquad c_4(R) = c_{40} - \frac{J_1}{4\pi D N_a R}$$

(25a–d

The equations (24, 25) can be solved iteratively. The rate constants in the equations are taken from the work of Kernohan [18] and Jonsson et al. [20].

The solution shows that the hydration and dehydration processes catalyzed by carbonic anhydrase ar not diffusion controlled in the presence of a buffer system. In fig. 2 it is shown how the flow depends on the diffusion coefficient and it is seen from the curve that as long as $D > 5 \times 10^{-10}$ the processes are quite in dependent of the diffusion. Fig. 3 shows how the con centrations c_1, c_2 and c_4 at the enzyme surface depends on the diffusion coefficient. It is seen that the CO_2 concentration is almost identical to the bulk con centration for $D > 5 \times 10^{-10}$ m^2 s^{-1}. The flow given eq. (24) does show the same pH-dependence as does

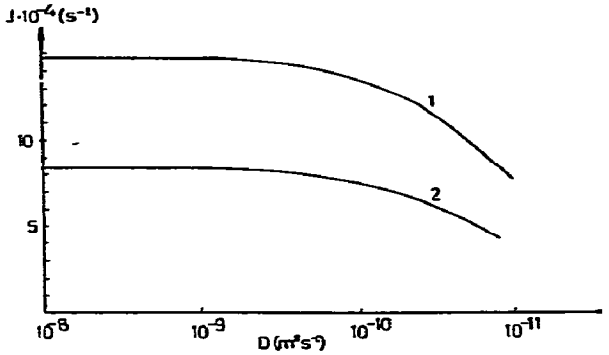

Fig. 2. The reaction rate per enzyme (J) as a function of the diffusion coefficient (D). Curve 1 = hydration and 2 = dehydration. (Scheme [VIII]).

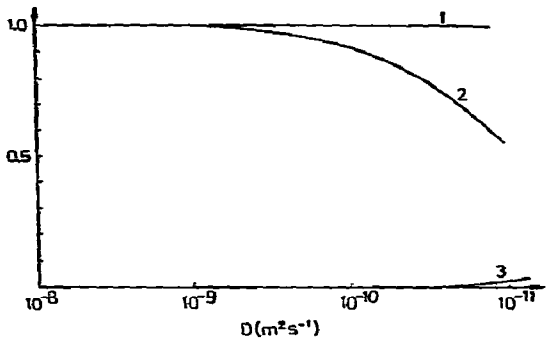

Fig. 3. The concentrations $c_i(R)$ at the enzyme surface as functions of the diffusion coefficient. Curve 1 = $c_1(R)/c_{10}$, 2 = $c_4(R)/c_{40}$ and 3 = $c_2(R)/c_2(\text{eq}) = c_2(R)\cdot[H]/K_1\cdot K_2\cdot c_1(R)$. (Scheme [VIII]).

the experimental curves of Steiner et al. (see fig. 4). If $J_1(R)$ is plotted as a function of $J_1(R)/c_{10}$ for different buffer concentration we obtain curves analogous to the ones obtained from experiment by Jonsson et al. (fig. 5).

4. Conclusions

It has been shown that in reversible enzyme reactions it is of importance to take into account both the diffusion of both reactants and products when the diffusion limit of the reaction rate is discussed. In the case of carbonic anhydrase this has consequence that several reasonable reaction schemes can be excluded on the basis that the observed rates are too large to be compatible with possible diffusion rates.

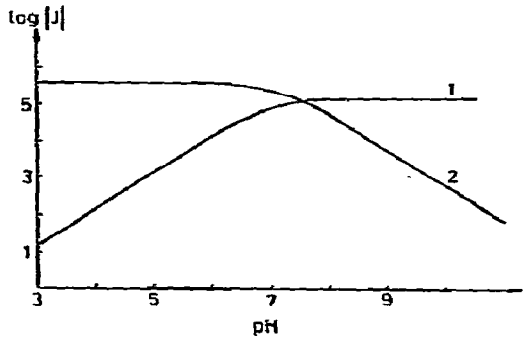

Fig. 4. The reaction rate per enzyme as a function of pH. Curve 1 = hydration and 2 = dehydration. (Scheme [VIII]).

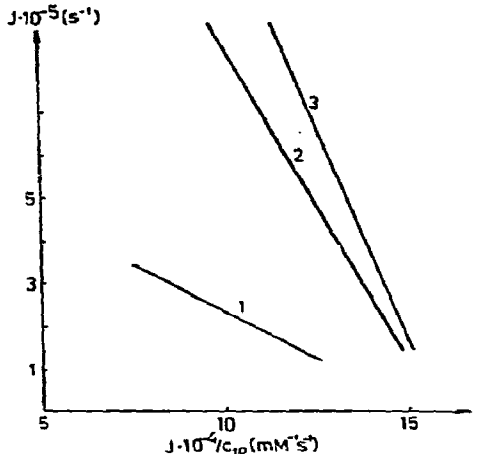

Fig. 5. Dependence of the rate on buffer concentration. 1 = 1 mM, 2 = 10 mM and 3 = 100 mM buffer with $pK_a = 6.2$. (Scheme [VIII]).

Of the investigated possibilities only the scheme with a direct reaction between buffer and the enzyme gave clearly consistent data. The reaction involving carbonic acid combined with an equilibrium between H_2CO_3 and HCO_3^- in the bulk phase can not be unequivocally ruled out on the basis of the present calculations. However, for this reaction scheme to be consistent an unlikely coincidence between a number of factors is required.

One should keep in mind that the present calculations produce upper limits to the diffusion controlled rates. The most restricting assumption that has been made is that of spherical symmetry. Through this assumption the diffusion limit can be overestimated by a factor of approximately ten [6,7]. If, however, the substrate binds to the enzyme surface this factor is greatly reduced [7]. The only mechanism that can lead to rates larger than those calculated above is attraction due to long range coulombic forces. In an aqueous medium with its high dielectric constant these forces are, however, quenched so that their contribution to the diffusion rate is rather small. Furthermore in the case of carbonic anhydrase the possible reactants H^+ and HCO_3^- have opposite charge so that if one of them is attracted the other is repelled.

Acknowledgement

Illuminating discussions with Sven Lindskog and Sture Forsén are gratefully acknowledged.

References

[1] M. Eigen and G.G. Hammes, Adv. Enzymol. 25 (1963) 1.
[2] R.A. Alberty and G.G. Hammes, J. Phys. Chem. 62 (1958) 154.
[3] M.V. Smoluchowski, Z. Physik. Chem. 92 (1917) 129.
[4] R.M. Noyes, J. Chem. Phys. 22 (1954) 1349.
[5] K. Solc and W.H. Stockmayer, J. Chem. Phys. 54 (1971) 2981.
[6] K. Solc and W.H. Stockmayer, Int. J. Chem. Kinet. 5 (1973) 733.
[7] C. Kuo-Chem and J. Shou-Ping, Scientia Sinica 17 (1974) 644.
[8] T.L. Hill, Proc. Nat. Acad. Sci. USA 72 (1975) 4918.
[9] M. Doi, Chem. Phys. 11 (1975) 107.
[10] M. Doi, Chem. Phys. 11 (1975) 115.
[11] B. Jönsson, G. Karlström, H. Wennerström, S. Forsén, B. Roos and J. Almlöf, J. Am. Chem. Soc. 99 (1977) 4628.
[12] S. Lindskog, L.E. Henderson, K.K. Kannan, A. Liljas, P.O. Nyman and B. Strandberg, The Enzymes 5 (1971) 587;
K.K. Kannan, B. Notstrand, K. Fridborg, S. Lövgren, A. Ohlsson and M. Petef, Proc. Nat. Acad. Sci. USA 72 (1975) 51.
[13] S. Lindskog and J.E. Coleman, Proc. Nat. Acad. Sci. USA 70 (1973) 2505.
[14] R.G. Khalifah, Proc. Nat. Acad. Sci. USA 70 (1973) 1986.
[15] S.H. Koenig and R.D. Brown, Proc. Nat. Acad. Sci. USA 69 (1972) 2422.
[16] D.M. Kern, J. Chem. Educ. 37 (1960) 14.
[17] H. Steiner, B.H. Jonsson and S. Lindskog, Eur. J. Biochem. 59 (1975) 253.
[18] J.C. Kernohan, Biochem. Biophys. Acta 96 (1965) 304; E. Magid, Biochem. Biophys. Acta 151 (1968) 236.
[19] R.G. Khalifah, J. Biol. Chem. 246 (1971) 2561.
[20] B.H. Jonsson, H. Steiner and S. Lindskog, FEBS Lett. 64 (1976) 310.