Biochimica et Biophysica Acta, 358 (1974) 178–192
© Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

BBA 67258

BUFFER-FACILITATED PROTON TRANSPORT

pH PROFILE OF BOUND ENZYMES

JEAN-MARC ENGASSER and CSABA HORVATH

Department of Engineering and Applied Science and Physical Science Section of School of Medicine, Yale University New Haven, Conn. 06520 (U.S.A.)

(Received December 27th, 1973)

SUMMARY

1. In heterogeneous systems conjugate acid base pairs, besides their conventionally accepted static role as buffers, can also play a dynamic role in facilitating proton transport. When protons are generated and consumed at different locations, the conjugate base binds the proton at the "source" and the resulting acid diffuses to the "sink".

2. Both the buffering capacity of an acid base pair and its ability to facilitate proton transport are maximum when its pK_A is close to the pH of the medium. The transport facilitating effect can be appreciable at much lower concentrations than those needed for buffering capacity.

3. The effect of proton transport has been theoretically investigated by using a model consisting of a H^+-producing enzymic surface reaction, which occur with bound enzymes both in vitro and in vivo.

4. The enhancement of proton transport is quantitatively expressed by the extent of transport facilitation, ξ. The interplay of facilitated proton diffusion and reaction kinetics is quantified by the proton modulus, μ.

5. At a given pK_A the magnitude of transport facilitation increases with the acid–base concentration and the pH of the solution. The pK_A value plays a dual role in determining both the concentration of available carriers and the affinity of the proton to the carrier.

6. Due to its high proton affinity OH^- can also facilitate proton transport already at pH 7, but in the presence of buffers its effect becomes significant only at higher pH values.

7. When the extent of transport facilitation sharply increases with the pH of the substrate solution, at relatively low buffer concentrations bound enzymes display sigmoidal pH profiles with sharp peaks. Previously reported experimental data with membrane bound enzymes can be interpreted in view of these theoretical findings.

8. It is postulated that buffers ubiquitous in biochemical and living systems can play an important role also by facilitating proton transport.

INTRODUCTION

Most biochemical and physiological experiments are carried out in buffered solutions. Buffers are also important components of living systems. So far the static role of buffers in maintaining the pH of the medium at a constant value has mainly been considered.

We suggest that in heterogeneous systems such as cells, membranes or bound enzymes, in which H^+ is generated, consumed or transported, buffers may also play a dynamic role in acting as carriers for the hydrogen ions, thus, facilitating their transport. This ability of buffer molecules to facilitate the molecular or convective diffusion of protons both in vitro and in vivo arises from their capability of binding H^+ reversibly. Although mediated transport by carriers is well established for many physiological processes (Davson and Danielli [1], Danielli [2], Wilbrandt and Rosenberg [3]) the role of ubiquitous buffering substances as proton carriers in the cellular milieu or in experiments involving heterogeneous systems has not yet been put forward.

Many enzymic reactions produce or consume H^+. When the enzyme is bound to membranes or solid matrices, the slowness of proton transport may limit the overall rate of the enzymic reaction and result in proton accumulation or depletion in the vicinity of the bound enzyme. Such a change in the catalyst microenvironment can greatly affect the enzyme activity, the membrane permeability or other properties of the system under consideration. Under these conditions the buffer-mediated transport of H^+ from or to the catalytic sites can attenuate the degree of proton accumulation or depletion, thus, causing changes in the system behavior.

Because of the complexities of the cellular milieu, the study of this particular effect in living systems would have been very difficult. On the other hand immobilized enzymes can serve as simple and convenient models to investigate this phenomenon when the activity of the enzyme is affected by small changes in the local pH and diffusion resistance. The kinetics of ester hydrolysis with immobilized enzymes has been studied by Goldman et al. [4] and they postulated that proton accumulation is responsible for profound changes in the catalytic behavior. In the following the effect of such proton accumulation in the absence and in the presence of buffers is theoretically investigated by using a surface reaction model. The results of this analysis, which are, at least qualitatively, also applicable to enzymes embedded in porous media, show that pH profiles previously reported in the literature for certain immobilized enzymes can be explained only by taking into account buffer facilitating proton transport.

MATHEMATICAL MODEL

List of symbols

A	dimensionless concentration of the base
$[A_0]$	total concentration of the acid–base pair
$[A^-]_b$	concentration of the base in the bulk solution
$[A^-]_s$	concentration of the base at the surface
$[AH]_b$	concentration of the acid–base pair in the bulk solution
$[AH]_s$	concentration of the acid–base pair at the surface
H	dimensionless H^+ concentration
H_b	dimensionless H^+ concentration in the bulk solution

H_s	dimensionless H^+ concentration at the surface
$[H^+]_b$	H^+ concentration in the bulk solution
$[H^+]$	H^+ concentration at the surface
h_A	transport coefficient for the base
h_{AH}	transport coefficient for the acid
h_H	proton-transport coefficient for unmediated diffusion
h_{HF}	facilitated proton-transport coefficient
K	dimensionless constant defined by Eqn 4
K_A	dissociation constant of the acid
K_1	kinetic parameter defined by Egn 1
K_2	kinetic parameter defined by Eqn 1
V	overall rate of enzymic surface reaction
V^*	kinetic parameter in Eqn 1

Greek letters

γ	dimensionless parameter defined in Eqn 12
γ'	dimensionless parameter defined in Eqn 13
μ	proton modulus
μ_F	proton modulus with facilitated proton transport
ξ	extent of transport facilitation
ξ_{OH}	extent of transport facilitation by OH

Multiphase enzyme kinetics in the absence of an acid–base pair

We assume that H^+ is generated in an irreversible reaction catalyzed by a surface-bound enzyme at the saturation rate. The actual pH profile of the enzyme is assumed to be bell-shaped and described mathematically by the following equation:

$$V = \frac{V^*}{1 + \frac{[H^+]_s}{K_1} + \frac{K_2}{[H^+]_s}} \tag{1}$$

where V is the overall rate of reaction, $[H^+]_s$ is the H^+ concentration at the surface, V^*, K_1 and K_2 are the appropriate kinetic parameters, which are independent of the H^+ concentration.

Due to the heterogeneous nature of the reaction, the H^+ concentration at the surface is larger than that in the bulk solution, $[H^+]_b$, because protons have to be transported away from the surface. Assuming that in the stirred solution the transport by diffusion and convection takes place uniformly over the surface, the rate of transport is expressed by the product of a proton transport coefficient, h_H, and the driving force given by the concentration difference: $[H^+]_s - [H^+]_b$. At steady state when the substrate and proton concentrations in the solution are maintained constant, the rate of the enzymic reaction generating H^+ is equal to the rate of H^+ transport away from the catalytic surface, so that

$$\frac{V^*}{1 + \frac{[H^+]_s}{K_1} + \frac{K_2}{[H^+]_s}} = h_H ([H^+]_s - [H^+]_b) \tag{2}$$

Thus, for any concentration of H^+ in the solution the H^+ concentration at the surface and the enzyme activity, V, can be calculated from Eqns 2 and 1 at given values of transport and kinetic parameters.

Multiphase enzyme kinetics in the presence of an acid–base pair

When a conjugate acid base pair is present in the solution, the base can reversibly bind a proton to yield the conjugate acid according to the following scheme:

$$A^- + H^+ \overset{K_A}{\rightleftharpoons} AH$$

where K_A is the acid dissociation constant. As the proton concentration is larger at the catalytic surface than in the bulk solution, the surface concentration of the acid $[AH]_s$, is larger than its bulk concentration, $[AH]_b$, while the surface concentration of the base, $[A^-]_s$, is smaller than its bulk concentration, $[A^-]_b$. The changing concentrations of the acid, base and H^+ in the solution adjacent to the surface are schematically illustrated in Fig. 1.

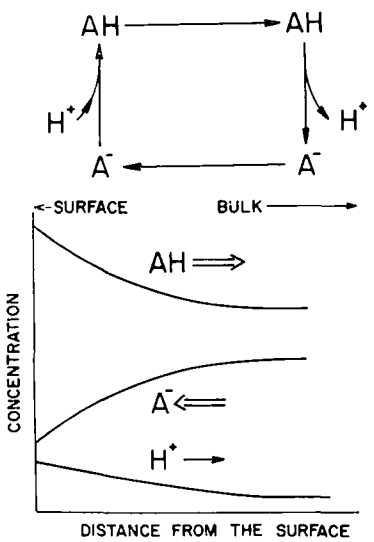

Fig. 1. Schematic illustration of the buffer-facilitated proton transport from the surface into the bulk solution and the concentration profiles of the species involved. Proton transport occurs both by diffusion of H^+ and by diffusion of AH.

Since the neutralization of A^- by H^+ and the dissociation of AH are usually very fast, the assumption of equilibrium between the three different species both at the surface and in the solution is conservative. Then we can write that

$$[A^-]_b\,[H^+]_b = K_A\,[AH]_b \tag{3}$$

$$[A^-]_s\,[H^+]_s = K_A\,[AH]_s \tag{4}$$

The H^+ generated at the surface can diffuse either freely or after reacting with A^-. In the latter case, the protons are carried by the buffer molecules into the bulk

solution. If H^+ is removed from the solution by a process such as neutralization or a consecutive enzyme reaction at the same rate as it is generated at the surface, the system is at steady state and $[H^+]_b$, $[A^-]_b$ and $[AH]_b$ are constant. Then the rate of H^+ generation must be equal to the total rate of proton transport, which is the sum of the transport rate of H^+ and that of AH. This is expressed by the following equation

$$\frac{V^*}{1 + \frac{[H^+]_s}{K_1} + \frac{K_2}{[H^+]_s}} = h_H ([H^+]_s - [H^+]_b) + h_{AH} ([AH]_s - [AH]_b) \quad (5)$$

where h_{AH} is the transport coefficient for the protonated base. Steady state also requires that the transport rate of A^- to the surface is equal to the rate of transport of AH into the bulk solution. Therefore, we can write that:

$$h_A ([A^-]_b - [A^-]_s) = h_{AH} ([AH]_s - [AH]_b) \quad (6)$$

where h_A is the mass transport coefficient for A^-.

From Eqns 3, 4, 5, 6 and 1 the H^+ concentration at the surface and thus the enzymic activity, V, can be calculated for different pH values in the bulk solution, assuming that the total acid–base concentration remains constant, i.e.

$$[A^-]_b + [AH]_b = [A_0] \quad (7)$$

where $[A_0]$ is the total acid–base concentration.

As shown in the Appendix the mathematical treatment is simplified by writing the equations in dimensionless form. The dimensionless parameters and variables thus obtained also facilitate the interpretation of the results of the numerical calculations presented in the following section. One of the dimensionless groups, μ, defined by

$$\mu = \frac{V^*}{h_H K_1} \quad (8)$$

is of particular importance. It is termed the proton modulus because its magnitude characterizes the interplay between the rates of proton generation and transport, therefore, expresses the relative importance of diffusion resistances for the proton.

THEORETICAL RESULTS

pH profiles without facilitated transport

When no proton-accepting species are involved in the transport of H^+ from the surface into the bulk solution, pH_s varies with pH_b at different values of μ as shown in Fig. 2. The magnitude of K_1 and K_2 is arbitrarily chosen 10^{-4} and 10^{-8}, respectively. As seen, the difference between the hydrogen ion concentration at the surface and in the bulk fluid, i.e., the magnitude of proton accumulation at the surface rapidly increases with increasing values of the proton modulus, μ.

The resulting pH profiles of the enzymic reaction are shown by plots of the normalized overall enzyme activity, V/V^*, against pH_b for different values of μ. Only for $\mu \leq 10^{-5}$ is the pH profile identical to that of the true enzymic reaction without diffusion limitations. At $\mu > 10^{-4}$, however, the enzyme activity plateaus at sufficiently

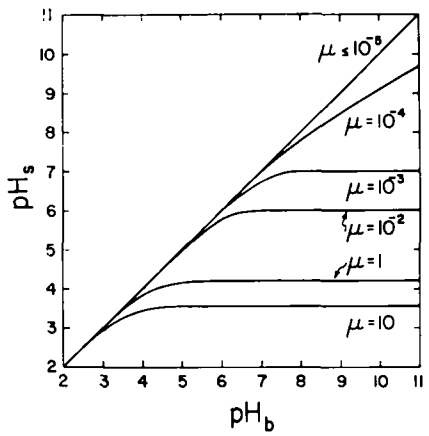

Fig. 2. Graph illustrating the pH at the surface, pH_s, for a proton-generating reaction, as a function of the pH of the bulk solution, pH_b, at different values of the proton modulus, μ, in the absence of proton carriers. When $\mu \leq 10^{-5}$ no proton accumulation occurs; at higher values of μ, i.e. with increasing diffusion resistance, the deviation from the diagonal illustrates the magnitude of proton accumulation at the surface.

high pH_b when the surface pH becomes independent of the bulk pH because of the accumulation of protons at the surface.

Accumulation of protons at the surface with facilitated transport

As it has been suggested in an earlier section the transport of hydrogen ions can be facilitated from the surface by proton-accepting species. In the model used for quantifying this effect we assume that the three transport coefficients involved, h_H, h_{AH} and h_A have equal values. This assumption can be justified despite significant

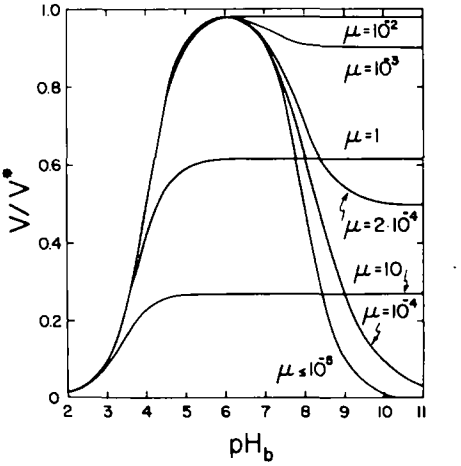

Fig. 3. pH profiles of a surface bound enzyme as a function of the pH of the bulk solution, pH_b, at different values of the proton modulus, μ. The curve for $\mu \leq 10^{-5}$ represents the pH profile of the enzyme without diffusion limitations.

differences between the diffusivities of H^+ and A^- individually. First, the transport coefficient is proportional to the corresponding diffusivity only if the transport takes place solely by molecular diffusion, but in a stirred system this dependence is mitigated (Levich [5]). Second, the transport of the two charged species H^+ and A does not take place independently due to the requirement of local electroneutrality. For instance, it has been shown that two species carrying the same number of charges have the same interdiffusion coefficient in the absence of electrical current (Helfferich [6]).

In Fig. 4 the pH at the surface is plotted as a function of pH_b in the presence of an acid–base pair having a pK_A value of 8 and a concentration of 10^{-2} M. When $\mu \geq 0.01$ protons accumulate at the surface when pH_b is low and pH_s tends to plateau

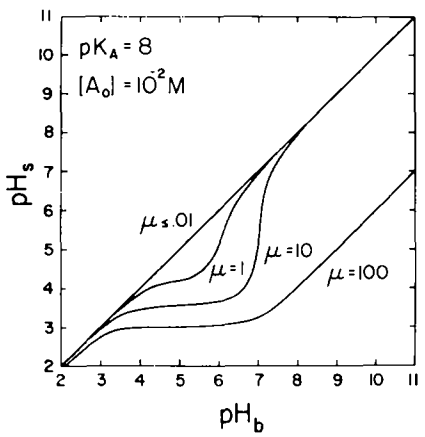

Fig. 4. Graph illustrating the pH at the surface, pH_s, for a proton generating reaction as a function of the pH of the bulk solution, pH_b, at different values of μ in the presence of 10^{-2} M acid–base pair having a pK_A value of 8. When $\mu < 10^{-2}$, no proton accumulation occurs; at higher values of μ, the deviations of the diagonal illustrates the magnitude of proton accumulation at the surface.

as in the absence of proton acceptor. The value of pH_s, however, does not remain constant but also increases when pH_b is further increased. This phenomenon can be explained as follows. At low pH, AH is practically undissociated so that the concentration of the proton acceptor, A^-, is too low to have an effect on proton transport. With increasing pH_b more and more carrier species are formed due to the dissociation of the weak acid so that the proton transport from the surface is increasingly facilitated. It is seen that for intermediate μ values, $\mu = 1$ and 10, pH_s increases faster than pH_b, thus, at high values of pH_b the concentration of A^- is sufficiently high to prevent any accumulation of H^+ at the surface.

pH profiles with facilitated proton transport

In view of these results the activity of the surface-bound enzyme is expected to be affected by proton acceptors in the solution if proton transport per se is relatively slow. Fig. 5 shows the dependence of the enzyme activity on the bulk pH at different μ values in the presence of a buffer having a pK_A value of 8 and a concentration of 10^{-2} M.

In this case the activity of the bound enzyme always decreases after reaching

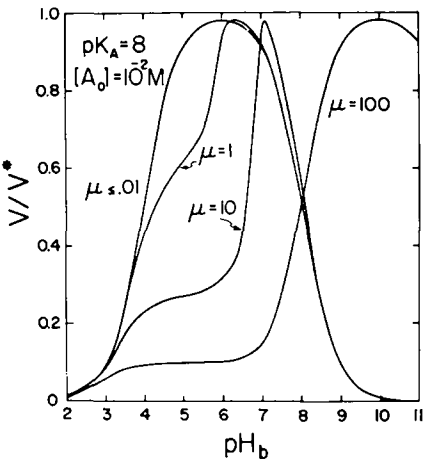

Fig. 5. pH profiles of a surface-bound enzyme as a function of the pH of the bulk solution, pH_b, at different values of μ in the presence of 10^{-2} M acid–base pair having a pK_A value of 8. The curve for $\mu \leq 10^{-2}$ represents the pH profile of the enzyme without diffusion limitations.

the maximum activity and vanishes at high enough bulk pH. On the other hand at sufficiently high μ the pH profile is S shaped before reaching the maximum. This dependence of the enzymic activity on the bulk pH is readily accounted for by the dynamic role of the acid–base pair. At low pH_b the concentration of A^- is too low to affect proton transport. As a result H^+ accumulates at the surface and the activity begins to plateau as in the absence of buffer. With increasing pH_b, however, the generation of A^- results in a sharp decrease in $[H^+]_s$, therefore, the enzymic activity sharply increases as shown in Fig. 5. The activity reaches its maximum value when $[H^+]_s$ equals 10^{-6} and then decreases with the further increase in pH_b.

The effect of the concentration and the pK_A of the buffer on the pH profile at $\mu = 1$ is shown in Fig. 6. Since A_0 and K_A determine the maximum possible and actually available concentration of the proton carrying species, A^-, respectively, both have a significant influence on the pH profiles.

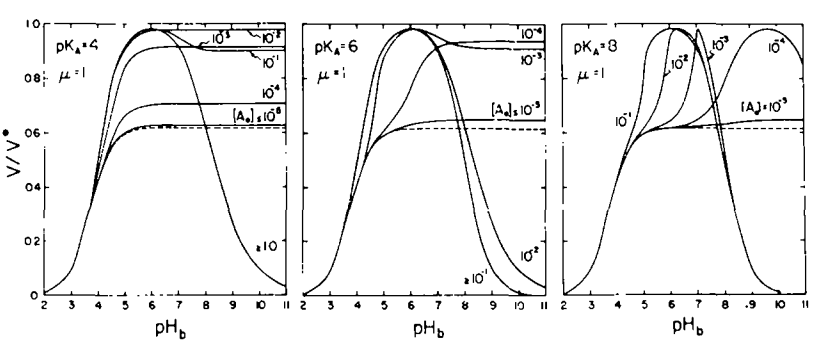

Fig. 6. Effect of the pK_A and the concentration of the buffer $[A_0]$ on the pH profile of a surface-bound enzyme at $\mu = 1$.

Quantitative interpretation of transport facilitation

The quantitative interpretation of the previously discussed results is possible by introducing a parameter, the extent of transport facilitation, ξ, which expresses the relative increase of proton transport in the presence of A . It is defined by

$$\xi \quad \frac{h_{AH}([AH]_s - [AH]_b)}{h_H([H^+]_s - [H^+]_b)} \tag{9}$$

Two limiting behavior of this function are readily derived

$$\xi \cdot \frac{h_{AH}}{h_H}\frac{A_0}{K_A} \qquad \text{at } pH_s \text{ and } pH_b \gg pK_A \tag{10}$$

$$\xi \cdot = \frac{h_{AH}}{h_{AH}}\frac{A_0 K_A}{[H^+]_b\,[H^+]_s} \qquad \text{at } pH_s \text{ and } pH_b \ll pK_A \tag{11}$$

In order to demonstrate the effect of A_0 and pK_A on ξ in a wide range of pH_b, the ξ values obtained by numerical calculation are plotted in Fig. 7 as a function of pH_b with A_0 and pK_A as the parameters. Of particular interest is the dependence of ξ on the pK_A of the buffer. At low pH_b a buffer having a low pK_A value and at high pH_b a buffer of high pK_A value is the most effective in facilitating proton transport. This behavior can be predicted from the limiting analytical expressions in Eqns 10 and 11, which indicate that ξ is proportional to K_A at small pH_b, but inversely proportional at high pH_b. In addition Fig. 7 and Eqn 10 illustrate that at high pH_b the extent of

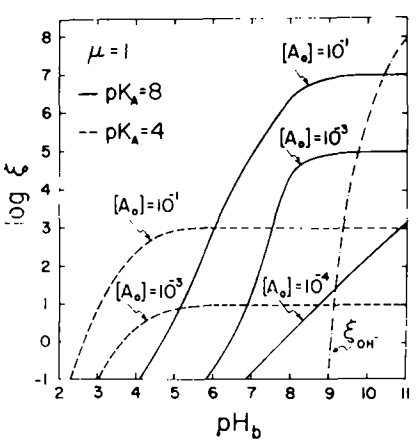

Fig. 7. Graph illustrating the dependence of the extent of transport facilitation, ξ, on the bulk pH at different molarities, $[A_0]$, and pK_A values of the buffer. The curves were obtained assuming that μ = 1. The dash-dot line shows the extent of transport facilitation by OH^-, ξ_{OH}.

facilitated transport can be significant even at very small concentrations of a buffer having a large pK_A. For instance a 10^{-4} M buffer of pK_A 8 yields a ξ value of 10^4 at high bulk pH.

In the presence of an acid base pair h_H is no longer the coefficient for the total transport of protons. The effective transport coefficient h_{HF}, which relates the total

rate of proton transport to the concentration difference of H^+, is defined by the following relation:

$$h_H([H^+]_s - [H^+]_b) + h_{AH}([AH]_s - [AH]_b) = h_{HF}([H^+]_s - [H^+]_b) \quad (12)$$

Then in view of Eqn 9 the coefficient for facilitated proton transport is given by

$$h_{HF} = h_H(1 + \xi) \quad (13)$$

It follows that the interaction between proton generation and facilitated proton transport is characterized by the effective proton modulus, μ_F, which, according to Eqns 8 and 13, is related to the modulus in the absence of proton carriers, μ, by

$$\mu_F = \frac{\mu}{1 - \xi} \quad (14)$$

Eqn 14 quantitatively expresses the fact that in heterogeneous system where protons are generated the transport facilitating effect of a proton acceptor manifests itself in a reduction of the diffusion resistance for protons by a factor $(1 + \xi)^{-1}$.

Facilitated proton transport by OH^-

Since OH^- ions bind protons reversibly and are always present in aqueous solutions they also have to be considered as proton carriers. When H^+ is generated at the surface, there is a flux of OH^- from the bulk solution to the surface where H^+ is neutralized, and the water diffuses away from the surface. The magnitude of facilitated transport by OH^- can be calculated by considering OH^- as the conjugate base of water whose concentration and K_A is taken as 55.5 M and $1.82 \cdot 10^{-16}$, respectively. The extent of transport facilitation by OH^-, ξ_{OH}, in this case also depends on the bulk pH as shown for $\mu = 1$ in Fig. 7. It is seen that ξ_{OH} is negligibly small below pH_b 9 but then rapidly increases with the pH and reaches a value of 10^6 at pH_b 10.

However, when the diffusion resistance is small, i.e. $H^+_b \approx H^-_s$, the value of ξ_{OH} can be significant also at lower pH values. It is seen from the limiting expression in Eqn 11 that when h_H and h_{OH} are assumed equal, ξ_{OH} is inversely proportional to $[H^+]^2_b$, and the maximum possible value of ξ_{OH} is 1, 10^2 and 10^4 at pH 7, 8 and 9, respectively. Therefore, even though the OH^- concentration is very small, proton transport can be greatly enhanced by a small increase in pH_b slightly above 7 because of the very high pK_A of water. Of course, when another proton acceptor is present, it participates together with OH^- in proton transport and their relative contributions can be estimated from the data presented in Fig. 7.

Optimum pK_A of the buffer for proton transport

The dependence of ξ on pK_A as shown in Fig. 7 as well as in Eqns 10 and 11 suggests that there is an optimum pK_A value for the acid–base pair which yields maximum ξ at a given pH_b. This is illustrated in Fig. 8 by the plots of ξ against pK_A for different pH_b values at $A_0 = 10^{-2}$ M and $\mu = 1$. It is seen that the extent of transport facilitation is the greatest when the pK_A of the buffer is the same as the pH of the solution. Calculations with μ values greater than unity have shown that the optimum pK_A slightly decreases with respect to pH_b, and this shift increases with μ.

These results suggest that not only the buffering capacity of a given acid–base

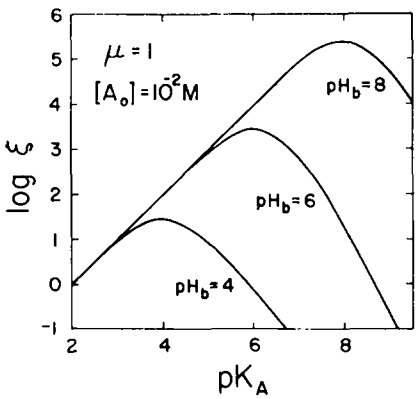

Fig. 8. Illustration of the effect of pK_A on the extent of transport facilitation, ξ, at different pH values in the bulk solution, pH_b. The buffer concentration, $[A_0]$, is 10^{-2} M and the modulus, μ, is unity.

pair but also its proton transporting facility is the greatest when the pK_A of the buffer is near the pH of the solution. This can be understood by the double role of the pK_A value of the buffer, which expresses both the propensity of the buffer to make anions available for proton transport and the resistance of hydrogen ions to combine with buffer anions. The optimum pK_A value represents a compromise between a desirable low pK_A to make a large fraction of the buffer available as carrier and a desirable high pK_A to provide a high affinity of H^+ to the carrier species at the surface.

DISCUSSION AND CONCLUSIONS

Comparison of the theoretical results with experimental data obtained with immobilized enzymes

Goldman et al. [4] and Silman [7] studied the kinetic behavior of papain immobilized on collodion membranes in the hydrolysis of *N*-benzoly-L-arginine ethylester (BAEE) and *N*-benzoyl-glycine ethylester (BGEE). One of the products in both reactions is H^+. Experiments were carried out in the absence as well as in the presence of 0.1 M phosphate and 0.4 M Tris buffer.

In the absence of such buffers the pH profile of the immobilized papain in BAEE hydrolysis was found to be "S-shaped, flattening out in the neutral pH region and then rising sharply at alkaline pH values up to and above pH 9.6" (Silman [7]). In the presence of buffer, however, the pH profiles of both the soluble and immobilized papain were bell shaped with the maximum activity near pH 6. The anomalous curves obtained with the immobilized enzyme in the absence of buffer have been explained by postulating "that the changed pH dependence resulted from a lowering of the local pH in the vicinity of the enzyme, caused by generation of hydrogen ions within the membrane by enzymic ester hydrolysis. Thus, as the external pH was raised, the increased rate of enzymic activity would cause a decrease in local pH which would tend to decrease the observed rate. This is a form of negative feedback and leads to the flattening of the pH activity curve in the neutral pH range" (Silman [7]).

According to the calculated curves shown in Fig. 3, the experimentally found flattening of the pH profile in the neutral region can indeed be explained by proton

accumulation in the microenvironment of the immobilized enzyme. Limitations in proton transport, however, cannot account for the observed sharp increase in activity between pH 7 and 9 if no buffer or other proton acceptor was present. In view of the theoretical curves shown in Fig. 6, however, the obtained S-shaped profile can be explained by the presence of $5 \cdot 10^{-3}$ M cysteine ($pK_A = 8.4$) and $2 \cdot 10^{-3}$ M EDTA (pK_A values = 6.2 and 10.3) in the reaction mixture. Despite their relatively low concentration, the dynamic role of these substances cannot be neglected in the absence of phosphate or Tris buffer. They can increase the effective proton transport coefficient by more than three orders of magnitude as seen in Fig. 7.

The pH profiles obtained in the hydrolysis of *N*-benzoyl-glycine ethylester (Goldman et al. [4]) at high buffer concentrations were bell shaped for both the immobilized and soluble papain with the peak at pH 7. On the other hand, in the "absence" of buffer the activity reaches a constant value between pH 4 and 6, increases sharply at pH values up to 8.5 then decreases rapidly with further increase of pH. This curve exhibits a plateau followed by a sharp peak at increasing pH_b as the pH-profiles which are shown in Figs 5 and 6 for a buffer of $pK_A = 8$. Therefore, the experimental results can again be explained by the transport facilitating effect of cysteine and EDTA.

The hydrolysis of acetylcholine by membrane bound acetylcholine esterase from *Electrophorus electricus* was studied by Silman and Karlin [8] in the absence and presence of added buffer. The results were similar to those obtained when *N*-benzoyl-L-arginine ethylester was hydrolyzed with immobilized papain. Again the shape of the curve in the "absence" of buffer can conveniently be explained by proton accumulation and the presence of proton acceptors such as EDTA, which was used in the preparation of the membrane.

Dynamic role of buffers in heterogeneous biochemical systems

Buffers have been used in biochemical experiments to maintain the pH constant. Other proton accepting species have also been frequently used for specific purposes. In heterogeneous mixtures, when proton transport is directly or indirectly involved, the presence of such compounds can have a drastic effect on the experimental results, as was previously illustrated by the pH profiles. Of course, the dynamic role of buffers is not restricted to enzymic reactions. Bishop and Richards [9] have reported that in the acid–base titration of crystalline β-lactoglobulin crosslinked with glutaraldehyde it took several hours to reach the endpoint. In these experiments the diffusion of H^+ in the insoluble protein particles was assumed to be the rate limiting step because the binding of proton to the various functional groups of the protein proceeds with a half time of a few microseconds. It has been found, however, that by adding a small amount of acetate to the heterogeneous mixture the time of equilibration was reduced to 2 or 3 min (F. Richards, private communication). Similarly rapid equilibration has been observed in studies by Rupley [10] on the ionization of the tyrosyl residues in methemoglobin crystals in strong buffer solutions. These findings suggest that buffer facilitated proton transport can have profound significance in reducing the time needed to achieve equilibrium when the rate of equilibration is limited by the diffusion of H^+.

Facilitated proton transport in living systems

Many metabolic processes produce H^+ by heterogeneous reactions in the cellular milieu, which are ultimately excreted, for example, via the lungs and the kidneys in the human body. It is well known that the pH of extra- and intracellular fluids is maintained constant by buffer systems despite the large metabolic acid production.

In our view, the buffering species can play a dual role also in living systems. The maintenance of constant pH in such heterogeneous media requires both high buffering capacity and efficient proton transport facility. Biological buffers consist of proton donors and acceptors with a pK_A close to the physiological pH, thus, possess both properties, which can explain their remarkable efficiency according to this study. It is possible that a smaller buffer concentration is required to keep the pH constant in a complex heterogeneous system such as a living organism due to buffer facilitated transport than that one would expect from taking only the buffering capacity into account. In the hypothetical limit of infinitely fast proton transport, no buffering capacity would be needed to maintain the pH constant at a steady state.

Proton acceptors participate at least in the following three transport processes: convective proton transport in the circulatory system, proton translocation through membranes and intracellular diffusion.

It has been established that buffers such as bicarbonates, phosphates, and hemoglobin serve not only to hold the pH of the blood plasma at 7.4 but also to carry protons from one part of the body to another and finally to excretion.

The movement of protons across cellular membranes is usually facilitated by proton acceptors which are lipid soluble. According to Mitchell's [11] chemiosmotic theory, this phenomenon could be responsible for the uncoupling of oxidative phosphorylation assumed to be driven by transmembrane proton gradients. The uncouplers are weak aromatic acids such as 2,4-dinitrophenol, which are most effective at pH values near their pK_A to carry protons through membranes (Hopfer et al. [12]).

The results of the theoretical analysis presented here are probably most applicable to proton transport in intracellular fluids which contain a large number of buffering substances such as phosphates and bicarbonates. Typical intracellular pH values are 6.8 and 7.0 in animals. According to this study, proton transport would occur predominantly by buffer carriers because their intracellular concentration is at least four orders of magnitude larger than that of H^-. Fig. 7 shows that at pH 7 about 100 times more protons are transported by a buffer of pK_A 7 at a concentration of 10^{-2} M than as H^+ even if they diffuse 100 times faster than the carrier species. This also supports the assumption that no significant proton gradients exist in the cytoplasm. It is intriguing to postulate that in view of the facilitated proton transport there is a net flow of both the protonated species from the intracellular fluid to the cell wall and the proton acceptors in the reverse direction. The significance of such shuttle mechanism in the cytoplasm, however, could only be assessed by determining the rate of intracellular proton generation as well as the nature and concentration of the individual carrier species.

APPENDIX

The H^+ concentration at the surface for different pH values in the solution has been calculated as follows.

In the absence of an acid–base pair

The dimensionless H^+ concentration, H, is defined as

$$H = \frac{[H^+]}{K_1} \tag{A-1}$$

Then according to Eqn 2, H_s is obtained by solving the following third order equation

$$H_s^3 + (1 - H_b)\, H_s^2 + (K - H_b - \mu)\, H_s - KH_b = 0 \tag{A-2}$$

where K is defined by

$$K = \frac{K_2}{K_1} \tag{A-3}$$

and μ, the proton modulus, is given by

$$\mu = \frac{V^*}{h_H\, K_1} \tag{A-4}$$

In the presence of an acid-base pair

In addition to the above defined dimensionless quantities, we introduce the dimensionless base concentration, A, defined as

$$A = \frac{[A^-]}{K_1} \tag{A-5}$$

Then, Eqns 3–7 yield the following fourth order equation for H_s

$$\begin{aligned} &\gamma H_s^4 + (1 + \gamma - \gamma H_b + \gamma' A_b)\, H_s^3 + (1 - \gamma\mu - H_b + \gamma K - \gamma H_b + \gamma' A_b \\ &- \gamma' A_b H_b)\, H_s^2 + (K - \mu - H_b - \gamma K H_b + \gamma' K A_b - \gamma' A_b H_b)\, H_s \\ &- (KH_b + \gamma' A_b H_b K) = 0 \end{aligned} \tag{A-6}$$

where

$$\gamma = \frac{h_{AH}}{h_A} \cdot \frac{K_1}{K_A} \tag{A-7}$$

$$\gamma' = \frac{h_{AH}}{h_H} \cdot \frac{K_1}{K_A} \tag{A-8}$$

Both Eqns A-2 and A-6 have been solved numerically by the Newton's iteration method to obtain H_s as a function of H_b and the pertinent parameters of the system.

ADDENDUM

Recently Gutknecht, J. and Tosteson, D.C. [13] demonstrated that the transport of salicilic acid from the bulk solution to a membrane could be greatly augmented by the addition of buffers. In our opinion the dynamic role of buffers is also applicable to buffer-facilitated transport of weak acids as well as that of weak bases since both involve proton transport as shown in a forth coming paper (Engasser, J.-M. and Horvath, C., submitted for publication)

ACKNOWLEDGEMENT

This study was supported in part by a Grant No. RR 00356 from the National Institutes of Health, U.S. Public Health Service. J.-M.E. is the recipient of a Yale University Fellowship.

REFERENCES

1 Davson, H. and Danielli, J. F. (1952) The Permeability of Natural Membranes, 2nd edn, Cambridge University Press, Cambridge and New York
2 Danielli, J. F. (1954) Proc. Symp. Colston Res. Soc. 7, 1–14
3 Wilbrandt, W. and Rosenberg, T. (1961) Pharmacol. Rev. 13, 109–183
4 Goldman, R., Kedem, O., Silman, H. T., Caplan, S. R. and Katchalski, E. (1968) Biochemistry 7, 486–500
5 Levich, V. G. (1962) Physicochemical Hydrodynamics, p. 39, Prentice-Hall, Englewood Cliffs
6 Helfferich, F. (1962) Ion Exchange, p. 269, McGraw-Hill, New York
7 Silman, H. T. (1969) J. Gen. Physiol. 54, 50s–57s
8 Silman, H. T. and Karlin, A. (1967) Proc. Natl. Acad. Sci. U.S. 58, 1664–1668
9 Bishop, W. H. and Richards, F. M. (1968) J. Mol. Biol. 33, 415–421
10 Rupley, J. A. (1964) Biochemistry 3, 1524–1530
11 Mitchell, P. (1961) Nature 191, 144–148
12 Hopfer, U., Lehninger, A. L. and Thompson, T. E. (1968) Proc. Natl. Acad. Sci. U.S. 59, 484–490
13 Gutknecht, J. and Tosteson, D. C. (1973) Science 182, 1258–1261