Summary

A simple and painless microtechnique for measuring interstitial-fluid pressure is described. We agree with Guyton that this pressure is normally negative. Dehydration and edema were studied in various animals by means of subcutaneous and peritoneal probes, and the hydrostatic compensation against tilting was studied in large snakes. Fluid pressure was followed in dehydrating muscles and electric organs; the measurements show an abrupt increase in tension when the water content reaches 70 to 80 percent. This increase is attributed to packing of the structural elements. These measurements were made as a sequel to similar studies of negative pressure in the drowned forest of the Amazon. They demonstrate that the parameters in the two systems are the same, but that the negative pressures in plants are some 10^4 times greater than those in animals.

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Facilitated Proton Transfer in Enzyme Catalysis

It may have a crucial role in determining the efficiency and specificity of enzymes.

Jui H. Wang

A question frequently asked concerning enzyme action is, When a substrate molecule is bound at the active site of an enzyme, is the susceptible bond already distorted or under strain so that it is rendered more reactive? Recent x-ray data on the lysozyme-tri-*N*-acetylglucosamine complex suggest that when a larger substrate is bound to this enzyme there may be considerable distortion in the susceptible section of the substrate molecule (*1*). On the other hand, infrared studies show that the CO_2 molecule bound at the active site of carbonic anhydrase is definitely not distorted or under appreciable strain (*2*). These observations show that, although the "strain theory" (*3*) might be applicable in some cases, it cannot be the general explanation of enzyme catalysis. There is also the alternative theory of enzyme action based on the activation entropy effect, according to which enzyme catalysis is only a special case of general acid-base catalysis, having the particular advantage that the activation step does not involve a large decrease in entropy since the responsible acid and base groups are already nearby. While this activation entropy effect is undoubtedly an important factor, it is generally believed that enzymes must have additional characteristics which enable them to carry out their remarkable function. In this article I suggest that facilitated proton transfer along rigidly held hydrogen bonds (*4*) may play a crucial role in determining the efficiency and specificity of many enzymes. For clarity, let us examine these possibilities by considering selected examples.

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Carbonic Anhydrase

Carbonic anhydrase contains one firmly bound zinc ion per enzyme molecule. It catalyzes the hydration of CO_2 to HCO_3^- and the reverse dehydration of HCO_3^-. Accurate difference infrared spectrometry shows that CO_2 bound at the active site of carbonic anhydrase exhibits an infrared absorption peak at wave number 2341 cm^{-1}, due to the asymmetric stretching of this linear molecule. Since this wave number is very close to the corresponding values for dissolved CO_2 (2343.5 cm^{-1} for CO_2 dissolved in water, 2340 cm^{-1} for CO_2 dissolved in methanol, and 2336 cm^{-1} for CO_2 dissolved in benzene) it has been concluded that the CO_2 at the active site is neither coordinated to the Zn(II) nor appreciably distorted, but is loosely bound to a hydrophobic surface or cavity of the protein, as in clathrate compounds (*2*). The infrared studies also show that the inhibitor azide ion is coordinated to the Zn(II) of the enzyme, and that the binding of a single azide ion at this Zn(II) prevents the binding of CO_2 at the specific CO_2 site mentioned above. Since the binding of inhibitors has not been observed to lead to gross conformational change in this enzyme (*5*), it was concluded that the specific CO_2 site must adjoin the Zn(II) so that the ligand azide can protrude at least partly into that site to interfere sterically with the binding of CO_2.

Nitrate and bicarbonate were found, in these infrared studies, to displace both the azide from the Zn(II) and the CO_2 from its specific binding site. But

since the specific CO_2 site is merely a hydrophobic surface or cavity which loosely binds nonpolar molecules as CO_2 or N_2O in preference to polar water molecules or ions, we must conclude that the HCO_3^- is coordinated to the Zn(II) through its negatively charged oxygen atom in such a way that its relatively neutral oxygen atom and OH group are placed at the specific CO_2 site, as illustrated by structure I in Fig. 1 (*2*).

These results show that, in the dehydration reaction represented by the lower arrow in Fig. 1, proton transfer must accompany the breaking of the C–O bond, since we already know that only CO_2 is to be left in the hydrophobic binding site. Conversely, because of microscopic reversibility, it must be the OH^- on the Zn(II) which attacks the bound CO_2 and converts the latter to HCO_3^- in the reverse hydration reaction represented by the upper arrow in Fig. 1.

Although the foregoing conclusions are consistent with earlier suggestions (*6*), with kinetic data (*7*), and with the results of recent titration (*8*) and fluorescence (*9*) studies, two important riddles regarding the catalytic mechanism remain unsolved.

1) The value of first-order rate constant k_2 for the enzyme-catalyzed hydration step in Fig. 1 is 4×10^5 sec^{-1} at *p*H 7 and 25°C (*7*); this is 10^7 times the rate of hydration of CO_2 in the absence of a catalyst. The observed bimolecular rate constant for the reaction of OH^- and CO_2 at 25°C is $\sim 8 \times 10^3$ sec^{-1} M^{-1} (*7*). Using this latter value, we may estimate the pseudo-first-order rate constant for a hypothetical system in which a given OH^- ion is placed next to a CO_2 molecule. The estimated pseudo-first-order rate constant is $\sim 8 \times 10^3$ $(10^3/10)$ $(2/4) = 4 \times 10^5$ sec^{-1}. Because of the numerical uncertainties in this estimate, the exact value may be debatable, but its order of magnitude is significant. That this estimated value is of the same order of magnitude as the observed k_2 for carbonic anhydrase is surprising indeed, because the free OH^-, with $K_a = 10^{-15.7}$ as the acid dissociation constant of its conjugate acid, is a much stronger base than the OH^- coordinated at the Zn(II) of carbonic anhydrase (*6*), with $K_a = 10^{-7.1}$. Although the ratio of the nucleophilic reactivities of free and coordinated OH^- need not be equal to the ratio of their K_a values, for very similar reacting groups one would not expect these ratios to differ very much in order of magnitude. In other words, the OH^- coordinated to the Zn(II) of carbonic anhydrase reacts faster by several orders of magnitude than one would expect on the basis of a pure activation entropy effect. Therefore, the enzyme must have additional means of expediting the reaction. But what are the additional means?

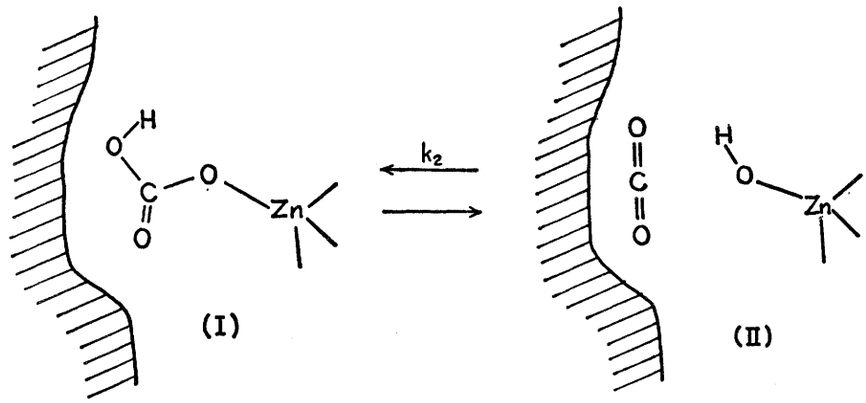

Fig. 1. Catalytic mechanism of carbonic anhydrase.

2) The foregoing conclusion that proton transfer must accompany C–O bond breaking or bond formation is a very vague one. Specifically, we want to know whether the proton transfer precedes, is concerted with, or immediately follows the breaking or formation of the C–O bond. Are all three processes of proton transfer—namely, pretransition-state proton transfer, concerted proton transfer, and posttransition-state proton transfer—important enough to be considered, and, if so, what is the path of transfer in each process?

Unfortunately, in spite of the in-

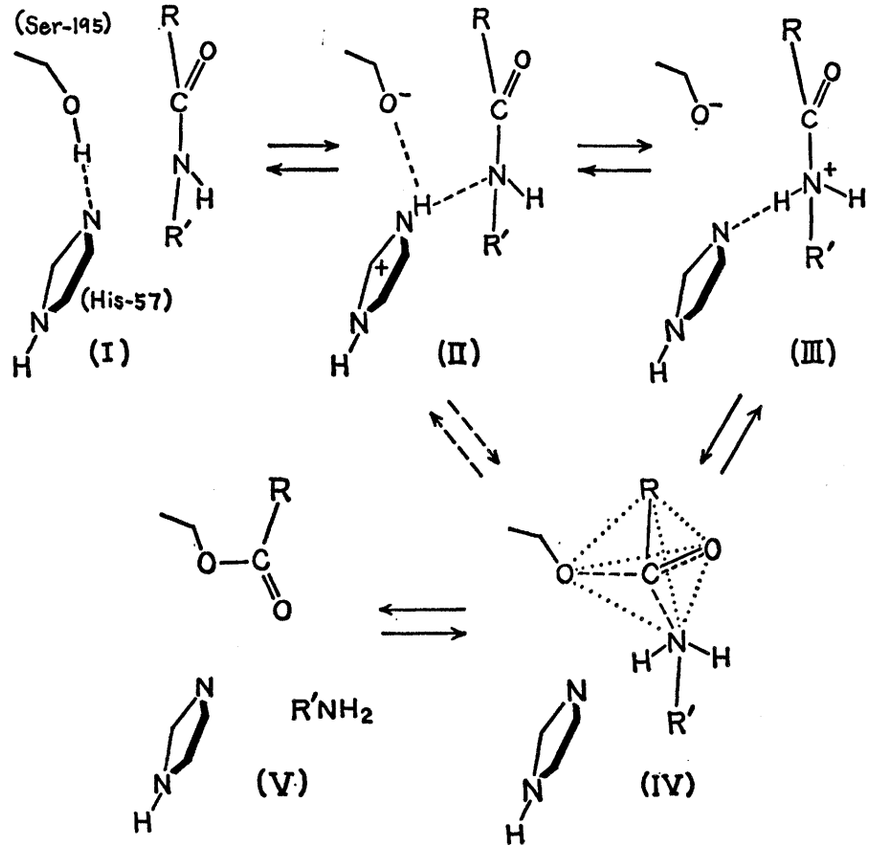

Fig. 2. Mechanism of the acylation step in the chymotrypsin-catalyzed hydrolysis of anilides or peptides.

teresting kinetic studies that have been made of carbonic anhydrase with other substrates (*10*), the experimental information seems still insufficient to allow us to reach a definite conclusion on these problems. On the other hand, the catalytic hydrolysis of a large number of substrates by α-chymotrypsin has been systematically and thoroughly investigated in many laboratories, under a variety of conditions. A careful examination of the existing data on chymotrypsin might, one would hope, throw some light on the nature of proton transfer in enzyme catalysis.

Chymotrypsin

α-Chymotrypsin catalyzes the hydrolysis of a large number of compounds according to the following scheme:

$$E + S \rightleftarrows ES \rightleftarrows \cdots\cdots \rightleftarrows E\text{–}\overset{\overset{O}{\|}}{C}\text{–}R + P_1 \rightleftarrows E + P_1 + P_2 \quad (1)$$

where E is the enzyme, S is the substrate, and ES is the enzyme-substrate complex, which transforms, through one or more steps, first to the acyl enzyme

$$E\text{–}\overset{\overset{O}{\|}}{C}\text{–}R$$

and splits off the product P_1, then splits off the second product, P_2, and regenerates the enzyme (*11*). Studies of amino acid sequences (*12*) show that in the acyl enzyme the acyl residue is attached to the OH group of serine-195 of α-chymotrypsin. Fast kinetic measurements suggest that this active serine may be hydrogen-bonded to a basic imidazole group (*13*). Recent x-ray data indicate probable hydrogen bonding between the active serine-195 and the basic imidazole group of histidine-57 (*14*).

In the hydrolysis of *p*-nitrophenyl esters and mixed acid anhydrides, the deacylation step—that is, the splitting off of P_2 and regeneration of the active enzyme—is rate-limiting. But in the hydrolysis of proteins, peptides, amides, anilides, and normal esters, the acylation step is rate-limiting. Let us first consider the chymotrypsin-catalyzed hydrolysis of the C–N bond, since presumably such catalysis is the principal function of chymotrypsin in nature.

Careful studies of the *p*H-dependence of the chymotrypsin-catalyzed hydrolysis of amides show that only one basic imidazole group is involved in the catalysis (*15*). On the other hand, the catalysis definitely involves protonation of the substrate, since the measured values of log k_2 bear a linear relationship to the values of pK_a of the protonated anilides, as predicted from the Hammett relationship $\log (K_a)_i/(K_a)_o = \rho\sigma$, where the parameter σ is characteristic only of the substituent and the parameter ρ is characteristic of the type of reaction under consideration (*16*). The only apparent way to reconcile these two sets of observations is to assume that the proton which is added to the nitrogen atom of the substrate came from the OH group of serine-195 via the nitrogen atom of histidine-57. A plausible path for this essential proton transfer is discussed below.

Let us assume that a good substrate RCONHR′ is bound to the enzyme in such a stereospecific way that the susceptible C–N bond of the substrate is placed in juxtaposition to the serine-histidine hydrogen bridge of the enzyme, and that the plane of the imidazole group of histidine-57 is roughly perpendicular to the plane determined by the basic nitrogen atom of the imidazole, the oxygen atom of serine-195, and the nitrogen atom of the substrate (*17*), as illustrated by structure I in Fig. 2. In view of the distribution of the outer σ- and π-electrons around the basic nitrogen atom of this imidazole, one expects structures I, II, and III in Fig. 2 to be in relatively fast protonation equilibrium via the electronic charge of this nitrogen atom. Complex III in Fig. 2 is expected to be highly reactive, since it contains an alkoxide group in juxtaposition to the carboxyl carbon atom of an already protonated anilide. Let us consider the rate of hydrolysis along the path

$$I \rightleftarrows II \rightleftarrows III \rightleftarrows IV \rightarrow V$$

in solutions where the concentration of the product $R'NH_2$ is negligible. To simplify the notation, let us define the first-order rate constants k_f, k_b, and k_a by the following equivalent reaction scheme

$$I \underset{k_b}{\overset{k_f}{\rightleftarrows}} III \xrightarrow{k_a} V \quad (2)$$

According to Eigen and his co-workers (*18*), the second-order rate constant for the recombination of H^+ and OH^- in ice at −10°C is 0.86×10^{13} sec^{-1} M^{-1}; this is 70 times as fast as the corresponding process in water at 25°C. From this value we estimate the pseudo-first-order rate constant for the transfer of an excess proton of a hydronium ion in ice to a given water molecule among its four nearest neighbors to be (0.86×10^{13}) (55.5) (0.9) (1/4) (3/4), or $\approx 10^{14}$ sec^{-1}. This unusually high value is, according to Eigen and DeMaeyer (*4*), due to facilitated proton transfer along rigidly held H bonds in ice. In fact, for a hydronium ion held next to a hydroxide ion in an ice lattice, we may, in view of the absence of any restoring force, expect the rate of proton transfer from the former to the latter to be even faster. Since the thermodynamically favorable transition

$$III \xrightarrow{k_b} I$$

also represents proton transfers along preformed H bonds, we may infer that k_b is of the same order of magnitude. Making use of the relationship

$$\frac{k_f}{k_b} = \frac{(K_a)_{Ser}}{(K_a)_{SH^+}} \quad (3)$$

where $(K_a)_{Ser}$ and $(K_a)_{SH^+}$ represent the acid dissociation constants of serine-195 and the protonated substrate, respectively, we obtain

$$k_f \approx k_b\,(K_a)_{Ser}/(K_a)_{SH^+} \approx 10^{14}\,(10^{-13})/(10^{0.5}) \approx 10^{0.5} > 1 \text{ sec}^{-1}$$

if we choose a typical anilide with $pK_a \approx -0.5$ as the substrate. Since this value of k_f is larger than the measured values of k_2 for most anilides (*16, 17*), the proton-transfer

$$I \xrightarrow{k_f} III$$

cannot be the rate-limiting step in the above reaction scheme. Consequently it is justifiable to treat the catalytic hydrolysis of these substrates approximately as a pretransition-state protonation problem and to calculate the relative rates of hydrolysis of structurally similar anilides as follows (*17*):

$$\frac{[III]}{[I]} = \frac{k_f}{k_b + k_a} \approx \frac{k_f}{k_b} \quad (4)$$

$$\text{Rate} = k_2[I] = k_a[III] \approx k_a\,(k_f/k_b)\,[I]$$

or

$$k_2 \approx k_a\,(K_a)_{Ser}/(K_a)_{SH^+} \quad (5)$$

For the enzyme discussed above, $(K_a)_{Ser}$ is a constant, and for a series of structurally very similar substrates, k_a may be assumed to be approximately constant. Therefore Eq. 5 gives

$$\log (k_2)_t - \log (k_2)_o = (pK_a)_t - (pK_a)_o = \rho(\sigma_t - \sigma_o) \quad (6)$$

for two substrates represented by the subscripts i and o, respectively. This result is entirely consistent with experimental data on the chymotrypsin-catalyzed hydrolysis of acetyl-L-tyrosyl anilides (*16, 17, 19*).

In addition, by combining the kinetic data for the hydrolysis of a series of acetyl-L-tyrosyl anilides in H_2O mixtures with the titration data for the corresponding acetanilides in protonated and deuterated mixtures, respectively, it is possible, from the treatment presented in the foregoing paragraph, to predict the k_2 values for the corresponding acetyl-L-tyrosyl anilides in D_2O mixtures. The predicted values are, within the limits of experimental uncertainty, in agreement with the directly measured values (*19*). These results suggest that for most anilides, and also for amides and peptides, which are even stronger bases, treatment as essentially a pretransition-state protonation problem provides an adequate description of the catalytic process, and that consequently for these substrates the transformation path

$$\text{I} \rightleftarrows \text{II} \rightleftarrows \text{IV} \rightarrow \text{V}$$

in Fig. 2 may be neglected in this treatment.

This approximation breaks down in the case of benzoyl-L-tyrosyl-nitroanilides, where not only is the substrate a much weaker base but the strong electron-withdrawing property of the p-NO_2 group weakens the susceptible C–N bond by decreasing its partial double bond character; this results in a much higher value of observed k_2 (*20*), so that Eqs. 4 and 5 are no longer valid.

For the chymotrypsin-catalyzed hydrolysis of esters the situation becomes even more unfavorable to treatment as pretransition-state protonation, both because esters are weaker bases and because the susceptible C–O bonds are also more labile than the corresponding C–N bonds. Therefore, for the hydrolysis of alkyl esters, the direct transformation of II to IV through the concerted and posttransition-state protonation mechanisms illustrated in Fig. 2 becomes more important.

The general catalytic scheme for α-chymotrypsin is summarized in Fig. 3, where the general substrate is represented by RCOXR′. For the hydrolysis of p-nitrophenyl esters, XR′ = $OC_6H_4NO_2$, no protonation of substrate is necessary, since at pH > 7 the resulting p-nitrophenylate ion, p-$NO_2C_6H_4O^-$,

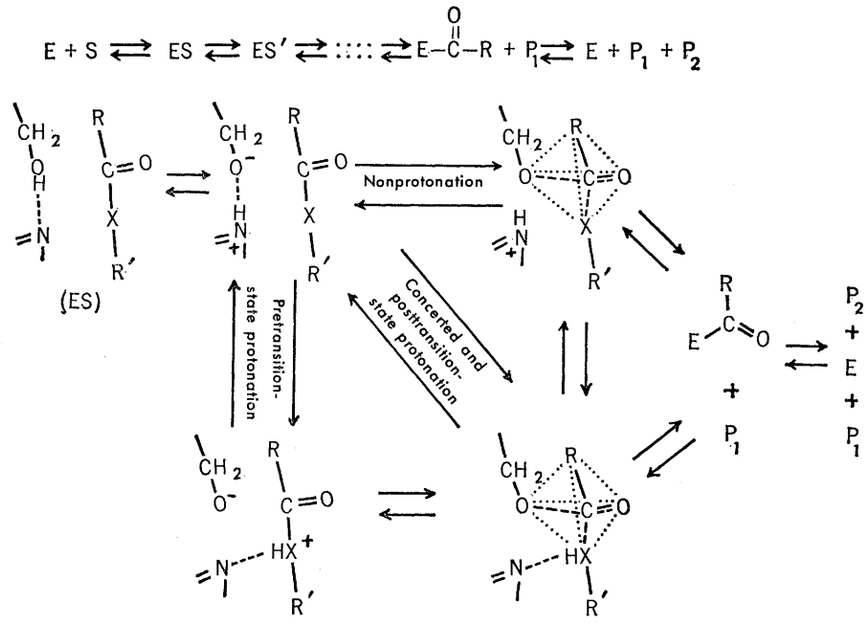

Fig. 3. General reaction scheme for chymotrypsin catalysis.

might not even be protonated in solution. Consequently the hydrolysis can take place rapidly through the upper, nonprotonation path. In fact, for this case the acylation step becomes so rapid that it is no longer rate-limiting (*11*). Nevertheless, in all three of the reaction paths of Fig. 3, facilitated proton transfer along the rigidly held H bonds always occurs during the transformation of the enzyme-substrate complex I to the active-form complex II. From there on the paths differ according to the nature of the substrate.

Specificity

The proposed mechanism also suggests an attractive interpretation of the unsettled question of substrate specificity of enzymes (*21*). In the case of α-chymotrypsin, the substrates which are most efficient are presumably those in which the specificity group R in Fig. 2—for example, L-phenylalanyl, L-tyrosyl, or L-tryptophanyl residue—interacts with the enzyme and stabilizes the H bonds crucial for facilitated proton transfer. By analogy, R in Fig. 2 may also be taken to represent the L-arginyl or L-lysyl residue of the efficient substrate in tryptic hydrolysis, presumably via the same catalytic mechanism (*22*).

Recently two groups of workers have independently succeeded in replacing the active serine residue of subtilopeptidase A (subtilisin) by an SH group without either configurational or conformational change (*23*). The "chemical mutant" so obtained has been christened thiol-subtilisin. It is one-third as active as subtilisin in catalyzing the hydrolysis of p-nitrophenyl acetate (NPA), almost inert toward acetyl-L-tyrosine ethyl ester, and completely inert toward natural proteins. By contrast, subtilisin hydrolyzes proteins 10^2 to 10^3 times as fast as it hydrolyzes NPA, and hydrolyzes acetyl-L-tyrosine ethyl ester 3 to 4 times as fast as NPA (*23*).

This surprising observation cannot be reconciled with the usual activation entropy theory of general acid-base or "push-pull" catalysis. While the concentration of –SH and –OH groups at the respective active sites of these two proteins, subtilisin and thiol-subtilisin, cannot be very different, the reactivities should greatly favor thiol-subtilisin. This should be the case since not only does the mercaptide ion have a nucleophilic action 10^2 to 10^3 times as fast as that of the alkoxide ion but, at pH 7 to 8, the concentration of RS^- is higher than that of RO^- by a factor of $(10^{-9})/(10^{-13})$, or $\sim 10^4$. Thus, on the basis of the activation entropy theory we would expect thiol-subtilisin to be 10^6 to 10^7 times as active as subtilisin—an expectation in complete disagreement with the facts!

On the other hand, if we assume that subtilisin catalyzes through a facilitated proton transfer mechanism similar to that depicted in Fig. 2 for chymotrypsin, we would expect the

replacement of an oxygen atom (van der Waals diameter, 2.80 angstroms; covalent diameter, 1.32 angstroms; single bond angle, 105 degrees) by a sulfur atom (van der Waals diameter, 3.70 angstroms; covalent diameter, 2.08 angstroms; single bond angle, 92 degrees) at the active center to disrupt the rigidly and accurately held H bonds in the natural enzyme-substrate complex which are required for facilitated proton transfer from the active OH group to the susceptible nitrogen atom of the substrate. For a substrate such as NPA, which does not need protonation, the effect of replacing the active OH group in subtilisin by an SH group may not be very pronounced, since at *p*H 7 to 8 the equilibrium concentration of the very reactive mercaptide group may be high enough to catalyze the hydrolysis of the substrate at a moderate rate. But for normal alkyl esters, and particularly for proteins where protonation of the leaving moiety of the substrate is essential, such a disruption of the crucial H bonds could completely wipe out facilitated proton transfer, with the consequence that the enzyme-substrate complex I (see Fig. 2) cannot reach its transition state IV fast enough, even though the free energy of IV in the modified system may not be very different from its free energy in the natural system.

Ribonuclease

Recent x-ray results on ribonuclease A (*24*) and ribonuclease S (*25*), in combination with the data on amino acid sequence (*26*), generated a three-dimensional structure for ribonuclease which is consistent with most of the chemically deduced structural information (*27*). In particular, the structure shows that histidine-12 and histidine-119 are both very close to the active site. With this information, it is possible to construct a catalytic mechanism based similarly on facilitated proton transfer, as illustrated in Fig. 4.

The mechanism for the cyclization step in ribonuclease catalysis, as illustrated in Fig. 4, is quite similar to the mechanisms previously suggested by workers in several laboratories (*28*), except with respect to the following:

1) The basic histidine residue and the 2′-OH group in Fig. 4 are assumed to play roles in ribonuclease catalysis similar to those of histidine-57 and serine-195, respectively, in chymotrypsin catalysis, and facilitated proton transfer can take place as illustrated in the transformations I ⇄ II, II ⇄ III, III ⇄ IV, and possibly IV ⇄ V.

2) The initially protonated nitrogen atom of another histidine or lysine side chain is assumed to be hydrogen-bonded to a negatively charged oxygen atom to form a salt linkage, not to the neutral oxygen atom attached to the next nucleotide R. This assumption is supported by the observations that substrates stabilize the enzyme against denaturation, whereas triesters of phosphoric acid are not affected by the enzyme (*27*).

3) The intermediates III and IV are included in Fig. 4 to make the catalysis consistent with the elegant results obtained by Westheimer's group on the hydrolysis of small phosphates and phostonates (*29*). Protonation is assumed to assist the recipient oxygen atom to reach an apical position in the trigonal-bipyramidal configuration III, and deprotonation returns it to the equatorial position, in IV.

Figure 4 is otherwise self-explanatory. To find the mechanism of the decyclization step, all one need do is replace the HOR in configuration V by H_2O and follow the reaction backward.

Ribonuclease is known to catalyze specifically the formation and breakdown of the 2′ O–P bond of ribose residues with a pyrimidine group (*Py* in Fig. 4) attached to it. In chymotrypsin catalysis, the essential specificity group (the phenyl, 4′-hydroxyphenyl, or 3′-indolyl group of the susceptible L-amino acid residue) is three atoms away from the newly formed C–O bond in the acyl-enzyme intermediate. Similarly, for ribonuclease, the pyrimidine group is three atoms away from the newly formed P–O bond in the cyclic intermediate. If the function of the specificity group in chymotrypsin catalysis is to stabilize the crucial H bonds by secondary interactions with the enzyme, one may wonder if the pyrimidine group in ribonuclease catalysis plays a similar crucial supporting role without itself participating directly in the chemical reaction.

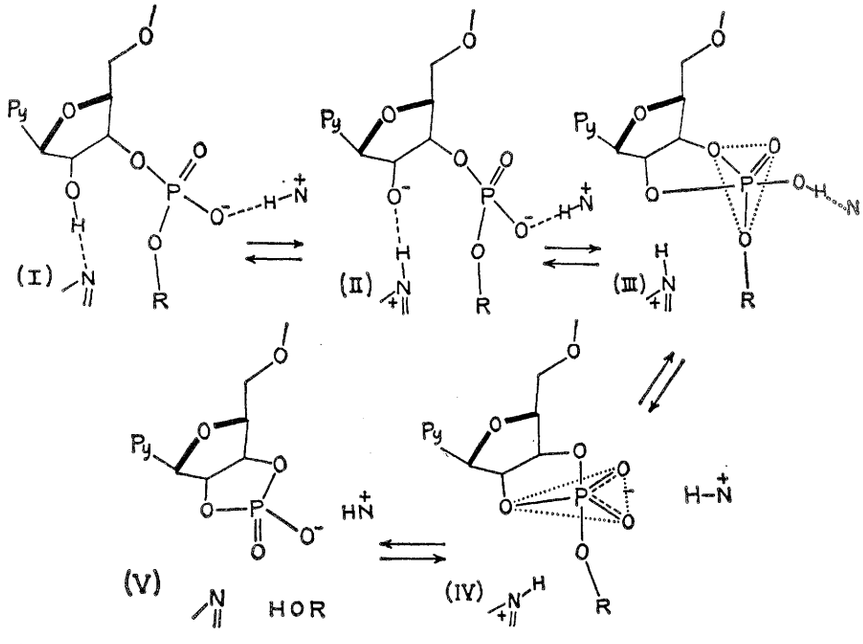

Fig. 4. Cyclization step in ribonuclease catalysis. The dotted triangles represent equatorial planes in the trigonal-bipyramidal conformation. *Py* represents a pyrimidine group. The tertiary nitrogen atom represents the basic nitrogen atom of the imidazole group of a histidine residue. The other nitrogen atom belongs either to another histidine residue or to a lysine residue.

Alcohol Dehydrogenase

Alcohol dehydrogenases of both yeast and liver are zinc enzymes which catalyze the following reaction (*30*) in a stereospecific manner (*31*):

$$CH_3CHO + NADH + H^+ \rightleftarrows CH_3CH_2OH + NAD^+ \quad (7)$$

The competition of imidazole with substrates for binding by the enzyme suggests that the substrates may be bound to the Zn(II). The displacement of

bound NAD^+ by *p*-mercuribenzoate and the protection of the enzyme from iodoacetamide by NAD^+ suggest the presence of a sulfhydryl group in close proximity to the bound NAD^+ (*30*).

Since, in media of low dielectric constant, 1-methyl-nicotinamide and donor anions are known to form stable ion pairs with characteristic charge-transfer spectra (*32*), one would expect that the binding of NAD^+ by alcohol dehydrogenase might result in the formation of a similar ion pair involving the positively charged nitrogen atom of NAD^+ and a negatively charged sulfur atom of the adjacent mercaptide group of the enzyme. If one assumes that the bound ethanol is coordinated to the Zn(II), with one of the hydrogen atoms of its C-1 atom oriented for stereospecific transfer to the C-4 atom in the nicotinamide ring of the coenzyme (*31*) and with its methyl group still free to rotate (*33*), one arrives at the arrangement illustrated by structure I in Fig. 5.

In structure I of Fig. 5, the OH group of the bound ethanol is shown hydrogen-bonded to the mercaptide group of the protein. Facilitated proton transfer along this H bond brings structure I into rapid equilibrium with structure II. Structure II should be thermodynamically very unstable, because of its net positive charge immersed in a medium of relatively low dielectric constant, and hence tends to decrease, through inductive effect, the electron density at the C-4 atom, as indicated by the arrows. Meanwhile the activated oxygen atom of the alkoxide ion in II tends to form an additional bond with its own carbon atom, and thereby to weaken the adjacent C–H bond. These two effects in II could effectively cooperate to assist the hydride ion transfer which changes II to III. Conversely, in the reverse hydrogenation reaction, III could change to II by hydride ion transfer and then transform to I by facilitated proton transfer.

Since the hydride and proton transfers illustrated in Fig. 5 are linked processes (*34*), there is a reciprocal relation between them. In other words, neither process has to precede the other. Consequently, the reaction path I $\rightleftarrows$ IV $\rightleftarrows$ III shown in Fig. 5 should be just as satisfactory as the path I $\rightleftarrows$ II $\rightleftarrows$ III.

A similar mechanism may be suggested for L-glutamate dehydrogenase, in which the NH_2 group of the substrate replaces the OH group of ethanol in Fig. 5. In addition, the observed esterase activity of D-glyceraldehyde-3-phosphate dehydrogenase after the removal of NAD^+ (*35*) and the identification of the active SH group in this enzyme by means of radioactive labels (*36*) are also consistent with the present interpretation. These observations suggest that dehydrogenase may catalyze through a mechanism which has much in common with the mechanisms for hydrases and hydrolytic enzymes.

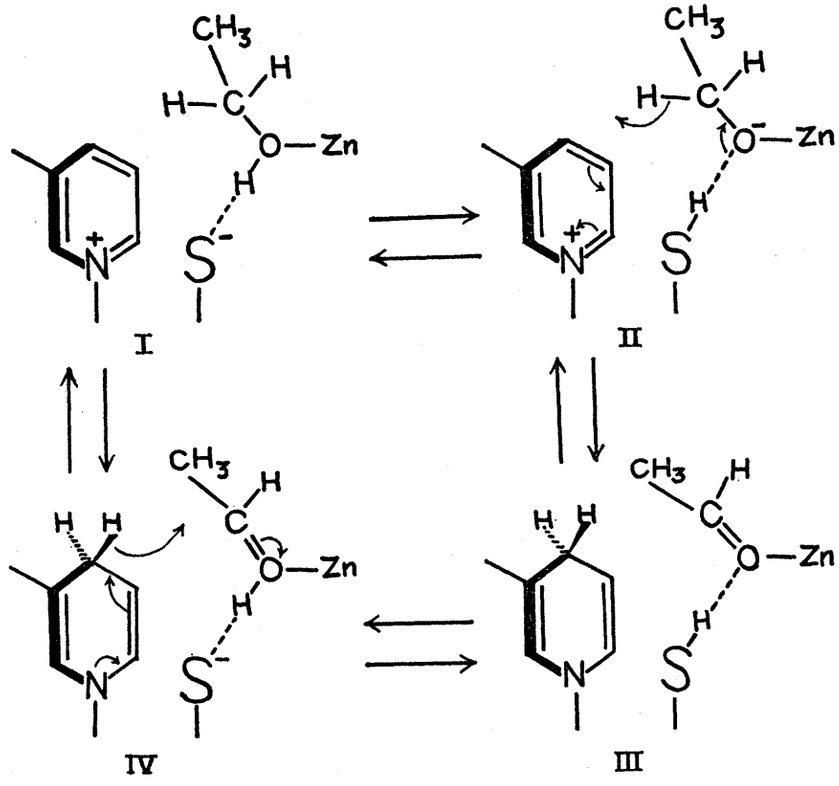

Fig. 5. Possible catalytic mechanism for alcohol dehydrogenase.

Conclusion

Ever since Fischer first developed his famous "lock and key" analogy (*37*), the secret of enzyme action has posed an intellectual challenge to scientists in several fields. The crux of this problem may not be resolved for some time, but any attempt to seek a broad catalytic principle which transcends the idiosyncracies of individual enzymes is likely to accelerate our rate of progress toward the truth. Two of the most fruitful examples of such attempts are the activation entropy theory inspired by the work of Swain and Brown (*38*) and the strain theory of Quastel (*3*), which has recently been revived by the exciting work of Phillips' group (*1*).

As an additional attempt, the present article proposes that facilitated proton transfer along rigidly and accurately held H bonds in the enzyme-substrate complexes, similar to the transfer mechanism in ice, discovered by Eigen and his co-workers (*4, 18*), may play a crucial role in enzyme catalysis. In other words, enzymes catalyze not only by lowering the free energy of the transition state (or states), as assumed in all previous theories, but also by enabling the system to reach the transition-state (or states) faster through facilitated proton transfer along strategically fixed H bonds. The available evidence concerning carbonic anhydrase, α-chymotrypsin, trypsin, thiolsubtilisin, ribonuclease, and alcohol dehydrogenases seems to support the proposed mechanism, although decisive information is still wanting. If the present interpretation proves to be correct, it will give us a new dimension for understanding the efficiency and specificity of enzyme action and even more appreciation of the advantage of using proteins to make enzymes.

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Natural Environment of Early Food Production North of Mesopotamia

Climatic change 11,000 years ago may have set the stage for primitive farming in the Zagros Mountains.

H. E. Wright, Jr.

In recent years the pace and detail of archeological exploration in the Near East have been so great that the chronology and locale of domestication of plants and animals are becoming fairly well established. At the same time, from paleoecological studies we are learning more about the environmental changes during this critical period in man's cultural evolution. A major factor in both these developments is the growing inventory of radiocarbon dates, without which the necessary refinement of the chronology would be impossible. With this increased body of facts and carefully controlled inferences, the question of climatic determinism—the effects of climatic change on cultural development—deserves reexamination.

Cultural Sequence

A compilation of radiocarbon dates from Near Eastern prehistoric sites (*1*) provides a useful chronological listing of the cultural stages of the transitional period from food-collecting to food-producing (Table 1), and other summaries discuss the regional developments (*2*).

Before the phase of terminal food-collecting, cultural evolution was slow, and the radiocarbon chronology is less well established. The Zarzian stage of terminal food-collecting was preceded by the Baradostian, comparable in general technological level to the earlier part of the Upper Paleolithic of Europe. The Baradostian at Shanidar Cave in the Zagros Mountains of northeastern Iraq (Fig. 1) dates from 26,500 to $>$ 34,000 years old, and Solecki (*3*) reports a stratigraphic unconformity between this level and the Zarzian level above—supposedly resulting from absence of occupation of the site for at least 15,000 years. However, more recent work in nearby Iran by Howe at Warwasi and by Smith at Ghar-i-Kar suggests that the Zarzian developed directly out of the Baradostian without any cultural hiatus (*4*).

The types of subsistence can be partially reconstructed from artifact types and from the remains of plants

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