Proceedings of the National Academy of Sciences
Vol. 66, No. 3, pp. 874–881, July 1970

Directional Character of Proton Transfer in Enzyme Catalysis*

Jui H. Wang

KLINE CHEMISTRY LABORATORY, YALE UNIVERSITY, NEW HAVEN, CONNECTICUT

Communicated by Joseph S. Fruton, April 22, 1970

Abstract. Hydrogen bonding can facilitate proton transfer in certain directions and retard proton transfer in certain other directions. By assuming that directed proton transfer along strategically oriented hydrogen bonds in the enzyme-substrate complex plays an important role in determining the efficiency and specificity of the enzyme, we present a unified interpretation for the reported observations on carbonic anhydrase and α-chymotrypsin.

Introduction. It is generally agreed that enzyme catalysis owes its efficiency and specificity to the way in which the enzyme and its substrate fit each other. But how do we define fitness in precise terms and explain the enhancement in reaction rate? Here the opinions differ.

According to the proximity theory,[1] the main advantage of enzyme catalysis is that the reacting groups are already held together in the enzyme-substrate complex and consequently the activation step does not involve as large an entropy decrease as in most nonenzymatic reactions. When the rate-limiting step of a reaction in aqueous solution involves the attack at a hydrophobic group by a charged and hence strongly hydrated nucleophile, the activation energy also includes the free energy needed for partially dehydrating the nucleophile.[2,3] In such cases the observed proximity effect can be several orders of magnitude larger than that computed from the change in translational entropy of the reacting groups.[4] According to the *strain* theory,[5] when a substrate is bound at the active site in the enzyme-substrate complex, the susceptible bond is already distorted or under strain so that it is rendered more reactive. Observations on lysozyme[6] and a model catalyst[7] seem to support this possibility. On the other hand, many investigators feel that the chance of successfully developing a theory of enzyme action based entirely upon *proximity* and *strain* is rather slim. For example, in their study of the pepsin-catalyzed hydrolysis of synthetic peptides, Fruton and co-workers[8] found that the values of Michaelis constant (K_m) for the substrates Z-His-Phe-Phe-OEt, Z-His-Phe-Phe-NH_2, Z-Gly-His-Phe-Phe-OEt, Z-His-Gly-Phe-Phe-OEt, and Bz-Lys-Phe-Phe-OEt are approximately the same, but that the values of k_{cat} may differ by a factor of 50. The proximity factor in the pepsin-catalyzed hydrolysis of these substrates must be essentially the same in each case, since all five substrates have approximately the same value of K_m = 0.4 ± 0.15 mM. If we attempt to account for the kinetic data in terms of *strain*, we have to assume that the substrate with the largest k_{cat} is under the greatest

strain. But since the free energy required for straining the susceptible bond came from enzyme-substrate interaction, we must assume that the original interaction energies are such that after subtracting the widely different strain energies, exactly the same net amount, within ± 0.3 Kcal mol^{-1}, is left in each case so that they all have the same K_m value—a highly improbable assumption! Furthermore, if we measure the catalytic efficiency of an enzyme at very dilute substrate concentrations by k_{cat}/K_m, then the effects of *proximity* and *strain* tend to cancel each other. This is because if the free energy for straining substrate i is ΔG_i, then it will increase both k_{cat} and K_m by approximately the same factor, $\exp[\Delta G_i/(RT)]$.

A third important factor is *orientation*.[9] In the hypothetical case that the productive orientation of each of the n crucial chemical bonds of a reacting system spans 1% of a 4π solid angle, then the reaction rate for the whole system held at the right orientations could be $\sim 10^{2n}$ times faster than at random orientations. As an eminent example, we suggested in 1967 that facilitated proton transfer[10,11] along strategically oriented hydrogen bonds in the enzyme-substrate complex may play an important role in enzyme catalysis. Since many relevant new results have been reported by others during the last 2 yr, it now seems worthwhile to reexamine this hypothesis more critically.

Directed Proton Transfer in Aqueous Systems. Eigen and co-workers[12,13] discovered that in ice, where all the H_2O molecules are tetrahedrally hydrogen bonded to their neighbors, proton transfer can take place rapidly and extensively without molecular reorientation. The observed mobility of proton in ice at $-10°C$ as a result of *directed proton transfer* under optimum conditions is 70 times as high as that in water at 25°C. According to Eigen and co-workers,[13] the second-order rate constant (k_R) for the recombination reaction $H_3O^+ + OH^- \xrightarrow{k_R} 2H_2O$ in ice at $-10°C$ is 0.86×10^{13} M^{-1} sec^{-1}. It is of interest to note that in dilute systems k_R is really a measure of the average rate of proton transfer from H_3O^+ to H_2O and from H_2O to OH^-, respectively, since once the H_3O^+ and OH^- are together, the transfer of proton from H_3O^+ to OH^- should be even faster and hence cannot be the rate-limiting step. For this reason, the pseudo-first-order rate constant k_1' for a H_3O^+ ion to transfer a proton to anyone of its neighboring H_2O molecules should be related to k_R by the approximate relationship $k_1' \approx [H_2O]\, k_R$. Using $[H_2O] = 50$ M for ice, we obtain $k_1' \approx 4 \times 10^{14}$ sec^{-1}.

In the opposite case where molecules are hydrogen bonded in an unfavorable direction, extremely slow proton transfer rates may result. For example, Blout and co-workers[14] observed a direct correlation between the helical content of poly-α, L-glutamic acid, and the percentage of their amide hydrogen atoms which were not readily exchangeable with water. According to Berger and co-workers,[15] the rate of amide H-atom exchange between *N*-methylacetamide and water in neutral or weakly acidic solutions is determined by the following proton transfer reaction: $CH_3CONHCH_3 + OH^- \rightleftarrows CH_3CONCH_3^- + H_2O$. If we accept this conclusion, we may interpret the observation of Blout and co-workers in terms of *directed proton transfer* as follows. In the random coil form, it is possible for any one of the peptide N-H groups to be hydrogen bonded to OH^- ions, and consequently *directed proton transfer* from N-H to OH^- can lead to rapid H-

atom exchange. But in the helical form, most of the amide hydrogen atoms are already hydrogen bonded to the carbonyl groups. Therefore, *directed proton transfer* from these N-H groups to nearby OH^- ions becomes impossible without first breaking the hydrogen bonds of the helix, and consequently the rate of H-atom exchange becomes very slow in spite of the fact that these amide H atoms are in van der Waals contact with H_2O molecules or OH^- ions.

These observations show that hydrogen bonding can facilitate proton transfer in a certain direction and retard proton transfer in another direction. Since many enzymatic reactions involve proton transfer, it appears likely that *directed proton transfer* along hydrogen bonds in the enzyme-substrate complex may play an important role in determining the *efficiency* and *specificity* of enzyme catalysis.

Catalytic Mechanism of Carbonic Anhydrase. Carbonic anhydrase contains one tightly bound Zn(II) ion per enzyme molecule. Difference infrared spectra show that the CO_2 at the active site in the enzyme-substrate complex is neither coordinated to the Zn(II) nor appreciably distorted, but is loosely bound to a hydrophobic surface or crevice of the protein, as in clathrate compounds.[16] The infrared data also show that the HCO_3^- is bound at the active site by coordination to the Zn(II) through its negatively charged O-atom in such a way that its relatively neutral O atom and OH group are placed at the specific CO_2 site as illustrated by structure **3** in Figure 1. These results show that in the

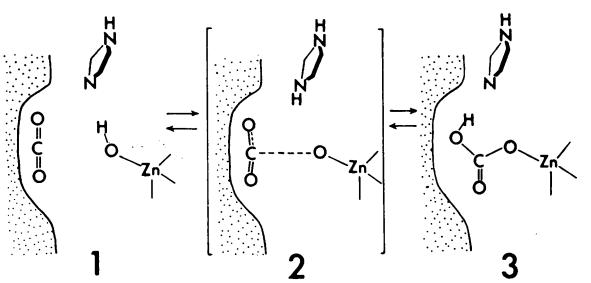

FIG. 1.—Catalytic mechanism of carbonic anhydrase.

dehydration reaction **3** → **1** illustrated in Figure 1, proton transfer must accompany the breaking of the C-O bond, since it is already known from the infrared data that only CO_2 is to be left in the hydrophobic binding site. Therefore, 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 **1** → **3**.

The observed first-order rate constant of the reaction **1** → **3** of Figure 1 is 4×10^5 sec^{-1} at pH 7 and 25°C. An approximate computation for the analogous reaction in a hypothetical system containing a free OH^- ion and a CO_2 molecule in juxtaposition yielded a first-order rate constant of the same order of magnitude.[11] This is rather surprising because the free OH^-, with a dissociation constant of $10^{-15.7}$ for its conjugate acid, is a much stronger base than the OH^- coordinated to the Zn(II) of carbonic anhydrase, with $K_a = 10^{-7.1}$ for $\geq ZnOH_2$. Although the ratio of nucleophilic reactivities of free and coordinated OH^- ions 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 much in their order of magnitude. In other words, the OH^- coordinated to the Zn(II) of carbonic anhydrase reacts faster by a factor of $\sim 10^8$ than what is expected from a simple proximity effect. Inasmuch as OH^- in water and OD^- in deuterium oxide seem to react with CO_2 approximately the same speed,[17] it seems unlikely that the solvation effect can raise the rate by a factor of $\sim 10^8$. Therefore the enzyme must have additional means of expediting the reaction. In addition, the path of proton transfer in the catalysis by carbonic anhydrase is still unknown. We want to know whether the proton transfer is facilitated by the participation of a certain functional group of the protein, and, if so, what the identity of this group and the nature of its participation are.

A conceivable way of combining the activation of the OH^- bound to the Zn(II) and the facilitation of proton transfer in a single molecular process consistent with the foregoing mechanism is to assume that this OH^- is hydrogen bonded either directly or through another water molecule to a strategically located basic imidazole group of the protein as illustrated in Figure 1. Directed proton transfer changes the enzyme-substrate complex **1** to the reactive intermediate **2**, which could rapidly react to form **3** as illustrated in Figure 1. Since the $O^=$ coordinated to the Zn(II) in **2** is expected to be a much better nucleophile than the OH^- of the Zn(II) in **1**, we may regard the transformation **1** → **2** as the mechanism by which the participating basic group of the protein directs proton transfer and activates the $\geq$ZnOH group. An imidazole group appears to be the best candidate to play such a role, not only because it is the strongest base among all functional groups of the protein which may remain unprotonated at neutral pH, but also because the distribution of its lone-pair and π-electrons makes its basic N atom particularly suited to relay the proton to the substrate. In the absence of such a participating basic group of the protein, the corresponding proton transfer must be directed toward a water molecule which is too weak a base to receive the proton sufficiently rapidly, or toward an OH^- ion which may not be present at a sufficiently high concentration at the active site of the enzyme-substrate complex to be effective. For similar reasons, the carboxylate group could play an analogous role in some other enzymes.

Catalysis by α-Chymotrypsin. The extraordinary nucleophilic reactivity of Ser-195 in α-chymotrypsin[18,19] has been related, on the basis of chemical and kinetic studies, to its possible hydrogen bonding to a basic imidazole group.[20-22] Recent x-ray data[23,24] at pH 4.2 show that the OH group of Ser-195 may indeed be hydrogen bonded to the imidazole group of His-57 in neutral solutions. Careful studies of the pH dependence of the chymotrypsin-catalyzed hydrolysis of amides show that only one basic imidazole group is involved in the catalysis.[25,26] On the other hand, the catalytic hydrolysis of anilides by chymotrypsin definitely involves protonation of the substrate, since the rate of hydrolysis increases with the basic strength of the anilide and the measured values of log k_2 bear a linear relationship to the values of pK_a of the protonated anilide.[37,38] An apparent way to reconcile these two sets of observations is to assume[10] that the proton which is added to the substrate came from the OH group of serine-195 via the basic N atom of histidine-57. A plausible path[11] for this essential proton

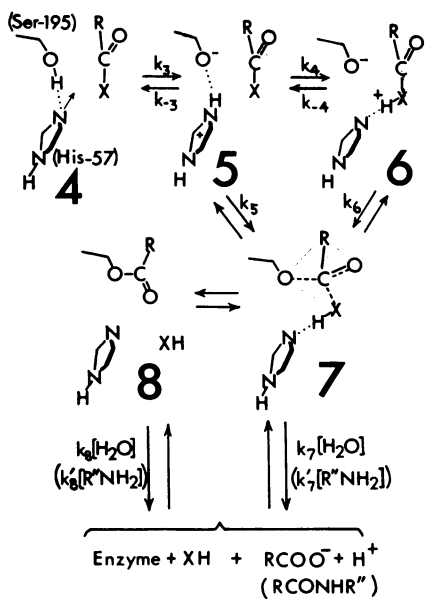

FIG. 2.—Catalytic mechanism of α-chymotrypsin.

transfer is illustrated in Figure 2 for a general substrate represented by RCOX (X = OR′ for carboxylic esters; X = NHR′ for amides, anilides and peptides).

It is assumed in Figure 2 that a good substrate RCOX is bound to the enzyme in such a stereospecific way that the susceptible C-X bond of the substrate is placed in juxtaposition to the Ser-195· · · · ·His-57 hydrogen bridge of the enzyme, and that the plane of the imidazole group of His-57 is roughly perpendicular to the plane of the paper which is determined by the basic N atom of the imidazole, the O atom of Ser-195 and the C-X bond of the substrate as illustrated by structure **4**. The distribution of the lone-pair electrons and π-electrons around the basic N atom of this imidazole for the imidazole plane perpendicular to and coplanar with the paper is illustrated qualitatively by (*B*) and (*A*), respectively, in Figure 3. It is apparent from Figure 3 that if the imidazole plane of His-57 is roughly perpendicular to the plane of the paper as illustrated by (*B*), then the transfer of proton from Ser-195 to the substrate should be much faster than in the coplanar arrangement (*A*), because the average electron density along the proton transfer path in (*B*) is considerably higher than that in (*A*).

If we assume that the first-order rate constants for the *directed proton transfer* in the thermodynamically favorable reactions **6** → **5** and **5** → **4** are of the same order of magnitude as that for the transfer of excess protons in ice, i.e., $\sim 10^{14}$ sec^{-1}, then the rate constants for proton transfer in the opposite direction are given by $k_3 \approx 10^{14}$ (K_3) and $k_4 \approx 10^{14}$ (K_4), where K_3 and K_4 are the equilibrium constants for the reactions **4** ⇄ **5** and **5** ⇄ **6**, respectively. Estimating the pK_a's of the OH group of Ser-195 and the imidazolium group of His-57 to be 13 and 7, respectively, we obtain $k_3 \approx 10^{14}$ $(10^{-13})/(10^{-7}) = 10^8$ sec^{-1}, which is much faster than the observed over-all rate constant k_2 for chymotrypsin catalysis and hence cannot be rate-limiting. In the present approximation, we have neglected the net effect of dielectric environment and ion-pair interactions on the pK_a's.

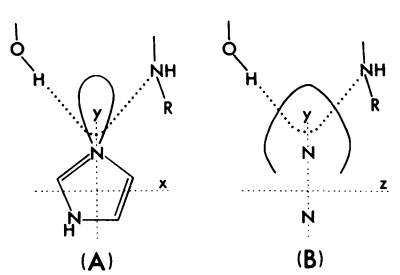

FIG. 3.—Distribution of the lone-pair electrons and π-electrons around the basic N atom of His-57 at the active center of chymotrypsin-substrate complex. The solid curves represent the contour surface of electron density due to the lone-pair and π-electrons near the basic N atom. The broken curves represent possible paths of proton transfer.

Compound **5** may either change directly to the tetrahedral intermediate **7** by passing through a transition state which involves the nucleophilic attack by the serine alkoxide group at the carboxyl C atom of the substrate concerted with or immediately followed by proton transfer to the leaving group X, or change first to **6** by pretransition-state protonation and then to **7** by passing through a transition state which involves the nucleophilic attack at an already protonated substrate as illustrated in Figure 2. **7** can then be hydrolyzed either by reaction with H_2O concerted with the departure of HX and followed by the liberation of the Ser-OH, or by reacting with H_2O after HX has completed its departure, i.e., at the acyl-enzyme stage **8**. According to this mechanistic scheme, the rate of the acylation step in the α-chymotrypsin-catalyzed hydrolysis of alkyl esters, anilides, amides, and peptides in dilute aqueous solutions may be approximated by the equation

$$\text{Rate} = k_5\,[\mathbf{5}] + k_6\,[\mathbf{6}], \tag{1}$$

and consequently the rate for the acylation step $\mathbf{4} \xrightarrow{k_2} \cdots \rightarrow \mathbf{7}$ or **8** is determined by

$$k_2 \approx k_5K_3 + k_6K_3K_4. \tag{2}$$

Since **6** should be more reactive than **5**, we expect k_6 to be much larger than k_5 for most substrates. On the other hand, for substrates with extremely weak base properties the additional factor K_4 may reduce the steady-state concentration of **6** so drastically that the second term on the right-hand side of Eq. 2 may become negligible as compared to the first term.

For the hydrolysis of esters, kinetic considerations show that the maximum theoretical value of the second term in Eq. 2 is smaller than the observed values of k_2 by several orders of magnitude. Therefore the hydrolysis of alkyl esters by α-chymotrypsin must take place by the concerted or posttransition-state protonation path $\mathbf{5} \rightleftarrows \mathbf{7} \rightleftarrows \mathbf{8}$. Experimental data[29] indicate that $k_2(\approx k_5K_3)$ decreases as the base strength of the substrate increases. For the hydrolysis of anilides, experimental data[27,28] show that $k_2 \approx k_6K_3K_4$ and that it increases proportionally with the base strength of the substrate. Therefore the hydrolysis of anilides by α-chymotrypsin must take place mainly by the pretransition-state protonation path $\mathbf{5} \rightleftarrows \mathbf{6} \rightleftarrows \mathbf{7} \rightleftarrows \mathbf{8}$. Since amides and peptides are stronger bases than anilides, the pretransition-state protonation path should be even more favorable.

Theoretically not only the acyl-enzyme **8** but a whole continuous range of configurational intermediates between **7** and **8** of Figure 2 can react with H_2O. For simplicity, the two rate constants k_7 and k_8 can be defined such that the total rate of liberation of $RCOO^-$ is given by $d\,[RCOO^-]/dt = [H_2O](k_7\,[\mathbf{7}] + k_8\,[\mathbf{8}])$. Here the second term in parentheses represents the usual acyl-enzyme mechanism; i.e., the already protonated leaving group, XH, moves spontaneously to infinity and leaves behind the acyl-enzyme **8** to be hydrolyzed at a subsequent step. The first term does not represent the direct nucleophilic attack of the tetrahedral intermediate **7** by H_2O. Rather, it represents the reaction of H_2O with the whole continuous range of configurational intermediates between **7** and **8**, each with its

C-X bond already stretched beyond the normal bond length but before the intermediate has attained its planar acyl-enzyme configuration. Similarly in the presence of an amine $R''NH_2$, the same continuous range of intermediates can also react with it at a rate equal to $d[RCONHR'']/dt = [R''NH_2](k'_7\,[7] + k'_8\,[8])$. Since the steady-state value of [7]/[8] for a peptide substrate should be larger than that for the corresponding ester substrate, we generally expect the two substrates to have different values of the partition ratio $[RCONHR'']/[RCOO^-]$.

Bent Hydrogen Bond. Ordinarily when a serine OH group is hydrogen bonded to the basic N atom of the imidazole group of a histidine residue, the system minimizes its free energy by approaching a conformation in which the O—H bond and the symmetry axis of the lone-pair electrons of the basic N atom (represented by the thin-lined arrow in structure **4** of Fig. 2) are colinear. In such a conformation the proton tends to oscillate almost exclusively between the oxygen and nitrogen atoms. In order to increase the probability that the proton of Ser-195 will be transferred, after its arrival to the basic N atom of His-57, to the bound substrate, we postulated[10,11] a bent hydrogen bond at the active center of α-chymotrypsin-substrate complex as illustrated by **4** in Figure 2. Because of the distribution of electronic charge around this N atom, the proton in such a bent hydrogen bond will have a better chance of being transferred to the leaving group of the bound substrate molecule. If this happens and if the resulting nucleophilic alkoxide group can swing around to attack the carboxyl C atom of the substrate either before as well as during substrate protonation (for esters) or after substrate protonation (for peptides), the formation of a tetrahedral intermediate, which leads to the products, can readily take place.

But what keeps this crucial Ser-195···His-57 hydrogen bond in the bent form? We had no idea about the correct answer when the proposal was made.[10] Shortly afterwards, Blow, Birktoft, and Hartley[24] made the important discovery that the imidazole-NH group of His-57 is hydrogen bonded to the carboxylate group of Asp-102. If the protein conformation at the active center of the enzyme-substrate complex is such that this imidazole group cannot form stable hydrogen bonds at both of its N atoms, then the strong linear Asp-102···His-57 hydrogen bond could force the His-57···Ser-195 hydrogen bond into the bent conformation.[30] It may be worthwhile to emphasize that the structure with the bent hydrogen bond in Figure 2 was proposed for the active center in the chymotrypsin-substrate complex with possible conformation changes, e.g., induced fit[31] and strain, already having taken place. The proposal does not require this hydrogen bond to be bent in the free enzyme.

Efficiency and Specificity. In view of the fact that although each molecule in liquid water is already hydrogen bonded to its neighbors, the slight additional improvement in the alignment of hydrogen bonds which takes place upon freezing is sufficient to increase the rate of proton transfer by a factor of 10^2, one can readily appreciate the advantage of accurately maintaining the crucial hydrogen bonds during enzyme catalysis. Presumably *directed proton transfer* along these hydrogen bonds in the enzyme-substrate complex may enable the system to reach with sufficient speed certain particularly effective intermediates (e.g., **7** in

Fig. 2) which are ordinarily not readily accessible. A good substrate and the enzyme should fit each other in exactly the right way to induce and stabilize these strategically oriented hydrogen bonds for *directed proton transfer* in the enzyme-substrate complex. If this interpretation is correct, it will give us a new dimension for understanding the *efficiency* and *specificity* as two inseparable factors of enzyme action, and even greater appreciation of the intricate relationship between molecular structure and biological function.

The author thanks Drs. D. M. Blow, J. S. Fruton, R. Henderson, J. R. Knowles, P. A. Lyons, and L. Parker for their valuable criticisms concerning this manuscript.

* This work has been supported by a research grant (GM 04483) from the U.S. Public Health Service.

[1] Swain, C. G., and J. F. Brown, Jr., *J. Amer. Chem. Soc.*, **74**, 2538 (1952).

[2] Jencks, W. P., *Catalysis in Chemistry and Enzymology* (New York: McGraw-Hill, 1969), p. 20.

[3] Wang, Jui H., in *Proceedings of the Symposium on CO_2* (Haverford), 24th International Congress in Physiological Sciences (1968), p. 31.

[4] Bruice, T. C., and U. K. Pandit, these PROCEEDINGS, **46**, 402 (1960).

[5] Haldane, J. B. S., *Enzymes* (London: Longmans, 1930).

[6] Blake, C. C. F., L. N. Johnson, G. A. Mair, A. C. T. North, D. C. Phillips, and V. A. Sarma, *Proc. Roy. Soc., Ser. B*, **167**, 378 (1967).

[7] Wang, Jui H., *J. Amer. Chem. Soc.*, **77**, 822, 4715 (1955); *Accounts Chem. Res.*, **3**, 90 (1970).

[8] Hollands, T. R., I. M. Voynick, and J. S. Fruton, *Biochemistry*, **8**, 575 (1969).

[9] Koshland, D. E., Jr., *J. Theor. Biol.*, **2**, 75 (1962).

[10] Wang, Jui H., and L. Parker, these PROCEEDINGS, **58**, 2451 (1967).

[11] Wang, Jui H., *Science*, **161**, 328 (1968).

[12] Eigen, M., and L. DeMaeyer, *Proc. Roy. Soc. (London)*, **A247**, 505 (1958).

[13] Eigen, M., L. DeMaeyer, and H.-Ch. Spatz, *Ber. Bunsenges.*, **68**, 19 (1964).

[14] Blout, E. R., C. deLozé, and A. Asadourian, *J. Amer. Chem. Soc.*, **83**, 1895 (1961).

[15] Berger, A., A. Loewenstein, and S. Meiboom, *J. Amer. Chem. Soc.*, **81**, 62 (1959).

[16] Riepe, M. E., and Jui H. Wang, *J. Amer. Chem. Soc.*, **89**, 4229 (1967); *J. Biol. Chem.*, **243**, 2779 (1968).

[17] Smith, E. L., *Fed. Proc.*, **8**, 581 (1949); Davis, R. P., *J. Am. Chem. Soc.*, **80**, 5209 (1958).

[18] Hartley, B. S., and B. A. Kilby, *Biochem. J.*, **56**, 288 (1954).

[19] Schaffer, N. K., L. Simet, S. Harshman, R. R. Engle, and R. W. Drisko, *J. Biol. Chem.*, **225**, 197 (1957); Osterbaan, R. A., and M. E. Adrichem, *Biochim. Biophys. Acta*, **27**, 423 (1958); Hartley, B. S., *Nature*, **201**, 1284 (1964).

[20] Gutfreund, H., and J. M. Sturtevant, these PROCEEDINGS, **42**, 719 (1956).

[21] Cunningham, L., *Science*, **125**, 1145 (1957).

[22] Bender, M. L., *J. Amer. Chem. Soc.*, **84**, 2580 (1962); Bender, M. L., and F. Kezdy, *J. Amer. Chem. Soc.*, **86**, 3704 (1964).

[23] Matthews, B. W., P. B. Sigler, R. Henderson, and D. M. Blow, *Nature*, **214**, 652 (1967).

[24] Blow, D. M., J. J. Birktoft, and B. S. Hartley, *Nature*, **221**, 337 (1969); Steitz, T. A., R. Henderson, and D. M. Blow, *J. Mol. Biol.*, **46**, 337 (1969).

[25] Himoe, A., and G. P. Hess, *Biochem. Biophys. Res. Commun.*, **23**, 234 (1966).

[26] Bender, M. L., M. J. Gibbian, and D. J. Whelan, these PROCEEDINGS, **56**, 833 (1966).

[27] Inagami, T., S. S. York, and A. Patchornik, *J. Amer. Chem. Soc.*, **87**, 126 (1965).

[28] Parker, L., and Jui H. Wang, *J. Biol. Chem.*, **243**, 3729 (1968).

[29] Zerner, B., and M. L. Bender, *J. Amer. Chem. Soc.*, **85**, 356 (1963).

[30] Wang, Jui H., in *Proceedings of the International Symposium on Structure-Function Relationship of Proteolytic Enzymes*, Copenhagen, June 16–18, 1969 (Amsterdam: Elsevier, in press).

[31] Koshland, D. E., Jr., these PROCEEDINGS, **44**, 98 (1958).