I–IV, 46 may be classified into one of these six types: (1) $B(OR)_3$—compounds 1 to 11, Table I; (2) $ClB(OR)_2$—compounds 1 to 8, Table II; (3) $ROBCl_2$—compounds 9 to 13, Table II; (4) BR_3—compounds 1 to 3, Table III; (5) $C_6H_5B(OR)_2$—compounds 1 to 7, Table IV; (6) $C_6H_4CH_3B(OR)_2$—compounds 8 to 19, Table IV. If the values for R_{obs} are plotted against n, where n is the number of carbon atoms in a single alkyl group, the following graph is produced.

		$R = an + b;$	a	R_{CH_2}
(1)	$B(OR)_3$	$R_{obsd} = 14.04n + 10.05;$	$14.04 = 3 \times 4.68$	
(2)	$ClB(OR)_2$	$R_{obsd} = 9.18n + 14.27;$	$9.18 = 2 \times 4.59$	
(3)	$ROBCl_2$	$R_{obsd} = 4.92n + 15.73;$	$4.92 = 1 \times 4.92$	
(4)	BR_3	$R_{obsd} = 14.14n + 6.04;$	$14.14 = 3 \times 4.71_3$	
(5)	$C_6H_5B(OR)_2$	$R_{obsd} = 9.34n + 34.10;$	$9.34 = 2 \times 4.67$	
(6)	$C_6H_4CH_3B(OR)_2$	$R_{obsd} = 9.42n + 38.94;$	$9.42 = 2 \times 4.71$	

Since the R_{obs} values for isomeric compounds show no systematic deviations (the n-isomers have partly larger, partly smaller values than the i-isomers), the average value was used in the plot. The fact that almost all of the points fall directly on straight lines may be taken as an indication of the reliability of the values for the density and refractive index as given in the report.[6] From the slopes and intercepts of the straight lines shown, one can derive a set of equations of the type

The resulting increment R_{CH_2} agrees, except in the series (3), within 0.07 cc. with the value 4.64 cc. observed for long homologous series. These equations may therefore be used to calculate the molar refractions for any organoboron compounds of the six types mentioned without the need of density and/or refractive index data.

The accuracy of equations 3 and 4 is questionable, since the derivation of the latter is based on only three points (see graph) and for the former the experimental points deviate relatively more from the straight line than for the other equations.

Acknowledgment.—The authors gratefully acknowledge the advice and helpful suggestions given by Professor Kasimir Fajans of the University of Michigan, Ann Arbor, Michigan.

NEWARK, N. J.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE]

The Influence of the Ionization of a Group in the Substrate Molecule on the Kinetic Parameters of Enzymatic Reactions

BY CARL FRIEDEN

RECEIVED MARCH 28, 1958

Equations have been derived for the single substrate case to include the effects of the ionization of a group in the substrate molecule upon the over-all rate of an enzymatic reaction. These equations have been described in terms of changes in the maximum velocity and Michaelis constant of the reaction. It is shown that there are several distinguishable kinetic cases even when the effects of substrate ionization are complicated by those of groups in the enzymatically active site. These cases may be distinguished by differences in plots of kinetic constants as a function of pH for the forward and reverse reaction. Provided that enough data are available and that the assumptions made in the derivations are correct, it is possible to tell whether (1) one of both ionic forms of the substrate are utilizable or (2) if only one form is utilizable, whether the other form is a good or poor competitive inhibitor.

The substrates for many enzymes contain groups which ionize in the pH range most frequently investigated. Since most substrate molecules are of low molecular weight the change in charge associated with the change in degree of ionization of substrate probably will affect the substrate-enzyme interaction in some way and thus will influence the rate of the over-all reaction. The changes in the over-all reaction may in turn be attributed to changes in the kinetic parameters, that is, the maximum velocity and Michaelis constant of the reaction. There has been little attention paid to the effect of substrate ionization on over-all rates of enzymatic reactions. Undoubtedly, much of this lack arises from the already complicated pH-dependence of the kinetic parameters due to enzymatic ionizations alone.[1-6] However, under suitable conditions, equations describing the over-all reaction rate in the presence of an ionizable substrate may be derived. Provided that there is some difference either in the binding of two different ionized forms of substrate to the enzyme or in the rate of breakdown of the two different enzyme–substrate complexes there will always be some effect of the substrate ionization on the kinetic parameters of the reaction. The derived equations show that changes in enzyme-substrate interaction and breakdown due to changes in the ionization state of the substrate molecule may be detected from a complete study of the kinetic parameters as a function of pH for the forward and reverse reaction. The ionization constant of the substrate must, of course, be known.

There are two major assumptions made in this paper: first, that the enzyme contains either 0, 1 or 2 ionizable groups which control the pH dependence of the reaction rate in a "total" way. It will be assumed that there are no more than two such groups. Such an assumption does not seem unlikely in view of the fact that this appears to be the situation for many enzymes as exemplified by fumarase.[6] Secondly, it is assumed that hydrogen ion equilibria are established rapidly.

(1) L. Michaelis and H. Davidson, *Biochem. Z.*, **35**, 386 (1911).
(2) L. Michaelis and H. Pechstein, *ibid.*, **59**, 77 (1914).
(3) J. B. S. Haldane, "Enzymes," Longmans, Green and Co., London, 1930.
(4) R. A. Alberty and V. Massey, *Biochim. et Biophys. Acta*, **13**, 347 (1954).
(5) M. Dixon, *Biochem. J.*, **55**, 161 (1953).
(6) C. Frieden and R. A. Alberty, *J. Biol. Chem.*, **212**, 859 (1955).

The *p*H Dependence of the Kinetic Parameters Arising Only from Substrate Ionization.—It seems advisable to investigate the simplest mechanism possible which will include the effect of ionization of a group in the substrate molecule and derive the equations for the kinetic parameters pertaining to this mechanism. In this way, the effects of substrate ionization may be described clearly. This simple mechanism is for the case where the over-all rate of reaction is not influenced by the ionization of any groups in the enzyme itself. For this mechanism, one ionic form of the substrate is utilizable while the other may be either utilizable or act as a competitive inhibitor. This mechanism may be represented as

$$\begin{gathered} E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \underset{k_4}{\overset{k_3}{\rightleftarrows}} EP \underset{k_6}{\overset{k_5}{\rightleftarrows}} E + P \\ E + SH^+ \underset{k_8}{\overset{k_7}{\rightleftarrows}} ESH^+ \underset{k_{10}}{\overset{k_9}{\rightleftarrows}} EPH^+ \underset{k_{12}}{\overset{k_{11}}{\rightleftarrows}} E + PH^+ \\ S + H^+ \underset{}{\overset{K_{SH}}{\rightleftarrows}} SH^+ \\ P + H^+ \underset{}{\overset{K_{PH}}{\rightleftarrows}} PH^+ \end{gathered} \qquad (I)$$

where S, SH^+, P and PH^+ represent the two ionized forms of substrate and product, respectively. Since S and SH^+ and P and PH^+ are in rapid equilibrium, the rates of the reactions $ES + H^+ \rightleftarrows ESH^+$ and $EP + H^+ \rightleftarrows EPH^+$ are not independent of the other rate constants. The steady state[3,7] derivation for this mechanism shows that

$$v = V_F(E)_0/(1^+ K_F/[S]_t) \qquad (I,1)$$

where v is the initial velocity, V_F is the maximum initial velocity in the forward direction, K_F is the Michaelis constant in the forward direction and $[S]_t$ is the total substrate concentration, $[S] + [SH^+]$. For this mechanism, V_F, the maximum velocity in the forward direction, is given by

$$V_F = \frac{k_1k_3k_5B + k_7k_9k_{11}A((H^+)/K_{SH})}{k_1(k_3 + k_4 + k_5)B + k_7(k_9 + k_{10} + k_{11})A((H^+)/K_{SH})} \qquad (I,2)$$

where

$$A = k_2k_4 + k_2k_5 + k_3k_5$$

and

$$B = k_8k_{10} + k_8k_{12} + k_8k_{11}$$

The Michaelis constant for equation I,1 is given by

$$K_F = \frac{AB(1 + (H^+)/K_{SH})}{k_1(k_3 + k_4 + k_5)B + k_7(k_9 + k_{10} + k_{11})A((H^+)/K_{SH})} \qquad (I,3)$$

and the ratio of kinetic parameters is given by the equation

$$V_F/K_F = \frac{k_1k_3k_5B + k_7k_9k_{11}A((H^+)/K_{SH})}{AB\,(1 + (H^+)/K_{SH})} \qquad (I,4)$$

From these general expressions, it may be shown that the Michaelis constants for S, K_{ES} and for SH^+, K_{ESH}, are given by

$$K_{ES} = \frac{A(1 + (H^+)/K_{SH})}{k_1(k_3 + k_4 + k_5)} \qquad (I,5)$$

(7) E. L. King and C. Altman, *J. Phys. Chem.*, **60**, 1375 (1956).

$$K_{ESH} = \frac{B(1 + K_{SH}/(H^+))}{k_7(k_9 + k_{10} + k_{11})} \qquad (I,6)$$

and the maximum velocities for each form of substrate S and SH^+ are

$$V_{ES} = k_3k_5/(k_3 + k_4 + k_5) \qquad (I,7)$$

$$V_{ESH} = k_9k_{11}/(k_9 + k_{10} + k_{11}) \qquad (I,8)$$

As would be expected, the extent of influence of substrate ionization on the kinetic parameters is dependent upon both the ratio of the Michaelis constants for the two forms and the ratio of the rate of breakdown of the intermediate enzyme complexes.

(a) **If Both S and SH^+ Are Substrates.**—The situation that both S and SH^+ are substrates for the enzyme is described by equations I,2-I,4 for the forward reaction. Exactly symmetrical equations may be obtained for the reverse reaction. For this case where it is assumed that the enzyme ionizations have no influence upon the *p*H dependence of the initial velocity, it should be noticed that V_F, K_F and $V_F(1 + (H^+)/K_{SH})/K_F$ are all *p*H-dependent as a result only of substrate ionization. Since analogous equations may be derived for the reverse direction, the same conclusions must hold. From equation I,4, and the similar equation for the reverse reaction, plus the equation which relates the two equilibria $S \rightleftarrows P$ and $SH^+ \rightleftarrows PH^+$ (*i.e.*, $k_1k_3k_5/k_2k_4k_6 = K_{PH}k_7k_9k_{11}/K_{SH}k_8k_{10}k_{12}$), it may be shown that

$$K_{eq} = \frac{V_FK_R}{V_RK_F}\left(\frac{1 + (H^+)/K_{SH}}{1 + (H^+)/K_{PH}}\right) \qquad (I,9)$$

This equation which relates the over-all equilibrium constant to the kinetic parameters is known as the Haldane relation.[3] Although $V_F(1 + (H^+)/K_{SH})/K_F$ and $V_R(1 + (H^+)/K_{PH})/K_R$ may be *p*H-dependent due to substrate ionization, these functions must vary with *p*H in exactly the same way so that their ratio, the equilibrium constant, is independent of *p*H. Thus the Haldane relation is valid regardless of whether only one or both substrates are utilizable.[8]

(b) **If Only S Is a Utilizable Substrate.**—The case where only one ionized form of the substrate is utilizable is the one most often considered. For the situation that S is utilizable, $k_9 = k_{10} = 0$ in equations I,2-I,4 and these equations reduce to

$$V_F = \frac{k_1k_3k_5}{k_1(k_3 + k_4 + k_5) + k_7A((H^+)/k_8K_{SH})} \qquad (I,10)$$

$$K_F = \frac{A(1 + (H^+)/(K_{SH})}{k_1(k_3 + k_4 + k_5) + k_7A((H^+)/k_8K_{SH})} \qquad (I,11)$$

and

$$V_F/K_F = \frac{k_1k_3k_5}{A(1 + (H^+)/K_{SH})} \qquad (I,12)$$

where k_7/k_8 is the reciprocal of the binding constant for the inactive form of the substrate SH^+. Thus the extent of binding of the non-utilizable form of substrate will influence the value of the maximum velocity or the Michaelis constant, but will not influence their ratio. Since K_{ES}, the Michaelis constant for S, is defined by equation 1,5, it is clear that the extent to which the two forms of substrate are bound is the important factor. There

(8) The author is indebted to Dr. R. A. Alberty for pointing out this fact.

are, then, two possibilities

$$\frac{k_7(H^+)}{k_8K_{SH}} << \frac{k_1(k_2 + k_4 + k_5)}{A} \qquad (i)$$

that is where SH^+ is an exceedingly poor competitive inhibitor. Then

$$V_F = \frac{k_3k_5}{(k_3 + k_4 + k_5)} = V_{ES} \qquad (I,13)$$

$$K_F = \frac{A(1 + (H^+)/K_{SH})}{k_1(k_2 + k_4 + k_5)} = K_{ES} \qquad (I,14)$$

so that V_F and $K_F/(1 + (H^+)/K_{SH})$ are independent of pH.

$$\frac{k_7(H^+)}{k_8K_{SH}} \geqslant \frac{k_1(k_2 + k_4 + k_5)}{A} \qquad (ii)$$

that is where SH^+ is a good competitive inhibitor. In this case, both V_F and $K_F/(1 + (H^+)/K_{SH})$ are pH dependent although their ratio, $V_F(1 + (H^+)/K_{SH})/K_F$ is independent of pH. The extent to which the maximum velocity and Michaelis constant are affected by changes in the hydrogen ion concentration is dependent upon how good a competitive inhibitor the non-utilizable form of the substrate, SH^+, is with respect to the Michaelis constant for S.

Thus either the maximum velocity or Michaelis constant may be influenced by the substrate ionization. However, when only one ionized form of the substrate is utilized by the enzyme, the ratio $V_F(1 + (H^+)/K_{SH})/K_F$ is always independent of pH, *no matter how tight the binding of the non-utilizable form.* If both forms of substrate are utilizable, equation I,4 indicates that this ratio may be pH dependent. It is this difference which in favorable cases may permit the determination of the active form or forms of substrate.

(c) **If Only SH^+ Is a Utilizable Substrate.**—The case where only SH^+ is utilizable is exactly symmetrical with the case where only S is utilizable. For $k_3 = k_4 = 0$, equation I,1 becomes

$$V_F = \frac{k_7k_9k_{11}}{k_7(k_9 + k_{10} + k_{11}) + k_1B(K_{SH}/k_2(H^+))} \qquad (I,15)$$

and equation I,3 becomes

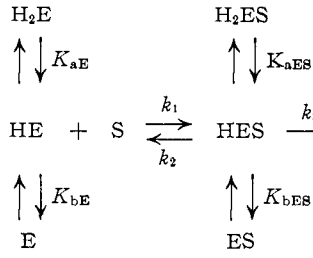
$$K_F = \frac{B(1 + K_{SH}/(H^+))}{k_7(k_9 + k_{10} + k_{11}) + k_1B(K_{SH}/k_2(H^+))} \qquad (I,16)$$

and their ratio is

$$V_F/K_F = \frac{k_7k_9k_{11}}{B(1 + K_{SH}/(H^+))} \qquad (I,17)$$

These equations are identical in form to equations I,10 and I,11 except that the ratio $(H^+)/K_{SH}$ has become $K_{SH}/(H^+)$. Thus if the enzyme active site contains no ionizable groups which influence the kinetic parameters, it is possible to tell which form of substrate is utilizable by a comparison of the pH dependence of equations I,12 and I,17.

The pH Dependence of the Kinetic Parameters Arising from Enzymatic Ionizations.—It is well known that most enzymes contain ionizable groups which appear to influence the reaction rate in a total way.[9,10] In many cases, there appear to be one or two such groups in the enzymatically active site of the protein. A brief review of the pH dependence of the kinetic parameters due to enzyme ionizations will be included here. It has been shown for the mechanism



(II)

that the form of the equation resulting from the steady-state derivation is

$$v = V_F(E)_0/(1 + K_F/[S]_t)$$

where

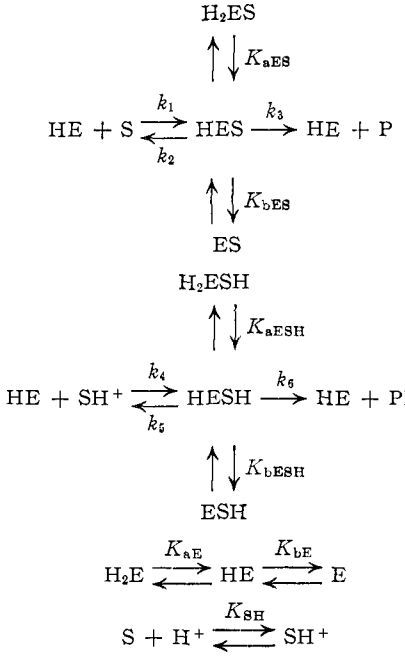
$$V_F = k_3/(1 + (H^+)/K_{aES} + K_{bES}/(H^+)) \qquad (II,1)$$

$$K_F = \frac{k_2 + k_3}{k_1}\left[\frac{1 + (H^+)/K_{aE} + K_{bE}/(H^+)}{1 + (H^+)/K_{aES} + K_{bES}/(H^+)}\right] \qquad (II,2)$$

$$V_F/K_F = \frac{k_1k_3}{k_2 + k_3}\Big/(1 + (H^+)/K_{aE} + K_{bE}/(H^+)) \qquad (II,3)$$

The complexes HEP, EP and H_2EP have been left out in this mechanism and mechanism III in order to simplify the equations. However, the expressions derived are identical in form to the more detailed mechanism. These expressions do not include irreversible enzyme denaturation due to pH changes. The equations show the pH dependence of the maximum velocity to be a function of the ionization constants of groups in the enzymatically active site when substrate is bound. On the other hand $V_F(1 + (H^+)/K_{SH})/K_F$ depends only upon the ionization of the groups in the enzyme where no substrate is bound to the active site.

The pH Dependence of the Kinetic Parameters Arising from Substrate and Enzyme Ionizations.—With substrate and enzyme ionization, mechanism II may be extended to give



(III)

(9) R. A. Alberty, *J. Cell. and Comp. Physiol.*, **47**, suppl. 1, 245 (1956).

(10) K. J. Laidler, *Trans. Faraday Soc.*, **51**, 550 (1955).

The over-all reaction again may be represented by the simple expression

$$v = V_F(E)_0/(1 + K_F/[S]_t)$$

However, the expressions which show the pH dependence of the maximum velocity and Michaelis constant have become much more complicated.

For the most general situation, the steady-state derivation of mechanism III yields this equation for the kinetic parameters

$$V_F = \frac{k_1k_3(k_5 + k_6) + k_4k_6(k_2 + k_3)(H^+)/K_{SH}}{k_1(k_5 + k_6)f_{ES} + k_4(k_2 + k_3)f_{ESH}(H^+)/K_{SH}} \quad (III,1)$$

$$K_F = \frac{(k_2 + k_3)(k_5 + k_6)f_E(1 + (H^+)/K_{SH})}{k_1(k_5 + k_6)f_{ES} + k_4(k_2 + k_3)f_{ESH}(H^+)/K_{SH}} \quad (III,2)$$

The ratio of kinetic parameters is then

$$V_F/K_F = \frac{k_1k_3(k_5 + k_6) + k_4k_6(k_2 + k_3)(H^+)/K_{SH})}{(k_2 + k_3)(k_5 + k_6)f_E(1 + (H^+)/K_{SH})} \quad (III,3)$$

where f_E, f_{ES} and f_{ESH} are defined as

$$f_E = 1 + (H^+)/K_{aE} + K_{bE}/(H^+)$$
$$f_{ES} = 1 + (H^+)/K_{aES} + K_{bES}/(H^+) \quad (III,4)$$
$$f_{ESH} = 1 + (H^+)/K_{aESH} + K_{bESH}/(H^+)$$

Thus, by way of comparison, equation II,1 would become

$$V_F = k_3/f_{ES}$$

As is true for mechanism I, symmetrical equations may be derived using product and it may be shown that the Haldane relation, equation I,9 holds at all pH values for this mechanism.[11] Assuming the mechanism to be correct, equation III,3 shows that the ratio $V_F(1 + (H^+)/K_{SH})/K_F$ represents the ionization of groups in the enzyme only if one of the forms of the substrate is non-utilizable.

(a) **If Both S and SH Are Active.**—In this most general case, equation III,3 shows that the ratio $V_F(1 + (H^+)/K_{SH})/K_F$ would be expected to show a pH dependence more complex than would be expected from enzyme ionization. Therefore, for mechanism III, $V_F(1 + (H^+)/K_{SH})/K_F$ would not be symmetrical with respect to pH. This mechanism, of course, assumes that the ionizing groups in the enzyme affect the enzyme activity in a total way. It is always possible that a complex pH dependence may result from a more complex type of ionization in the enzyme, and such results would be difficult to interpret. The pH dependence of both V_F and $K_F/(1 + (H^+)/K_{SH})$ may be very complex since both these parameters involve $(H^+)^2$ terms in the denominator. The situation for mechanism III is the same as for mechanism I with the additional influence of the enzyme ionization.

(b) **If Only S Is a Substrate.**—As in mechanism I, the equations for mechanism III become considerably simpler if it is assumed that $k_6 = 0$. For this case, the pH dependence of the ratio $V_F(1 + (H^+)/K_{SH})/K_F$ is due only to the ionization of groups in the enzyme. However, the pH dependence of the parameters V_F and $K_F/(1 + (H^+)/K_{SH})$ will be influenced by the extent of binding of the inactive form of the substrate relative to the Michaelis constant for the active form. As in mechanism I, two extreme cases may be considered.

(i) If SH^+ is an exceedingly poor competitive inhibitor, then V_F and $V_F(1 + (H^+)/K_{SH})/K_F$ will have the same type of pH dependence. The two curves need not be superimpossible but they will both be symmetrical. The same would be expected to hold true for the reverse direction.

(ii) If SH^+ is a good competitive inhibitor, that is if the second term in the denominator of equations (III,1) and (III,2) is important, the pH dependence of V_F will be different from that of $V_F(1 + (H^+)/K_{SH})/K_F$. Thus, it may be possible to distinguish between case (i) and (ii) on the basis of comparison of the pH dependence of these two plots.

It is possible that data for two enzymes described in the literature may be explained by the equations developed in this paper.

Roholt and Greenberg[12] have investigated the kinetic parameters of liver arginase in the forward direction at 25 and 35° at constant Mn^{++} concentration. These authors find that a plot of $V_F(1 + (H^+)/K_{SH})/K_F$ is symmetrical and bell-shaped at both temperatures indicating that the enzyme contains two ionizable groups which must influence the reaction in a total way and that the active form of the arginine is the zwitterion. However the maximum velocity as a function of pH is not symmetrical. Thus, a possible explanation for these data is that of the inactive forms of arginine, the acidic form is a good competitive inhibitor, thereby influencing the maximum velocity pH plots in a way which would not be expected to arise from the enzyme ionization alone.

The same explanation may also hold for the enzyme enolase where V_F and $V_F(1 + (H^+)/K_{SH})/K_F$ do not appear to have the same pH dependence.[13] These data, however, are complicated because the Haldane relation does not appear to hold unless it is assumed that the pK for the substrate and the product at the particular metal ion concentrations used are identical. From the data presented, this assumption does not seem likely.[14]

Discussion

The equations developed in this paper apply only to mechanisms for which the ionizable groups in the enzyme influence the enzymatic reaction in a total way. The equations have been derived assuming two such groups in the enzyme and enzyme–substrate complex but are applicable to the case where there is either one group or none. Such a type of mechanism seems reasonable in view of the fact that it appears to hold far many enzymes.[8,9]

The importance of substrate ionization upon the over-all rate of an enzymatic reaction appears to have been largely ignored. However, provided that the two ionized forms of the substrate or product are different is some way with respect to the enzymatic reaction, the kinetic parameters must be influenced by the change in degree of ionization of a group in the substrate or product molecule.

(11) The complete derivation for the mechanism involving the ionization of the HE, HES, HEP, HESH and HEPH complexes will be sent upon request.

(12) O. A. Roholt, Jr., and D. M. Greenberg, *Arch. Biochem. Biophys.*, **62**, 454 (1956).

(13) F. Wold and C. Ballou, *J. Biol. Chem.*, **227**, 313 (1957).

(14) F. Wold and C. Ballou, *ibid.*, **227**, 301 (1957).

Under the assumptions made, the derived equations indicate several kinetically different cases corresponding to different possible mechanisms in terms of the pH dependence of the kinetic parameters, the maximum velocity and Michaelis constant. These differences may be summarized as follows: if both ionized forms of substrate are utilizable, V_F and the ratio $V_F(1 + (H^+)/K_{SH})/K_F$ would be expected to vary with pH in a manner more complex than would be expected from the ionization of one or two groups in the active site of the enzyme molecule. On the other hand, when only one ionized form of substrate is utilizable, the two possibilities that the non-utilizable form of substrate be either a "good" or "poor" competitive inhibitor are kinetically distinguishable. For both these possibilities, $V_F(1 + (H^+)/K_{SH})/K_F$ *vs.* pH is a function only of the ionizable groups in the enzymatically active site which are responsible for activity and the pH dependence may be described easily by an equation similar to equation II,3. For the case of poor competitive inhibition, the maximum velocity represents only the ionization of groups of the active site of enzyme to which substrate is bound. However, for the case of good competitive inhibition, the maximum velocity will be influenced by the degree of ionization of the substrate and will therefore be more complex than would be expected.

In order that the equations derived in this paper be applicable, a large amount of kinetic data must be obtained. These data must be uncomplicated by interference due to substrate inhibition, substrate activation, irreversible enzyme denaturation, buffer effects due to different concentrations of anions or cations at different pH values and so forth. Thus, care must be exercised in the reinterpretation of data already presented in the literature. For example, the two enzymes arginase and enolase which have been discussed both involve metal ions for enzymatic activity. Although constant metal ion concentration was used, the mechanism of interaction of enzyme with the metal ions is not completely understood.

SAINT LOUIS, MISSOURI

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CORNELL UNIVERSITY]

The Elastic Properties of Elastin[1,2]

BY C. A. J. HOEVE[3] AND P. J. FLORY[3]

RECEIVED JUNE 2, 1958

Force–temperature measurements have been carried out on elastin (ox *ligamentum nuchae*) held at fixed elongation and immersed in glycol–water (3:7) mixture. The equilibrium degree of swelling of elastin in this mixture is independent of temperature, and the retractive force is directly proportional to the absolute temperature. It follows that $(\partial E/\partial L)_{TV} = 0$ for elastin and hence that the internal energy of the elastin chain is independent of its conformation. Contrary to previous studies on elastin, in which the influence of changes of swelling with temperature were overlooked, the thermoelastic behavior offers no indication whatever for crystallization on stretching at any elongation. The shape of the stress–strain curve is explained in terms of the morphology of native elastin; the abrupt rise in stress at high elongations is attributed to straightening out of the initially curled fibers of collagen which are associated with the native elastin.

Introduction

Elastin is an important constituent of various elastic tissues including ligaments, blood vessel walls and skin. It possesses a high extensibility combined with a low modulus not unlike that of rubber. Moreover the stress–strain curve for elastin, like that of rubber, swings upward sharply at high extensions[5]; the rise of the stress–strain curve is however more abrupt, and it occurs at somewhat lower elongations, as compared with vulcanized rubber. Thermoelastic studies[4–6] on elastin have yielded large positive stress–temperature coefficients even at low extensions. It has been inferred from this alleged deviation from *ideal* rubber elasticity that crystallization occurs on stretching. On the contrary, however, Astbury[7] found only an amorphous halo in the X-ray diffraction pattern of stretched elastin in which the collagen component had been destroyed.

The principles underlying rubber-like elasticity of amorphous polymers are of course well known. Progress recently has been made in the analysis of the thermoelastic behavior of partly crystalline polymers.[8] In extension of studies in this area, and especially those relating to fibrous proteins, it became of interest to examine elastin, and in particular to endeavor to resolve the apparent contradiction between the thermoelastic results and those of X-ray diffraction.

Theoretical

Insight into the nature of the molecular processes involved in elastic deformation may be gained by analysis of experimentally determined stress–strain–temperature results according to the thermodynamic equation of state for elastic deformation. This equation may be expressed as follows for a system subject to elongation at constant pressure

$$f = (\partial E/\partial L)_{PT} + T(\partial f/\partial T)_{PL} \qquad (1)$$

(1) Support of the National Science Foundation is gratefully acknowledged.

(2) Presented in September 1957 at the 132nd American Chemical Society Meeting, New York, N. Y.

(3) Mellon Institute, Pittsburgh, Pennsylvania.

(4) K. H. Meyer and C. Ferri, *Pflüger's Arch. ges. Phys.*, **238**, 78 (1936).

(5) E. Wöhlisch, *Kolloid Z.*, **89**, 239 (1939).

(6) E. Wöhlisch, H. Weitnauer, W. Grüning and R. Rohrbach, *ibid.*, **104**, 14 (1943).

(7) W. T. Astbury, *J. Intern. Soc. Leather Trades' Chem.*, **24**, 69 (1940).

(8) J. F. M. Oth, E. T. Dumitru, O. K. Spurr and P. J. Flory, THIS JOURNAL, **79**, 3288 (1957); J. F. M. Oth and P. J. Flory, *ibid.*, **80**, 1297 (1958).