in the presence of eserine, experiments carried out by Augustinsson and Nachmansohn[10] showed that the optimum was shifted only slightly with this inhibitor. The explanation for these seemingly contradictory results is probably that in the experiments of the latter workers the inhibitor was used under essentially irreversible conditions. This is possible because in the presence of the substrate a period of about 8 minutes is required before eserine and the enzyme equilibrate, forming the enzyme–eserine complex, while a longer period of 40 to 50 minutes is required for the dissociation of the latter.[12] The inhibition is therefore virtually irreversible if the enzyme and inhibitor are mixed before addition of the substrate, as was the case in the experiments of Augustinsson and Nachmansohn.[10]

In the experiments with prostigmine carried out by the latter workers,[10] the inhibitor and enzyme were incubated together for 1 hr. before addition of the substrate, as in the work with eserine. The behavior observed with prostigmine may therefore reflect both an effect of the type described by equation 2 and an irreversible inhibition producing a decrease in rate over the entire substrate range but resulting in no shift in substrate optimum. In this regard it is interesting to note that Wilson[22] used such a procedure of mixing prostigmine and the enzyme before adding the substrate in order to study the inhibition under non-competitive, that is essentially irreversible, conditions. Since a similar experimental procedure was used in the work with choline discussed above, it is concluded that an irreversible type of inhibition may contribute to the observed behavior of the system at high substrate concentrations.

(22) I. B. Wilson, *Biochim. Biophys. Acta*, **7**, 466 (1951).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF OTTAWA, OTTAWA, ONTARIO, CANADA]

Molecular Mechanisms for Hydrolytic Enzyme Action. III. A General Mechanism for the Inhibition of Acetylcholinesterase

BY RICHARD M. KRUPKA AND KEITH J. LAIDLER

RECEIVED AUGUST 12, 1960

General steady-state equations are derived for enzyme substrate–inhibitor systems in which reaction involves two intermediates, such as an addition (Michaelis) complex and an acyl enzyme. The inhibitor is assumed to combine with the free enzyme and the acyl enzyme, but not with the addition complex, and attachment of inhibitor to the acyl enzyme may or may not block deacetylation. It is shown that if the slow step is the transition from addition complex to acyl enzyme the inhibition is always competitive, and the same is always true if the inhibitor does not block deacylation. Non-competitive inhibition results if, and only if, the deacylation is the slow process and the inhibitor blocks deacylation. An experimental study of the inhibition of acetylcholinesterase action by choline, carbachol and eserine shows the behavior to be competitive even with acetylcholine as substrate, and the conclusion is that these inhibitors, unlike *cis*-2-dimethylaminocyclohexanol (Part II), do not block deacetylation. The structural requirements for the blocking of deacetylation are considered to be that the inhibitor contains in addition to its cationic center a center of high electron density; the molecule must also be sufficiently compact for the latter center to be able to interact with the acidic site on the enzyme without interfering with the acetyl group.

Introduction

It has been shown in the preceding papers[1] that substances containing a quaternary nitrogen atom can add on to the free and acetylated enzymes but not to the Michaelis complex. They can do so because the grouping in the enzyme to which they become attached, the anionic site, is free in the free and acetyl enzymes but is bound to the substrate in the Michaelis complex. If an inhibitor attached to the acetyl-enzyme can interact in a specific way with the esteratic site, deacetylation is blocked. For such interaction to occur the inhibitor must contain an appropriately placed electronegative function,[2] and it is believed that this is bound to the acid group in the esteratic site.[1a]

The effect of this behavior on inhibition caused by high concentrations of substrate was studied in part II.[1b] It was concluded that substrate inhibition results from addition of the substrate to the acetyl-enzyme rather than to the Michaelis complex. There is therefore now a need to develop the theory for cases of low substrate concentration and to decide if experimental data are in agreement with the theory under these conditions. The simplified reaction scheme, in which substrate inhibition is neglected, is shown in Fig. 1. As in the previous formulation[1b] the inhibitor adds on to the free and acetyl enzymes (E and ES′, respectively). Addition to the latter may or may not result in inhibition of deacetylation; that is, the factor a may be equal to zero or unity or some intermediate value.

Application of the steady-state treatment to this scheme gives rise to

$$v = \frac{k_2[\mathrm{E}]_0\bar{K}[\mathrm{S}]}{1 + K_i[\mathrm{I}] + \bar{K}[\mathrm{S}]\left\{1 + \frac{k_2(1 + \beta\bar{K}_i[\mathrm{I}])}{k_3(1 + a\beta\bar{K}_i[\mathrm{I}])}\right\}} \quad (1)$$

where $\beta\bar{K}_i = k_i'/(k'_{-i} + ak_3)$ and $\bar{K} = k_1/(k_{-i} + k_2)$.

If k_2 is much smaller than k_3, equation 1 describes the case of simple competitive inhibition, since the term

$$\frac{k_2(1 + \beta\bar{K}_i[\mathrm{I}]}{k_3(1 + a\beta\bar{K}_i[\mathrm{I}]}$$

is negligible under these conditions.

If k_2 is equal to or larger than k_3, equation 1 may lead to a non-competitive form of inhibition. If a is equal to unity, however, simple competitive inhibition results under any circumstances. Thus if $a = 1$, equation 1 becomes

$$v = \frac{k_2[\mathrm{E}]_0\bar{K}[\mathrm{S}]}{1 + K_i[\mathrm{I}] + \bar{K}[\mathrm{S}]\left(1 + \frac{k_2}{k_3}\right)} \quad (2)$$

This is the equation for pure competitive inhibition.

Non-competitive inhibition results if $a = 0$. In this case equation 1 becomes

$$v = \frac{k_2[\mathrm{E}]_0\bar{K}[\mathrm{S}]}{1 + K_i[\mathrm{I}] + \bar{K}[\mathrm{S}]\left\{1 + \frac{k_2}{k_3}(1 + \beta\bar{K}_i[\mathrm{I}])\right\}} \quad (3)$$

(1) R. M. Krupka and K. J. Laidler, (a) THIS JOURNAL, **83**, 1445 (1961); (b) *ibid.*, **83**, 1448 (1961).

(2) S. L. Friess and W. J. McCarville, *ibid.*, **76**, 1363 (1954).

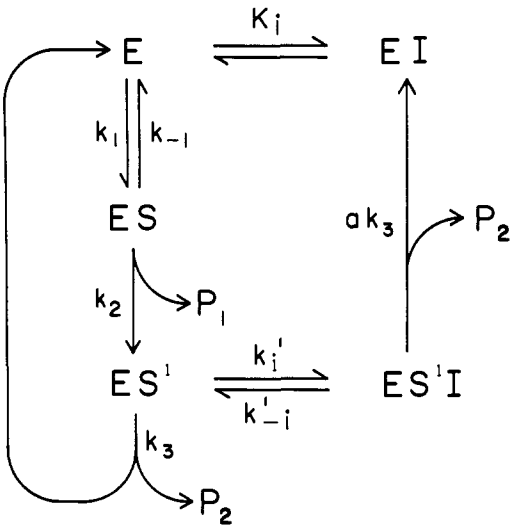


Fig. 1.—Reaction scheme in which the inhibitor combines with the free enzyme and the acyl enzyme but not the addition complex.

If $k_2 >> k_3$ and if $\beta\bar{K}_i = K_i$

$$v = \frac{k_2[E]_0\bar{K}[S]}{(1 + K_i[I])\left(1 + \frac{k_2}{k_3}\bar{K}[S]\right)} \quad (4)$$

The uninhibited rate under the same conditions is given by

$$v_0 = \frac{k_2[E]_0\bar{K}[S]}{1 + \frac{k_2}{k_3}\bar{K}[S]} \quad (5)$$

The ratio of uninhibited and inhibited rates is therefore

$$\frac{v_0}{v} = 1 + K_i[I] \quad (6)$$

This is the equation for simple non-competitive inhibition. If $\beta\bar{K}_i$ is not equal to K_i or if k_2 is not much greater than k_3, the inhibition will appear to be of the mixed type, partly competitive and partly non-competitive. For this type of inhibition plots of $1/v$ against $1/[S]$ for varying inhibitor concentrations intersect in the left-hand quadrant—that is, at negative values of $1/[S]$ and positive values of $1/v$—whereas for simple non-competitive inhibition the intersection is on the negative $1/[S]$ axis.

For other values of a, equation 1 may be written as

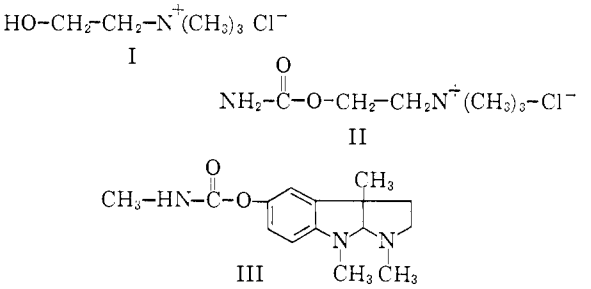
$$\frac{1}{v} = \frac{1}{[E]_0}\left(\frac{1}{k_2} + \frac{1}{k_3}\right) + \frac{\beta\bar{K}_i(1 - a)[I]}{k_3[E]_0(1 + a\beta\bar{K}_i[I])} + \frac{1 + K_i[I]}{k_2[E]_0\bar{K}}\frac{1}{[S]} \quad (7)$$

For $1 > a > 0$, a mixed type of inhibition is again observed, but in this case the plots of $1/v$ against $1/[S]$ fail to intersect at a single point; any two lines do, however, intersect in the left-hand quadrant of the graph. If $a\beta\bar{K}_i[I] << 1$, intersection is at a single point. When this is the case equation 7 becomes

$$\frac{1}{v} = \frac{1}{[E]_0}\left(\frac{1}{k_2} + \frac{1}{k_3}\right) + \frac{1}{k_2[E]_0\bar{K}[S]} + \left\{\frac{K_i}{k_2[E]_0\bar{K}[S]} + \frac{\beta(1 - a)\bar{K}_i}{k_3[E]_0}\right\}[I] \quad (8)$$

In this case plots of $1/v$ against $[I]$ are linear at constant $[S]$. It is seen from equation 7 that it may be a generally useful procedure to plot $1/v$ against both $1/[S]$ and $[I]$ since the former plot is linear, while the latter is not. Equations which resemble 7 in this respect have been derived on the basis of other inhibitory mechanisms by Segal, *et al.*[3]

Our experiments have been carried out with a view to testing the applicability of the reaction scheme shown in Fig. 1 and to determining values of the constants for various inhibitors. In particular, it was of interest to see whether there existed inhibitors which could become attached to the acyl enzyme, to form ES'I, but for which ES'I became deacylated as rapidly as ES'. The work was done with choline (I), carbachol (II) and eserine (III).



Experimental Procedure

Experiments were carried out as in Part I of this series.[1a] The choline chloride used was a product of Eastman Organic Chemicals, eserine salicylate of Merck and Co. Ltd., Montreal, and carbachol (carbamylcholine chloride) of the British Drug Houses (Canada) Ltd. Details of the substrate and inhibitor concentrations used are given in the captions of the figures. The inhibition of acetylcholine hydrolysis by all three inhibitors was studied, but the inhibition of N-methylaminoethylacetate hydrolysis was studied only with carbachol. Initial rates were measured, except with eserine. With this compound the degree of inhibition increased during the first 10 minutes of hydrolysis, and rate measurements were arbitrarily taken as the rates of reaction five minutes after the addition of the enzyme to the mixture of substrate and inhibitor.

Experimental Results

The inhibition of acetylcholine hydrolysis of carbachol, choline and eserine was shown to be of the simple competitive type, as was the inhibition of N-methylaminoethylacetate hydrolysis by carbachol (Figs. 2 to 5). Fig. 6 shows

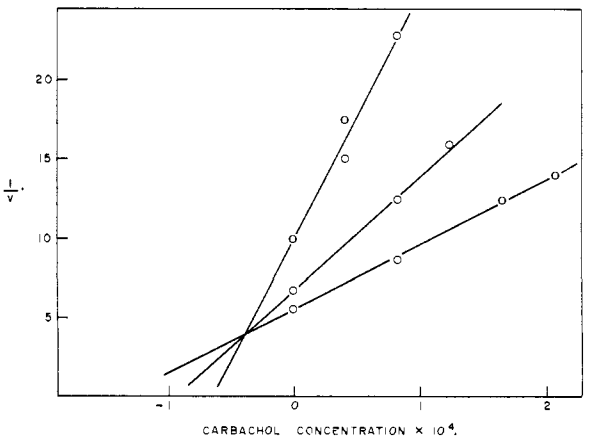


Fig. 2.—Plots of reciprocal of velocity (in the units 10^8 sec. mole^{-1}), against concentration of carbachol, for the hydrolysis of acetylcholine. The curves are for three concentrations of substrate, namely 2.03, 4.06 and 8.13 $\times$ 10^{-4} M.

(3) H. L. Segal, J. F. Kachmar and P. D. Boyer, *Enzymologia*, **15**, 187 (1952).

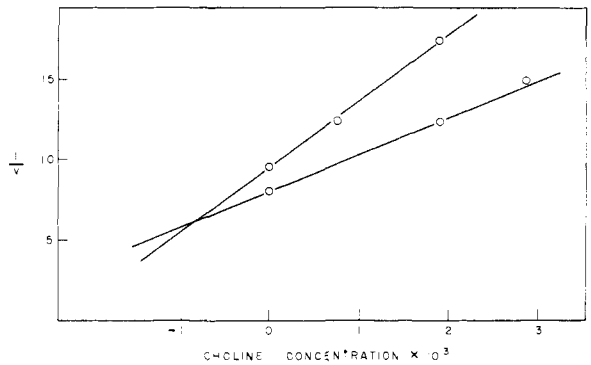


Fig. 3.—Plots of reciprocal of velocity (in the units 10^8 sec. mole^{-1}) against concentration of choline, for the hydrolysis of acetylcholine. The substrate concentrations are 4.11 and 8.22 × 10^{-4} M.

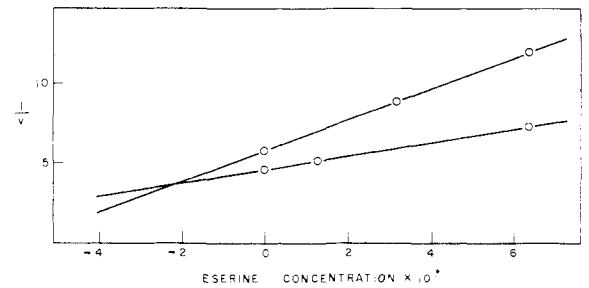


Fig. 4.—Plots of reciprocal of velocity (in the units 10^8 sec. mole^{-1}) against concentration of eserine, for the hydrolysis of acetylcholine. The substrate concentrations are 4.06 and 8.13 × 10^{-4} M.

a plot of relative values of the slopes of the lines in Figs. 2 to 5 against relative values of the reciprocal of the substrate concentration. The fact that in all cases these plots pass through the origin indicates that the inhibitors behave in a simple competitive way. The association constants were calculated to be 4.5 × 10^6, 3.9 × 10^4, and 1.2 × 10^3 for the inhibition of acetylcholine hydrolysis by eserine, carbachol and choline respectively. The constant for the inhibition of N-methylaminoethylacetate by carbachol was 2.6 × 10^4.

Discussion

The work of Cabib and Wilson[4] has shown that in the hydrolysis of acetylcholine by acetylcholinesterase, the rate constant k_2 is larger than k_3; with N-methylaminoethyl acetate, on the other hand, k_3 is larger than k_2. According to the theory it is only possible to observe non-competitive inhibition with substrates for which $k_2 \geqslant k_3$; simple competitive behavior is expected if $k_3 > k_2$. It is therefore not surprising that the inhibition of N-methylamino-ethyl acetate is of the simple competitive type.

The fact that choline, carbachol and eserine inhibit the hydrolysis of acetylcholine in a simple competitive manner shows that they do not appreciably inhibit deacetylation; as was previously shown, however, they do become attached to the acetylated enzyme.[1b]

In order to block deacetylation an inhibitor probably requires a site of high electron density[1a,2,4]; the carbonyl oxygen atom of carbachol may be such a site.[5] The electronegative locus

(4) I. B. Wilson and E. Cabib, THIS JOURNAL, **78**, 202 (1956).
(5) S. L. Friess, *ibid.*, **79**, 3269 (1957).

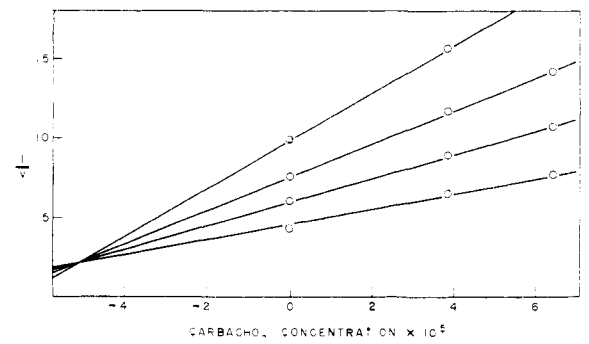


Fig. 5.—Plots of reciprocal of velocity (in the units 10^8 sec. mole^{-1}) against concentration of carbachol, for the hydrolysis of methylaminoethyl acetate. The substrate concentrations are 1.91, 2.86, 3.82 and 5.72 × 10^{-3} M.

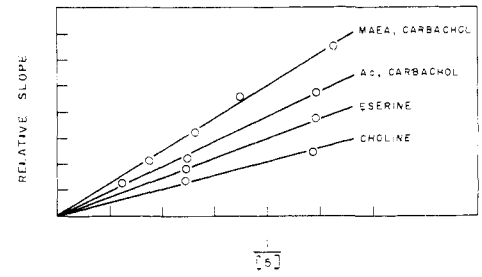


Fig. 6.—Plots of slopes of lines in Figs. 2–5 against the reciprocal of the substrate concentration

is believed to interact with the acid group of the catalytic center,[1a] which is not bound in either the free or the acetyl enzyme.[6] Friess and Baldridge[7] showed that in the most effective inhibitors of the type containing an electronegative grouping, the distance from the positive nitrogen atom to the electronegative locus is not greater than 2.5 Å. These facts suggest that in order for an inhibitor to interact with the esteratic site in the acetyl enzyme it must be sufficiently small and compact to approach the acidic group without interference from the acetyl grouping. It may be for this reason that carbachol, as well as the structurally similar acetylcholine, does not readily make contact with the acid group in the acetyl enzyme, while a rigid cyclic structure, such as *cis*-2-dimethylaminocyclohexanol, is able to do so.

In general it appears probable that inhibitors which interact with the basic group in the active center, such as carbachol, prostigmine and eserine, exhibit simple competitive behavior; on the other hand inhibitors which contain a site of high electronegativity and which may interact with the acidic group are known to show an apparently non-competitive behavior.[5,8] Since our *p*H studies[6] have shown that the basic group is involved in the deacetylation process, it is likely that the basic group is protected from contact with the inhibitor molecule by the acetyl grouping. The acidic group is apparently not so protected. Our inter-

(6) R. M. Krupka and K. J. Laidler, *Trans. Faradaday Soc.*, **56**, 1467 1477 (1960).
(7) S. L. Friess and H. D. Baldridge, THIS JOURNAL, **78**, 199 (1956).
(8) (a) D. S. Masterson, S. L. Friess and B. Witkop, *ibid.*, **80**, 5687 (1958); (b) S. L. Friess, E. R. Whitcomb, R. C. Durant and L. J. Reber, *Arch. Biochem. Biophys.*, **85**, 426 (1959).

pretation indicates, furthermore, that the acidic group must also be involved in deacetylation.

Implications of the Inhibition Theory. The Non-competitive Inhibition Constant.—In general the experimental value of the association constant for the binding of inhibitor to the acetyl enzyme is $\beta(1 - a)\bar{K}_i$, as is seen from equation 8. It is therefore not possible to separate the factors β and $(1 - a)$ experimentally. The experimental inhibitor constant thus reflects the strength of binding in the enzyme–inhibitor complex as well as the rate at which this complex reacts to give products. This conclusion should be true generally for inhibition studies.

The Relation of Inhibition Behavior to Substrate Concentration.—It was reported by Masterson *et al.*,[8a] that certain of the apparently non-competitive inhibitors of acetylcholinesterase (*e.g.*, 2-dimethylaminocyclohexyl trimethylammonium iodide) tend to exhibit simple competititve inhibition at low concentrations of substrate but non-competitive inhibition at high concentrations. It can be shown from the equation for the uninhibited rate and equation 3 that

$$\frac{v_0}{v} = 1 + \frac{[I]\left\{K_i + \frac{k_2}{k_3}\beta\bar{K}_i\bar{K}[S]\right\}}{1 + \bar{K}[S]\left(1 + \frac{k_2}{k_3}\right)} \qquad (9)$$

If [S] is low and $\beta\bar{K}_i$ is small the term $\frac{k_2}{k_3}\beta\bar{K}_i\bar{K}[S]$ may be small in comparison with K_i or with $\bar{K}[S]\left(1 + \frac{k_2}{k_3}\right)$. In this case the inhibition observed is competitive since the degree of inhibition is inversely related to [S]. At high [S], $\frac{k_2}{k_3}\beta\bar{K}_i\bar{K}[S]$ may be large relative to K_i and $\bar{K}[S]$ $(1 + k_2/k_3)$ large relative to unity. Here the inhibition is non-competitive since the degree of inhibition is independent of [S].

The Significance of the Michaelis Constant.—The present inhibition mechanism shows that non-competitive behavior may be due to a mechanism different from the usual form in which both E and EI may complex with S to form a potentially active intermediate. On the basis of the latter mechanism Morales[9] and Laidler[10] concluded that if an enzyme–substrate-modifier system exhibits non-competitive interaction, then, in general, its Michaelis constant is an equilibrium constant. The derivation of equations 3 and 4 based on the steady-state treatment shows that $\bar{K}$ (the reciprocal of the Michaelis constant) is not necessarily an equilibrium constant. On the basis of studies showing non-competitive inhibition of acetylcholinesterase, Friess[5] concluded that K_m was an equilibrium constant. In view of the theory developed here this conclusion is not justified.

The Slopes of $1/v$ against [I].—It is seen from equation 2 that for simple competitive inhibition in general the slopes of plots of $1/v$ against [I] for different substrate concentrations are inversely proportional to [S]; a plot of these slopes against the reciprocal of the substrate concentration therefore gives a straight line passing through the origin. For non-competitive inhibition in general (equation 3 and 8) such plots against the reciprocal of the substrate concentration give a straight line intersecting at a positive value of the slope axis. Intersection at a negative point on this axis might arise under certain circumstances. For example, for $a > 1$ but $a\beta\bar{K}_i[I] << 1$ such a negative intersection is obtained. A similar effect might be observed if an impurity in the inhibitor solution accelerated the rate-controlling step in the breakdown of the enzyme–substrate complex, or if the inhibitor, possibly by an effect on the properties of the reaction medium, caused such an acceleration. An example in which intersection is actually at a negative value is the inhibition of the glutamic aspartic transaminase system by isoniazid.[11]

General Conclusions.—The theory developed has the advantage of offering a general explanation for results reported in the literature on the inhibition of acetylcholinesterase by substances containing a quaternary nitrogen atom, applying to cases of both competitive and non-competitive behavior.[5,8] The action of the latter group of inhibitors differs from that of the former in only one respect. While both types complex with the anionic site in the free and acetylated enzymes, the non-competitive inhibitors also interact with a grouping—probably the acid group—in the esteratic site. As a result of this, deacetylation is blocked. Apparent non-competitive behavior is therefore explained by an essentially competitive mechanism, since the substrate and inhibitor complete for the same site on the enzyme surface. Mixed competitive and non-competitive inhibition is observed if $\beta\bar{K}_i$ is not equal to $\bar{K}_i$, if k_2 is not much greater than k_3 of if a is between zero and one. The theory may also apply to other hydrolytic enzymes. For example, non-comeptitive behavior was observed by Schwert and Eisenberg[12] in the benzoylarginine inhibition of the trypsin-α-benzoylarginamide system. Here the inhibitor closely resembles the substrate, suggesting that both should become attached to the same site on the enzyme surface. In this case the plots of $1/v$ against $1/[S]$ did not intersect at a single point in the left hand quadrant, suggesting that the system is described by equation 7 with a intermediate between zero and unity. It can be shown by substituting two values of the inhibitor concentration, $[I]_1$ and $[I]_2$, into 7, and solving for $\frac{1}{[S]}$ for a single value of $\frac{1}{v}$, that the point of intersection of two lines is given by

$$-\frac{1}{[S]} = \frac{k_2\bar{K}(1 - a)\beta\bar{K}_i}{k_3K_i(1 + a\beta\bar{K}_i[I]_1)(1 + a\beta\bar{K}_i[I]_2)} \qquad (10)$$

Equation 10 predicts that the intersection point is at smaller negative values of $1/[S]$ as the inhibitor concentration is increased. This is precisely the behavior which was observed by Schwert and Eisenberg, who were consequently led to describe the inhibition as of indeterminate type, being almost non-competitive in low concentrations of

(9) M. F. Morales, THIS JOURNAL, **77**, 4169 (1955).

(10) K. J. Laidler, *Trans. Faraday Soc.*, **52**, 1374 (1956).

(11) W. T. Jenkens, S. Orlowski and I. W. Sizer, *J. Biol. Chem.*, **234**, 2657 (1959).

(12) G. W. Schwert and M. A. Eisenberg, *ibid.*, **179**, 665 (1949).

benzoyl-L-arginine and approaching competitive inhibition with increasing concentrations of this inhibitor.

In the mechanism described here, more than one enzyme-substrate intermediate is involved. It is because part of the substrate molecule (the alcohol portion) is split off during the course of the reaction that, in a later intermediate in the sequence, part of the active center of the enzyme (in this case the anionic site) is free of bound substrate. This intermediate can therefore combine with a substance resembling the portion of the substrate which has been split off. If this substance interacts in a specific way with a region of the catalytic site which takes part in the further reaction of the intermediate, it may act as a non-competitive inhibitor, as has already been shown.

Proposed mechanisms for the non-competitive inhibition of enzymes such as lactic dehydrogenase[13] also invoke a sequence of several enzyme substrate intermediates. Here it is believed that the substrate migrates from one site on the enzyme surface to another during the course of the reaction. It is therefore possible for an inhibitor to become attached to this second site at the same time as the substrate is bound to the first. In this way further reaction is blocked, since the substrate is not free to migrate to the second site.

It may be significant that in both of these mechanisms a reaction sequence involving several steps is an essential part of the explanation for the non-competitive behavior. It is possible that multiple intermediates are a general feature of non-competitive inhibition.

Acknowledgment.—The authors are indebted to the Defence Research Board of Canada for their support of this work under Grant 9510-06.

(13) P. Ottolenghi and O. F. Denstedt, *Can. J. Biochem. Physiol.*, **36**, 1093 (1958); R. M. Krupka and K. J. Laidler, *ibid.*, **38**, 1185 (1960).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF OTTAWA, OTTAWA, ONTARIO, CANADA]

Molecular Mechanisms for Hydrolytic Enzyme Action. IV. The Structure of the Active Center and the Reaction Mechanism

BY RICHARD M. KRUPKA AND KEITH J. LAIDLER

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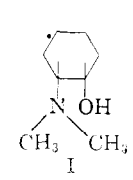
The kinetic results for acetylcholinesterase are briefly summarized, and on the basis of them a suggestion is made (Fig. 1) as to the structure of the active centre of the enzyme. Apart from the anionic site a similar structure is believed to exist in other hydrolytic enzymes. The way in which the substrate is held to the enzyme is deduced. A mechanism for acetylation is given, involving transfer of the acetyl group to the serine hydroxyl group. The acidic and basic sites are believed to play a role in this process, in which there is simultaneous making and breaking of several bonds. A mechanism for deacetylation is also proposed and involves both the acid and basic sites.

The enzyme acetylcholinesterase is a particularly convenient one for the purpose of arriving at a detailed reaction mechanism, owing to its possession of an anionic site. This interacts with cationic centers on substrates and inhibitors, and studies with molecules containing functional groups at known distances from these cationic centres have provided valuable evidence as to the positions of the active sites on the enzyme surface; the anionic centre may be used as an "origin" from which distances may be deduced. This mode of attack has been particularly exploited by Friess[1] and by Wilson.[2a] The deductions from their work combined with those in the preceding three papers[3] lead to a fairly clear-cut picture of the active center of the enzyme and of the way in which the various parts of the active center interact with the groups on substrates and inhibitor molecules. Our conclusions apply particularly to the acetylcholinesterase system, but there is considerable evidence, some of it referred to in Parts I–III of this series, that other enzymes behave in a very similar manner.

Structure of the Active Center.—The results described in the previous papers show that there are two distinct classes of inhibitors for hydrolytic enzymes; those of the first class block deacetylation, while those of the second do not. For the acetylcholinesterase system the inhibitors of the first class are exemplified by *cis*-2-dimethyl-aminocyclohexanol (I), while an example of the second class is prostigmine (II). All of the inhibitors investigated appear to become bound to the free enzyme and to the acyl-enzyme but not to the enzyme–substrate complex. A similar situation seems to exist with inhibitors for other hydrolytic enzymes, as exemplified by the discussion in Part III of the trypsin case. Kinetically the difference between the two



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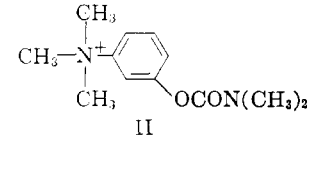

II

(1) (a) S. L. Friess and W. J. McCarville, THIS JOURNAL, **76**, 1363 (1954); (b) S. L. Friess, *ibid.*, **79**, 3269 (1957); (c) S. L. Friess and H. D. Baldridge, *ibid.*, **78**, 199 (1956); (d) D. S. Masterson, S. L. Friess and B. Witkop, *ibid.*, **80**, 5687 (1958); (e) S. L. Friess, E. R. Whitcomb, R. C. Durant and L. J. Weber, *Arch. Biochem. Biophys.*, **85**, 426 (1959).

(2) (a) I. B. Wilson and C. Quan, *ibid.*, **73**, 131 (1958). (b) That such a hydrogen bond occurs is strongly indicated by the results of Wilson and Quan[2a] who found that several compounds containing a hydroxyl group situated about 5 Å. from a tertiary nitrogen atom were very effective inhibitors. One fact that might appear to lead to the opposite conclusion is that the trimethylpropyl ammonium ion is as good an inhibitor as choline[4]; perhaps the end methyl group on the propyl radical interacts with some other site on the enzyme surface.

(3) R. M. Krupka and K. J. Laidler, THIS JOURNAL, **83**, 1445, 1448, 1454 (1961).

(4) I. B. Wilson, *J. Biol. Chem.*, **197**, 215 (1952).