ACTIVATION OF CHYMOTRYPSIN CATALYTED HYDROLYSES BY 9-AMINOACRIDINE*

Robert A. Wallace, Roger L. Peterson, Carl Niemann**
and George E. Hein

Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, California*** and Department of Chemistry, Boston University.

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In the course of an extensive investigation of aromatic compounds with α-chymotrypsin (Wallace, et. al., 1963), we noted that among a series of amino substituted acridines, one compound showed unusual behavior. Although acridine itself, as well as 1-, 2-, and 3- aminoacridines, inhibited the chymotrypsin-catalyzed hydrolysis of N-acetyl-L-valine methyl ester, with K_I values all approximately 2-3 X 10^{-4} M, the corresponding 9-amino isomer showed no detectable inhibition at concentrations up to 150 X 10^{-4} formal, the limit of its solubility at pH 7.9 under the reaction conditions. This anomalous result has led us to examine acridines as inhibitors of the bifunctional substrate, methyl hippurate.

As indicated in Fig. 1, both acridine and 2-amino-acridine act as competitive inhibitors of the hydrolysis of methyl hippurate. Inhibition constants calculated from the data in Fig. 1 give approximate values, K_I = 3 X 10^{-4} M for acridine and K_I = 2 X 10^{-4} M for 2-amino-acridine, in good agreement with the constants obtained previously using a trifunctional substrate. (Wallace, et. al., 1963) In contrast to this, 9-amino-acridine acts as an

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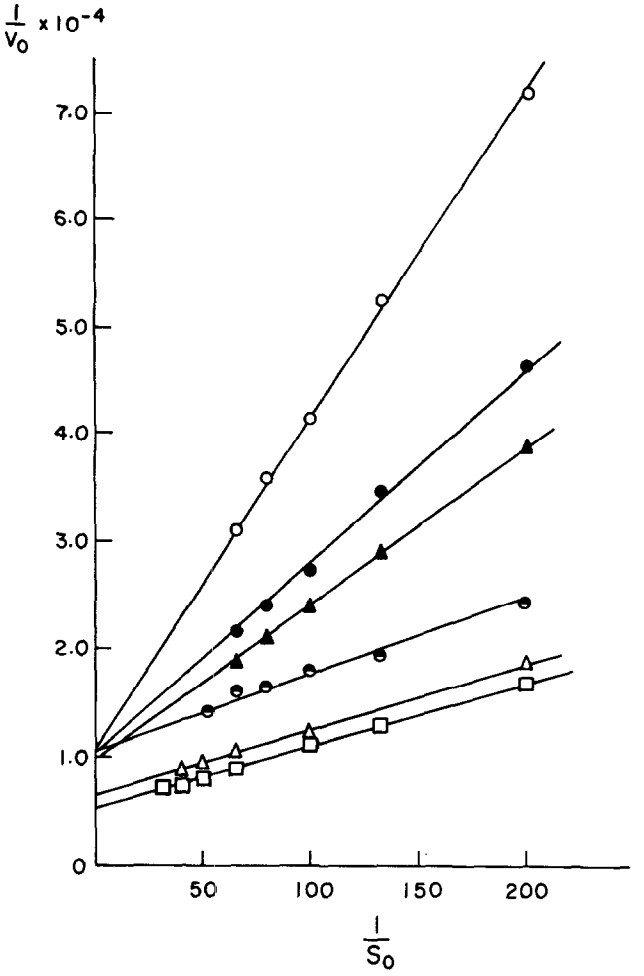

(Fig. 1) -- Effect of various acridines on the chymotrypsin catalyzed hydrolysis of methyl p-aminohippurate in aqueous solutions at 25.0 ± 0.1°, pH 7.90 ± 0.02 and 0.10 M with respect to sodium chloride with [E] = 1.0 mg. ml.-○- 2-amino-acridine (2.0 X 10^{-3} M),-●- acridine (0.4 X 10^{-3} M),-▲- 2-amino-acridine (0.2 X 10^{-3} M),-⊖- no added inhibitor or activator,-△- 9-amino-acridine (2.0 X 10^{-3} M),-□- 9-amino-acridine (6.0 X 10^{-3} M).

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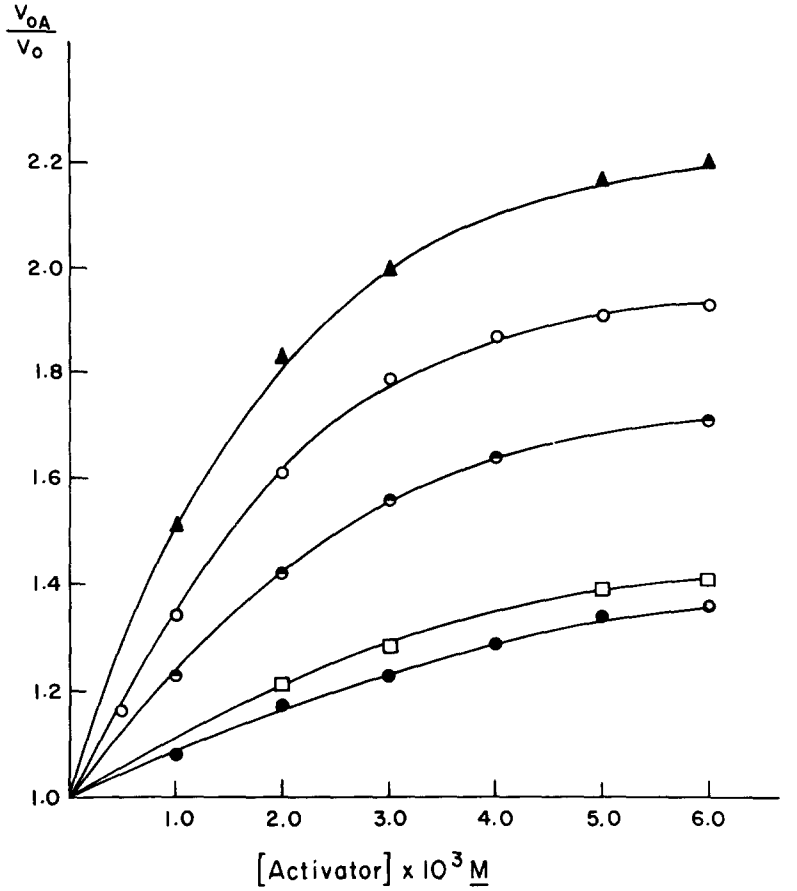
activator of the α-chymotrypsin-catalyzed-hydrolysis of methyl hippurate under the conditions indicated in Fig. 1.

Control experiments with 9-amino-acridine showed that, within the limits of experimental error, neither the base-catalyzed hydrolysis of

methyl hippurate at pH 7.9 (the substrate blank reaction) nor the enzyme blank reaction was affected by the activator.

The peculiar action of 9-amino-acridine was further examined as a function of activator concentration with a series of bifunctional substrates. Figure 2 shows the effect of 9-amino-acridine on the observed rate of enzyme-catalyzed hydrolysis of the methyl esters of N-acetyl, -furoyl, -benzoyl,



(Fig. 2) -- Relation between degree of activation and 9-amino-acridine concentration for bifunctional substrates in aqueous solution at 25.0 $\pm$ 0.1°, pH 7.90 $\pm$ 0.02 and 0.1 M with respect to sodium chloride with (E) = 1.0 mg. per ml. -▲- methyl phenaceturate (15.0 x 10^{-3} M), -○- methyl p-aminohippurate (5 x 10^{-3} M), -●- methyl hippurate (5 x 10^{-3} M), -□- methyl furouate (5 x10^{-3} M), -●- methyl aceturate (20 x 10^{-3} M).

-p-aminobenzoyl, and -phenacetyl glycine. In each case activation was observed. The amount of activation at any activator concentration plotted as initial rate in the presence of activator divided by initial rate in the absence of activator, $v_o \backslash v_o$, increased with the size of the acyl side chain, in the order listed above. The complex nature of the activation mechanism is suggested by the observation that neither single nor double reciprocal plots of the data in Fig. 2 yielded straightline relations for the entire concentration range.

Attempts to evaluate the experimental results quantitatively are rendered difficult by uncertainties concerning the nature of 9-amino-acridine in solution. In concentrations above 10^{-4} M, 9-amino-acridine shows evidence of forming molecular aggregates. This type of behavior, leading to the formation of ionic micelles, has been reported for this and other acridines. (Albert, 1951; Kruger, 1932; Steigmann, 1932) We have observed that aqueous solutions approximately 10^{-3} formal in 9-amino-acridine give a heavy blue precipitate with iodine-potassium iodide solution (Wagner's reagent) which disappears upon dilution with an equal volume of water. At substantially lower concentrations only a brown turbidity is obtained.

Further, the ultraviolet spectrum of aqueous solutions of 9-amino-acridine at pH 7.9 and in the presence of 0.1M NaCl shows dramatic changes with concentration which suggest the formation of several aggregated species in solution. Studies on the luminescence spectrum of 9-amino-acridine (Zanker and Rammensee, 1960, Levshin, 1956) have indicated the formation of dimeric or higher aggregates at concentrations above 10^{-3} M. Unfortunately, the status of 9-amino-acridine in aqueous solutions of relatively high ionic strength cannot be directly inferred from either of these studies. In the absence of information concerning the specific nature of the 9-amino-acridine species present, or of evidence that implicates any particular form as the activator, it is fruitless to attempt to derive kinetic expressions

that might correlate the observed behavior with a theoretical model.

The effect of ionic micelles on enzyme systems have been reported previously. Hofstee (1955) found that the activity of liver esterase and of two pancreatic esterases decreased suddenly at the critical micelle concentrations of the substrates used. His results, however, may simply reflect a change in effective substrate concentration, rather than an enzyme-micelle interaction. Wills (1952) observed that a colloidal soap micelle completely inhibits urease.

The phenomenon of activation in enzyme-catalyzed reactions by a third substance (" cofactor ") is not unusual. Activation of an enzymic reaction by an organic molecule that is not normally regarded as a coenzyme is, however, unusual. Theorell (1961) found that imidazole will activate liver alcohol dehydrogenase at high substrate concentrations. Foster (1961) observed that indole activates the solvolysis of acetyl chymotrypsin, although the overall effect on the hydrolysis of p-nitrophenyl acetate is one of inhibition. Inagami (1964) and Erlanger (1964) have reported the activation of certain trypsin catalyzed hydrolyses by small amines. Substrate activation of trypsin catalyzed reactions has also been noted. (Trowbridge, et. al., 1963)

The reported substrate activation of the chymotrypsin catalyzed hydrolysis of acetyl glycine methyl ester (Wolf and Niemann, 1959) has been shown to be erroneous (Wolf and Niemann, 1963, Ingles and Knowles, 1965).

Our data thus indicates the first report of the activation of a chymotrypsin catalyzed reaction by an organic molecule. The results are particularly significant because acridine derivatives are receiving increasing attention as "typical" inhibitors of chymotrypsin (Glazer, 1965).

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