sorption at 1120 cm.$^{-1}$,[3] the latter with two components which are approximately degenerate in free ATP^{4-}. The absorption which is shifted from 1120 to 1175 cm.$^{-1}$ by complex formation with Zn^{2+} is most likely one of these two asymmetric $-PO_3^{2-}$ stretching components. A splitting of the two asymmetric $-PO_3^{2-}$ vibrations by 50–60 cm.$^{-1}$ is also observed in other ligands containing $-PO_3^{2-}$ groups, *e.g.*, diphosphate, on complex formation with Zn^{2+} and also with Cu^{2+}.[4] This removal of degeneracy must be due to a reduction of the pseudo-C_{3v} symmetry of the terminal $-PO_3^{2-}$ group by its coordination to these metal ions. Significant information regarding the mode of binding in these complexes may therefore evolve from a closer study of their 1100–1200-cm.$^{-1}$ absorptions in aqueous solution.

Acknowledgment. Thanks are expressed to the Swiss National Foundation for Scientific Research for financial support of this research.

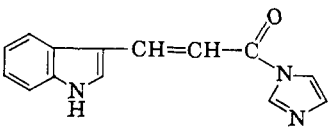
(3) W. Bues and H. W. Gehrke, *Z. anorg. allgem. Chem.*, **288**, 291 (1956).
(4) H. Brintzinger, *Helv. Chim. Acta*, **48**, 47 (1965).

H. Brintzinger
Institute of Inorganic Chemistry, University of Basel
Basel, Switzerland
Received January 18, 1965

Acyl Intermediates in the α-Chymotrypsin-Catalyzed Hydrolysis of Indoleacryloylimidazole

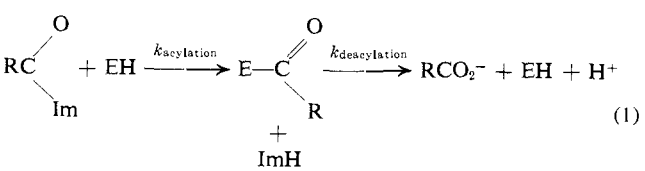
Sir:

The utility of acylimidazoles as acylating agents of serine proteases has been well established by Bender and co-workers.[1,2] Recently we have been concerned with the preparation of acyl-enzyme intermediates with strong absorptions in the ultraviolet, but outside of the absorption regions of the constituent tyrosine and tryptophan residues of the enzyme proteins. Toward this end the kinetics of the reaction of N-(indole-3-acryloyl)imidazole (I) with α-chymotrypsin



I

was investigated spectrophotometrically. A reaction intermediate with an absorption maximum (λ_{max} 360 mμ) lower than the acylimidazole reactant (380 mμ) and higher than the carboxylate product (313 mμ) was detected. When the course of reaction was followed at 350 mμ, there was an initial rise in optical density followed by a decline, suggesting a correspondence with the general pathway proposed by Bender and co-workers[3] (eq. 1).



(1)

A more detailed spectral analysis of the kinetics of the reaction of I with α-chymotrypsin revealed some quantitative additions to the acylation–deacylation mechanism of eq. 1. By following the reaction pathway at particular wave lengths and at various pH, a pH-dependent shift in the spectrum of the acyl-enzyme could be observed. These spectrophotometric changes are more pronounced with I than with cinnamoylimidazole because of the extremely high degree of susceptibility of the indoleacryloyl ultraviolet spectrum to the nature of the acyl substituents (and/or to the nature of the solvent environment).

When the reaction of I with α-chymotrypsin is followed spectrophotometrically near neutrality, two consecutive reactions can be distinguished. In this pH range the first reaction is too rapid to be followed with a conventional recorder. At lower pH, however, there is a measurable, and pH-dependent, rate for this first step. The second step in the reaction can be measured over a wide range of pH and concentrations and is clearly first order. The pH dependence of the rate constants is illustrated in Figure 1. The pH–rate profile for deacylation resembles that reported by Bender and co-workers[3,4] for various deacylation processes. It should be noted, however, that the apparent pK_A' for activation of this acyl-enzyme (7.7) is considerably higher than the various values quoted for other acyl-enzymes.[3] We have measured the apparent pK_A' for activation of cinnamoyl-chymotrypsin in the same buffer solvents and find a value of 7.15 pH, in excellent agreement with a previous report.[4] Hence the difference in pK_A' for the indoleacryloyl-enzyme is real and, we believe, significant.

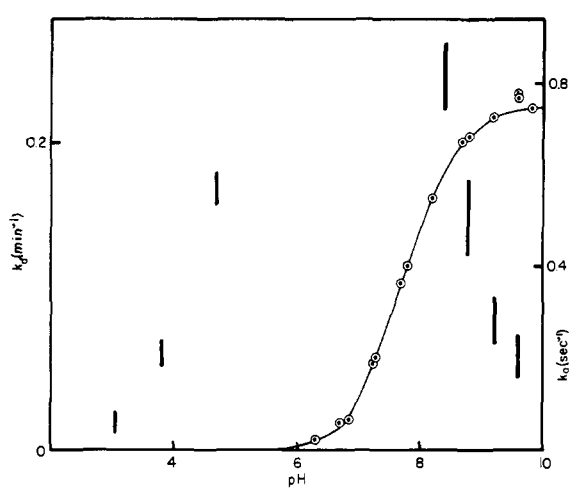


Figure 1. Rates of acylation and deacylation of α-chymotrypsin by indoleacryloylimidazole (I). ○. Deacylation rate (left-hand ordinate) of indoleacryloyl chymotrypsin in pyrophosphate and phosphate buffers, μ = 0.1 *M*. 25°. The solid line is calculated for pK_A' = 7.70. |, Acylation of chymotrypsin by I in acetate and pyrophosphate buffers under apparent pseudo-first-order conditions; $k = -d \ln [I]/dt$ (right-hand ordinate). $E_0 = 1 \times 10^{-4}$ *M*, $[I]_0 = 2 \times 10^{-5}$ *M*. The lack of precision in acylation rates is a consequence of the rapidity of the reaction.

(1) G. R. Schonbaum, B. Zerner, and M. L. Bender, *J. Biol. Chem.*, **236**, 2930 (1961).
(2) M. L. Bender, G. R. Schonbaum, and B. Zerner, *J. Am. Chem. Soc.*, **84**, 2540 (1962).
(3) M. L. Bender, *ibid.*, **84**, 2582 (1962).
(4) M. L. Bender, G. R. Schonbaum, and B. Zerner, *ibid.*, **84**, 2652 (1962).

Essentially the same sequence of rates and spectral changes can be observed in the reaction of I with the bacterial protease subtilisin, a protein which differs from chymotrypsin in secondary and tertiary structure and in sequence about the reactive serine.[5] The pH dependence of the rates in the subtilisin reaction are as in Figure 1, although the pK_A' is somewhat higher.

Since at low pH the deacylation rate of indoleacryloyl-chymotrypsin is significantly slower than the preceding acylation reaction, it is possible to measure the optical density of the acyl intermediate at any pH by the rapid addition of concentrated buffer to a low pH solution of this intermediate. The optical density at 380 mμ is pH dependent and is consistent, over the range pH 4.0–9.8, with that predicted for a weak acid acyl-enzyme intermediate with a single pK_A' of 7.68. This pK_A' is the same as the apparent pK_A' calculated for activation of the "deacylation" process (Figure 1).

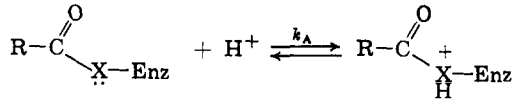
The existence of spectrophotometrically distinct acyl-enzyme acid–base conjugates (Table I) has been demonstrated. These conjugates might arise either

Table I. Spectral Properties of Indoleacryloyl Chymotrypsin

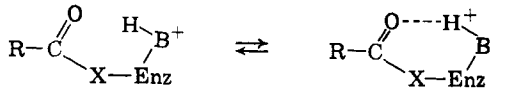
λ, mμ	$\epsilon \times 10^4$, cm.$^{-1}$ M^{-1} pH 4.0	pH 9.5
400	0.30	0.18
390	0.69	0.38
380	1.20	0.72
370	1.65	1.17
360	1.84[a]	1.56
353	1.78	1.63[a]
340	1.40	1.38
330	1.01	1.04
320	0.68	0.76

[a] λ_{max}.

from chemical coupling of the indoleacryloyl resonance chain with a proton (eq. 2) where pK_A or pK_{BH^+} = 7.7, or from a perturbation of the (solvent) environment of the chromophore upon protonation of a basic substituent of the protein. Equation 2a is incompatible



(2a)



(2b)

with the (presumed) acyl–serine ester linkage. In this regard, the dissimilarity of the spectrum of either of the acyl-enzyme conjugates with that of model acyl–serine peptide esters[6] (λ_{max} 330–335 mμ) should be noted.

N-(Indole-3-acryloyl)imidazole was prepared from the recrystallized carboxylic acid and imidazole *via* the mixed anhydride method. It was recrystallized three times from benzene, m.p. 190°. *Anal.* Calcd. for $C_{14}H_{10}ON_3$: C, 71.0; H, 6.8; O, 4.2; N, 17.9. Found: C, 70.7; H, 7.0; O, 4.4; N, 18.0.

A prior preparation from the carboxylic acid, imidazole and dicyclohexylcarbodiimide yielded a product (II, m.p. 182°) with identical elemental analysis and saponification equivalent. With this latter product, three steps in the reaction pathway could be detected kinetically, although the acyl-enzyme intermediate (following the first two steps) was kinetically and spectrophotometrically identical with that described herein. The extra step in the reaction sequence with II could be abolished, by incubation of the substrate in buffer, at a rate equal to its own rate of spectrophotometric change in the presence of buffered enzyme, as was pointed out to us by Professor M. Bender. Surprisingly, only a single hydrolytic step is observable in the "uncatalyzed" reaction of II in buffer, and this rate is identical with that obtained with the presumably clean product (I). We are indebted to Professor Bender for informing us of his observations prior to publication.

Acknowledgment. We wish to acknowledge research grants from the U. S. Public Health Service and the National Science Foundation.

Sidney A. Bernhard, Zohrab H. Tashjian
Department of Chemistry and Institute of Molecular Biology
University of Oregon, Eugene, Oregon
Received August 5, 1964

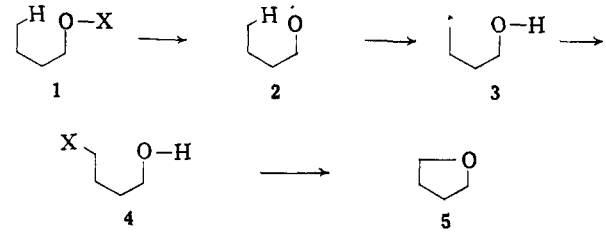
(5) F. Sanger and D. C. Shaw, *Nature*, **197**, 872 (1960).
(6) S. A. Bernhard, S. J. Lau, and H. Noller, *Biochemistry*, in press.

Silver-Catalyzed Decomposition of Hypobromites

Sir:

Recently a number of closely related methods for the *in situ* generation of hypohalites have been described and their reactions extensively studied.[1] Hypohalites can decompose in either an ionic or a radical manner, depending on experimental conditions.[1b] It has generally been recognized that the transformation of hypohalites (of type I) to oxides (of type 5) takes place through free-radical intermediates.[1] This is represented in Scheme I.

Scheme I



In a recent communication, Sneen and Matheny[2] have claimed that, in the presence of silver acetate (or silver oxide) and bromine, alcohols are transformed into oxides by an ionic mechanism. This transformation has been represented as

(1) (a) M. Akhtar and D. H. R. Barton, *J. Am. Chem. Soc.*, **83**, 2213 (1961); (b) M. Akhtar and D. H. R. Barton, *ibid.*, **86**, 1528 (1964); (c) C. Walling and A. Padwa, *ibid.*, **83**, 2207 (1961); (d) F. D. Greene, M. L. Savitz, H. H. Lau, D. Osterholz, and W. N. Smith, *ibid.*, **83**, 2196 (1961); F. D. Greene, M. L. Savitz, H. H. Lau, D. Osterholz, W. N. Smith, and P. M. Zanet, *J. Org. Chem.*, **28**, 55, (1963); (e) Ch. Meystre, K. Heusler, J. Kalvoda, and P. Wieland, *Experientia*, **17**, 475 (1961); *Helv. Chim. Acta*, **45**, 1317 (1962), and later papers. The work of the Ciba group is adequately reviewed: K. Heusler and J. Kalvoda, *Angew. Chem., Intern. Ed. Engl.*, **3**, 525 (1964), and M. Akhtar, *Advan. Photochem.*, **2**, 263 (1964).
(2) R. A. Sneen and N. P. Matheny, *J. Am. Chem. Soc.*, **86**, 3905 (1964); **86**, 5503 (1964).