The pH-Dependence of the Rate of Deacylation of α-Benzamido-*cis*-cinnamoyl-α-chymotrypsin

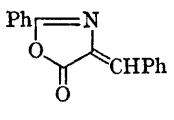
By K. BROCKLEHURST*

(*Department of Biochemistry and Chemistry, St. Bartholomew's Hospital Medical College, Charterhouse Square, London, E.C.*1)

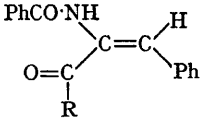
and K. WILLIAMSON

(*Department of Chemistry, John Dalton College of Technology, Chester Street, Manchester* 1)

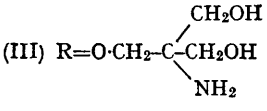
WE reported[1] that the decay of 4-*cis*-benzylidene-2-phenyl-2-oxazolin-5-one (I)[2] as measured by the fall in ultraviolet absorption at *ca.* 360 mμ on admixture of (I) with an excess of α-chymotrypsin is very rapid and that the pH-dependence of the rate of the subsequent slow deacylation of the acyl-enzyme (II) which was assumed to be formed, as measured by the fall in ultraviolet absorption at 310 mμ, in the pH range *ca.* 5·5—7·0 follows a rate equation for a single ionizing group required for activity in the base form, *i.e.*, $k = \bar{k}/(1 + [H^+]/K)$ where $\bar{k} = 130 \times 10^{-3}$ sec.$^{-1}$ and pK = 7·68. In the pH range *ca.* 7—9, however, the observed rate constants were found to be much less than those predicted by this rate equation. The experiments in this pH range were carried out in Tris buffer.



(I)



(II) R=Enzyme



(III) R=O·CH$_2$–C(CH$_2$OH)(NH$_2$)–CH$_2$OH

(IV) R=NH·C(CH$_2$OH)$_3$

We now report that when the measurements in the pH range *ca.* 7—10 are made in phosphate and borate buffers the rate of deacylation as measured by the fall in ultraviolet absorption at 310 mμ follows a rate equation of the above form where $\bar{k} = 154 \times 10^{-3}$ sec.$^{-1}$ and pK = 7·64. The pH-dependences of the rates of deacylation of α-benzamido-*cis*-cinnamoyl-α-chymotrypsin and *trans*-cinnamoyl-α-chymotrypsin are compared in Figure 1.

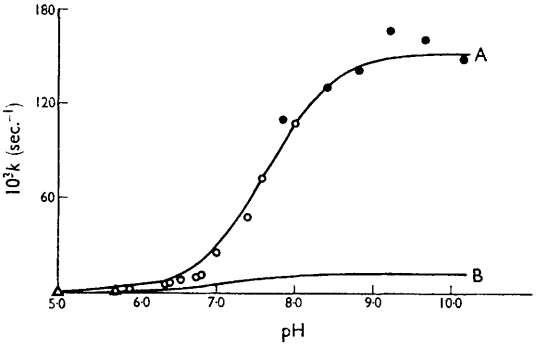


FIGURE 1. *Deacylation of* (A) α-*benzamido*-cis-*cinnamoyl-α-chymotrypsin and* (B) trans-*cinnamoyl-α-chymotrypsin.* (A): *buffers,* △ *Acetate;* ○ *phosphate;* ● *borate;* 4·8% *dioxan;* I = 0·1; 25·0°; *the points are experimental and the curve is theoretical for* $\bar{k} = 154 \times 10^{-3}$ *sec.*$^{-1}$ *and* pK = 7·64. (B) *is taken from Bender, Schonbaum, and Zerner*[3]; *the curve is theoretical for* $\bar{k} = 12{\cdot}5 \times 10^{-3}$ *sec.*$^{-1}$ *and* pK = 7·15.

Although the nonenzymic rate of disappearance of (I) in Tris buffer at pH 7·6 is greater than its rate of disappearance in phosphate buffer at the same

pH, this rate is small compared with the rate of disappearance of (I) in the presence of α-chymotrypsin

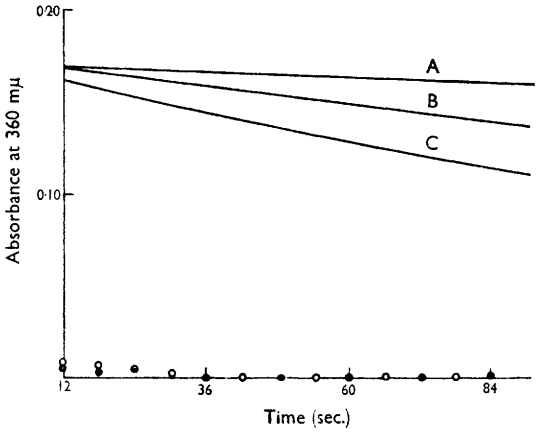


FIGURE 2. *Rates of decay of* 4-cis-*benzylidene*-2-*phenyl*-2-*oxazolin*-5-*one* (I): 4·8% *dioxan*; I = 0·1; 25·0°; [I] = 5·6 × 10^{-6}M; (A) *Phosphate buffer pH* 7·6; (B) *Tris buffer pH* 7·3; (C) *Tris buffer pH* 7·6; ○ *phosphate buffer pH* 7·6 + α-*chymotrypsin* (4 × 10^{-5}M); ● *Tris buffer pH* 7·6 + α-*chymotrypsin* (4 × 10^{-5}M).

in both types of buffer at this pH (see Figure 2). Preliminary experiments show that when the acylation of α-chymotrypsin by (I) is carried out in acetate buffer at pH 5·5, where deacylation is very slow (see Figure 1) and the subsequent deacylation of a sample of this acyl-enzyme is carried out in Tris buffer at pH 8 the observed rate of deacylation is much greater than that observed when both acylation and deacylation are carried out in Tris buffer at pH 8. These findings suggest that Tris is not merely lowering the rate of the deacylation step and that either Tris directs the acylation of α-chymotrypsin by (I) to a site which is different from that acylated by (I) in the other buffers or that α-chymotrypsin catalyzes the acylation of Tris by (I) and the absorbance change at 310 mμ represents the hydrolysis of either (III) or (IV).

These possibilities and also the nature of the secondary absorbance change at 310 mμ which results in a very slow fall in the infinity readings of the deacylation reactions are being investigated further.

(*Received, May 18th*, 1967; *Com.* 487.)

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[2] R. E. Buckles, R. Filler, and L. Hilfman, *J. Org. Chem.*, 1952, **17**, 233.
[3] M. L. Bender, G. R. Schonbaum, and B. Zerner, *J. Amer. Chem. Soc.*, 1962, **84**, 2540.