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## Studies on the action of carboxypeptidase B on polylysine

The hydrolysis of high molecular weight, synthetic polyamino acids by proteolytic endopeptidases depends dramatically in some instances on both the conformation and charge state of the substrate[1-3]. Chain length dependences are also known, *e.g.*, glutamyl-glutamic acid as well as glutamyl-glutamyl-glutamic acid is an extremely poor substrate for papain compared to polyglutamic acid[4]. Although exopeptidases are known to hydrolyze polyamino acids[5-7], little quantitative data have been reported. The action of carboxypeptidase B on poly-α-L-lysine is reported in this communication.

Porcine carboxypeptidase B (Worthington Biochem. Corp, COB6073) was used at a typical concentration of $5 \cdot 10^{-7}$ M. The specific activity, determined by the method of WOLFF, SCHIRMER AND FOLK[8], was 75 units/mg. Poly-α-L-lysine hydrobromide (Pilot Chemicals, Inc; wt. av. mol. wt. 110 000) was hydrolyzed at 25° in solutions 0.20 M in NaCl and 0.046 M in Tris buffer over the pH range 6.5–10. Above pH 10 the

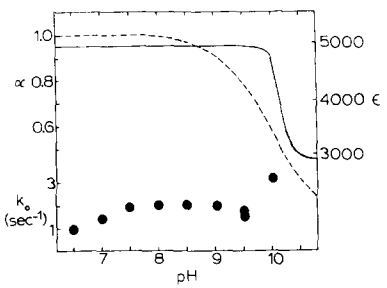



Fig. 1. The pH dependence of $k_0$ (●). Also shown is $\alpha$ (— — —), the fraction of the side chain amines as $NH_3^+$; and $\varepsilon$ (———), the molar (residue) absorptivity at 201 mμ.

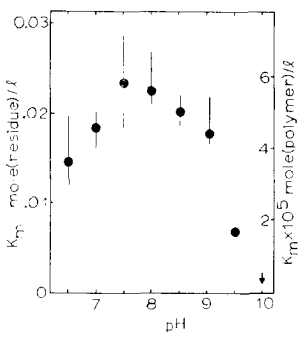
polymer is only slightly soluble. The reaction was followed in a Beckman DU spectrophotometer at 237 mμ. At this wavelength the absorptivity of the polymer is only slightly dependent on conformation, and is over 20 times the absorptivity of the monomer. As monomer is split off, the rate of hydrolysis may be calculated from the decrease in absorption. At pH 10.0 absorbance–time plots exhibited curvature indicative of enzyme inactivation. At other pH's such curvature was not observed. At a fixed pH, the substrate concentration was varied and the kinetic parameters $k_0$ and $K_m$ determined from a double reciprocal plot.

In Figs. 1 and 2, $k_0$ and $K_m$, respectively, are shown as a function of pH. Also in Fig. 1 are shown the low ultraviolet spectrum, indicative of polymer conformation, and the titration curve of the polymer[2]. In Fig. 3 is plotted the pH dependence of $k_0/K_m$.

The two kinetic parameters are seen to vary with pH in nearly the same manner from pH 6.5 through pH 9, similar to that observed for hippuryl-L-arginine hydrolysis (ref. 8; J. E. FOLK, personal communication). Above pH 9 $K_m$ decreases and at pH 10.0 only an upper limit was obtainable. At pH 10.0 about 45% of the side-chain amines are uncharged, whereas the polymer conformation is still predominantly random coil.

The changes are clearly not a result of changes in substrate conformation, nor is such a dependence likely to be observable in exopeptidase hydrolysis. Above pH 10.2 most of the residues are in the $\alpha$-helical conformation, but end residues still have a high probability of being in random conformations[9]. Conditions for observing a conformational dependence would be very difficult to achieve. The drop-off in $K_m$ above pH 9, though not substrate-conformation dependent, may be a result of the change in side chain charge on the substrate. Studies on chemically similar small substrates do not exist in this pH range for comparison.

In comparison to small substrates, polylysine is a poor substrate for carboxypeptidase B. At pH 8.0, for example, $\alpha$-*N*-benzoyl-L-lysyl-L-lysine is hydrolyzed with



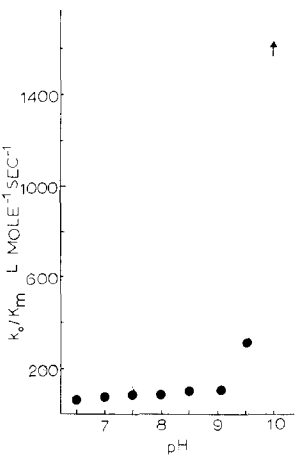




Fig. 2. The pH dependence of $K_m$ on a "moles of residues per l" basis and on a "moles of substrate molecules per l" basis, assuming a number average degree of polymerization of 400. At pH 10.0 only an upper limit of $K_m$ is shown.

Fig. 3. The pH dependence of $k_0/K_m$. A lower limit is shown at pH 10.0.

a $k_0$ and $K_m$ of 86 $sec^{-1}$ and $1.8 \cdot 10^{-4}$ M, respectively[8]. Small molecules are also competitive inhibitors, with L-lysine and $\alpha$-*N*-benzoyl-L-lysine having inhibition constants at pH 8.0 of $1.3 \cdot 10^{-2}$ M and $1.1 \cdot 10^{-3}$ M, respectively. Inasmuch as polyamino acids sometimes act as inhibitors in enzyme catalysis[10-12] one could postulate that $k_0$ is small because the long polymer chain has multiple sites for binding to the enzyme, but only binding at the C-terminal end can lead to hydrolysis. The substrate would be an autoinhibitor. Assuming each residue is a potential binding site, there are, on the average, 300–500 potential binding sites per substrate molecule, depending on what one assumes the molecular weight distribution to be. If each binding site is as strong or stronger than the one leading to hydrolysis, $K_m$ calculated on a "moles of substrate (polymer) per l" basis should be exceedingly small, while that calculated on a "moles of residues per l" basis should fall in the normal range. At pH 8.0, $K_m$ on a "moles of substrate" basis, is within a factor of three of the $K_m$ for *N*-benzoyl-lysyl-lysine while on a "moles of residues" basis it is very large, even larger than the inhibition constant for the weak inhibitor, L-lysine. Thus on the basis of available data, nonproductive

binding is probably not the basis for polylysine being a rather poor substrate for carboxypeptidase B.

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*Department of Chemistry,*
*University of Iowa,*
*Iowa City, Iowa (U.S.A.)*

DANIEL D. JONES*
WILMER G. MILLER**

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* Present address: Department of Biochemistry and Biophysics, University of Michigan, Ann Arbor, Mich., U.S.A.
** Present address: Department of Chemistry, University of Minnesota, Minneapolis, Minn., U.S.A.