discovered with the co-operation of Dr. R. Aschaffenburg, Reading, England. This discovery does not alter the acceptable nomenclature of α_s-*A* and α_s-*B*, and a detailed report of its occurrence is in preparation.

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Poly-α-amino-acids containing L-Glutamyl Residues as Substrates for Pepsin

In a study of the action of pepsin on various poly-α-amino-acids we have found that poly-L-glutamic acid as well as some amino-acid copolymers containing L-glutamic acid residues are readily hydrolysed by pepsin. Digestion was tested at 35° in 3 ml. solutions containing 12 mg of polyamino-acid[1] and 0·8 mg crystalline pepsin, adjusted to *p*H 2·3. Since most of the polypeptides investigated do not dissolve directly at acid *p*H, they were dissolved in the minimal amount of 0·1 N sodium hydroxide, and the required *p*H was obtained by adding 0·1 N hydrochloric acid. Hydrolysis was followed by measuring : (*a*) the uptake of acid in the *p*H-stat ; (*b*) the increase in the colour produced with ninhydrin[2] ; (*c*) the increase in Van-Slyke amino-nitrogen. Fair agreement was found in the results obtained by the foregoing three methods. The peptic digests were analysed by descending paper chromatography in *n*-propanol/water/concentrated aqueous ammonia (100 : 50 : 1 v/v). The chromatograms were run for 48 h, and were revealed with ninhydrin.

The following poly-α-amino-acids were resistant to peptic action : poly-L-aspartic acid, poly-L-lysine, poly-DL-alanine, poly-L-serine, poly-L-proline, poly-hydroxy-L-proline and the water insoluble poly-L-phenylalanine and poly-L-tyrosine.

Approximately 16 per cent of the peptide links of a poly-L-glutamic acid preparation, with an average degree of polymerization $n = 125$, were hydrolysed within 30 min. No marked increase in the extent of hydrolysis occurred on additional incubation for 24 h. From the uptake of hydrochloric acid at *p*H 2·3 an initial rate of hydrolysis of 3·0 μmoles peptide bond per min per mg of pepsin was calculated, assuming a negligible extent of ionization of the newly formed carboxyl groups. The rate of hydrolysis of poly-L-glutamic acid is thus very markedly greater than that of low molecular weight synthetic pepsin substrates[3].

Oligopeptides containing 2–9 glutamic acid residues could readily be detected on paper chromatograms of the peptic digests of poly-L-glutamic acid. High oligopeptides Glu_4 to Glu_9 appear in the incubation mixture at the initial stages of the enzymatic reaction (10–30 min of incubation). Further incubation results in their conversion into lower oligopeptides, triglutamic acid being the major product of exhaustive enzymatic hydrolysis. Synthetic α,L-glutamyl oligopeptides (Glu_2 to Glu_8)[4] were used as markers in these experiments.

Chromatographic analysis revealed that under the experimental conditions used, synthetic α- di-, tri-, and tetra-L-glutamic acids are essentially resistant to hydrolysis by pepsin. Penta-, hexa-, hepta- and octa-L-glutamic acids, however, are readily hydrolysed, mainly to triglutamic acid.

The rate of enzymatic hydrolysis of poly-L-glutamic ($n = 125$) at 35° decreased approximately 100-fold, on increasing the *p*H from a value of 2·3 to 4·5. The relatively low rate of peptic digestion of poly-L-glutamic acid at *p*H 4–4·5 is in accord with the recent findings of Simons *et al.*[5].

Copolymers of L-glutamic acid with D-glutamic acid, L-aspartic acid, L-lysine, L-alanine, L-tyrosine or L-phenylalanine were also tested for susceptibility to peptic digestion. Copolymers with relatively high L-glutamic acid content, such as L-Glu : D-Glu (molar residue ratio 11 : 1), L-Glu : L-Asp (9 : 1) or (5 : 1) and L-Glu : L-Tyr (9 : 1) were hydrolysed at a rate and to an extent similar to those of poly-L-glutamic acid. Copolymers with relatively low contents of L-glutamic acid were hydrolysed at slower rates and to lesser extents than poly-L-glutamic acid. The copolymers L-Glu : D-Glu (1 : 1), L-Glu : L-Asp (1 : 5) and L-Glu : L-Lys (1 : 5) were resistant to peptic digestion.

Since the high-molecular-weight poly-L-glutamic acid is readily hydrolysed by pepsin at *p*H values (*p*H 2·3–4·0) at which the substrate molecules possess a helical conformation[6], it seems that pepsin might act on polypeptides possessing a specific macromolecular stereochemical structure. A similar conclusion was reached recently by Simons *et al.*[5] from their investigation of the action of pepsin on poly-L-glutamic acid at *p*H 4·18.

The formation of the high oligopeptides at the initial stages of the enzymatic hydrolysis of poly-L-glutamic acid suggests that pepsin acts on the high-molecular-weight synthetic substrates by an 'intermediate' mechanism[7]. Our experimental findings rule out for pepsin a 'one by one' mechanism[7], in accord with the findings of Ginsburg and Schachman[8].

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