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The determination of aminotripeptidase activity by a rapid spectrophotometric method

Aminotripeptidase catalyses the hydrolysis of tripeptides at their N-terminal peptide bond, forming a dipeptide and a free amino acid[1]. The usual substrate for routine assay of tripeptidase is triglycine, and the reaction is usually followed by the titrimetric method of GRASSMANN AND HEYDE[2]. This method is time-consuming, and because the end-point fades, reproducible results are difficult to obtain.

Amino acids and peptides form coloured copper complexes[3]. It was discovered that triglycine forms a complex with a maximum absorbance at 550 mμ, while glycylglycine and glycine both form complexes with maximum absorbance at 640 mμ, suggesting that the decrease in absorbance at 550 mμ and the increase in absorbance at 640 mμ should both follow the hydrolysis of triglycine by aminotripeptidase.

Copper complexes were made by addition of 0.5 ml of test solution to 3 ml washed copper phosphate suspension[5], and centrifuging after standing for 5 min. Absorbances at 550 and 640 mμ were then measured. The colour reached a maximum after 5 min and was stable for at least 30 min.

Synthetic mixtures of triglycine with glycine *plus* glycylglycine representing varying degrees of hydrolysis of the tripeptide were made, and their absorption spectra determined (Fig. 1). The degree of simulated hydrolysis was directly related to decrease in absorbance at 550 mμ, and to increase at 640 mμ.

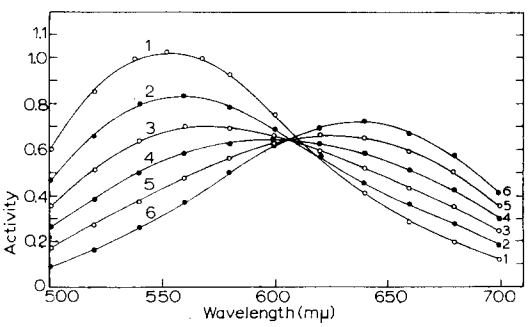
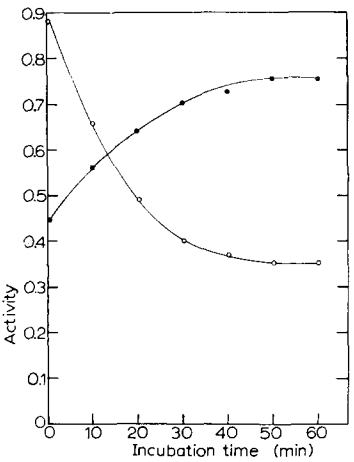


Fig. 1. Absorption spectra of copper complexes formed from mixtures of triglycine with glycylglycine and glycine. Various degrees of hydrolysis of triglycine were simulated by mixing 0.05 M triglycine and a solution 0.05 M with respect to both glycine and glycylglycine in varying proportions. Copper complexes were formed from 0.5 ml of each mixture by adding 3 ml of copper phosphate suspension, allowing to stand 5 min and centrifuging. Numbering of curves refers to the proportion of triglycine, as follows: Curve 1, 100%; Curve 2, 80%; Curve 3, 60%; Curve 4, 40%; Curve 5, 20%; Curve 6, 0% triglycine; respectively representing 0, 20, 40, 60, 80 and 100% hydrolysis.

Horse erythrocyte aminotripeptidase was prepared according to the methods of ADAMS, DAVIS AND SMITH[6]. 1.0 ml of the purified enzyme was incubated at 37°, pH 7.9 with 0.2 mmole Tris and 0.25 mmole triglycine in a total volume of 5 ml, and 0.5-ml samples were withdrawn at intervals to follow the course of hydrolysis by the change in absorbance of the copper complexes at 550 and 640 mμ (Fig. 2). There was no change in absorbance in control systems without enzyme, nor in controls without



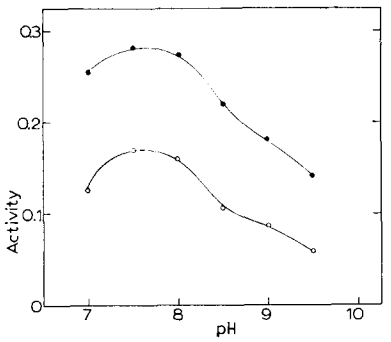


Fig. 2. Enzymic hydrolysis of triglycine followed by absorbance of copper complexes. Purified horse aminotripeptidase was incubated at 37° with triglycine at pH 7.9 as described in the text. Samples were withdrawn at intervals, and absorbance of the copper complexes determined at 640 mμ (full circles) and 550 mμ (open circles). 100% hydrolysis of the tripeptide had been achieved after approx. 50 min incubation, as can be seen.

Fig. 3. Effect of pH on hydrolysis of triglycine by aminotripeptidase. 0.5 ml of purified aminotripeptidase was incubated at 37° with 0.1 mmole Tris and 0.125 mmole triglycine in 2.5 ml total volume at several pH values. Immediately after adding enzyme and after 10 and 20 min 0.5-ml samples were withdrawn for copper complexing. Absorbance of copper complexes was measured at 550 and 640 mμ. The absorbance difference between zero and 20-min samples was the measure of activity. Open circles refer to absorbance at 640 mμ due to appearance of glycylglycine and glycine; full circles refer to 550 mμ, due to disappearance of triglycine.

triglycine. The pH optimum of the aminotripeptidase was easily and rapidly determined by this method using Tris buffer throughout, adjusting the pH of buffer and triglycine solutions beforehand by adding 1 M NaOH (Fig. 3). The decrease in triglycine and increase in hydrolytic products gave exactly similar values for the pH optimum, suggesting that the method could just as accurately be applied to the routine assay of aminotripeptidase using the decrease in absorbance due to copper complex at 550 mμ as the sole parameter.

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