Ion-determined specificity of peptidase activity

Among the peptidase present in animal tissues, certainly leucine aminopeptidase has been the most studied. This enzyme, identified first by LINDERSTROM-LANG[1], has been recently isolated and purified from swine kidney by SMITH *et al.*[2]; it has a wide specificity, as it hydrolyzes not only leucyl compounds but many other peptides and amides as well[3,4]. The presence of Mn^{++} (or Mg^{++}) is necessary in order to reach maximal activity; activation by these ions requires an incubation period at 38° C, and this period becomes shorter the more purified the preparation. L(—)leucyl-glycine (LG) is a typical substrate. Glycyl-L(—)leucine (GL) and Glycyl-glycine (GG) are among the peptides which are not specifically attacked by leucine aminopeptidase. They are believed to be hydrolyzed by two distinct dipeptidases.

It has been observed in a recent paper[5] that GL was rapidly hydrolyzed by a partially purified preparation of leucine aminopeptidase from human placenta, when the latter was not incubated beforehand with Mn^{++} at 38° C and without any ion added. Further observations[6] demonstrated that a distinct activity towards GG appeared when the same preparation of leucine aminopeptidase was incubated at 38° C with Co^{++} 0.001 *M*.

These results are confirmed in the present note with a more purified preparation of leucine aminopeptidase from swine kidney. Table I shows that the same enzymic preparation at the fourth stage of purification (see below) can hydrolyze GL, LG and GG under different experimental conditions. GL is the substrate most rapidly hydrolyzed when the enzyme is not incubated; incubation with Mn^{++} lessens GL hydrolysis and enhances that of LG and finally, incubation with Co^{++} lessens hydrolysis of GL and LG and enhances that of GG. No ion addition is necessary for hydrolysis of GL, as the latter is probably firmly bonded to the protein; however GL hydrolysis is also metal dependent, since Versene 0.001 *M* as well as dialysis against bidistilled water strongly inhibits it.

TABLE I

ACTIVITY OF PARTIALLY PURIFIED PREPARATION OF PEPTIDASE (STAGE IV) TOWARDS GL, LG AND GG

	Enzyme concn. mg for ml	*Time in min*	*Hydrolysis % without incubation*	*Hydrolysis % incub. for 15 min with 0.002 M $MnSO_4$*	*Hydrolysis % incub. for 10 min with 0.0005 M $CoCl_2$*
GL	0.007	7	41	11	22
		14	65	—	—
		21	85	34	37
		35	—	40	43
LG	0.007	7	10	29	8
		14	—	55	—
		21	33	74	12
		35	53	—	16
GG	0.014	7	11	8	22
		14	—	—	42
		21	18	10	54
		35	25	14	—

Buffer borate according to CLARK, 0.2 *M*, pH 8.8. Each flask contained: 0.3 ml buffer; 0.2 ml substrate, 0.25 *M*; 0.1 ml enzyme solution; 0.1 ml of metal solution (when added); made up to 1 ml with bidistilled water. In experiments with Mn^{++} and Co^{++}, these metals were added directly to the flasks and incubated with enzyme at 38° C; hydrolysis was then started by adding substrate.

The working hypothesis that only one enzyme is responsible for the activity towards the three different substrates, is confirmed by the fact that a purification of the enzyme of about fifty-fold does not really modify the relative rates of hydrolysis of GL, LG and GG. Table II.

The enzymic preparations used were prepared according to the method of SMITH[2] modified as follows:

Stage I. Fresh swine kidney was homogenized for two minutes with cold water (one volume), centrifuged and reextracted in the same manner; the supernatants were mixed together and the solution was used for the activity test. Total N = about 9 mg/ml.

Stage II. From stage I, acetone-dried powder was prepared as described elsewhere[7]; it was extracted twice with a double volume of Clark borate buffer 0.2 *M*, pH 8.8, for 20 minutes at

TABLE II

PROTEOLYTIC COEFFICIENTS (FIRST ORDER VELOCITY CONSTANT/mg PROTEIN N/ml) OF GL, LG AND GG IN FOUR DIFFERENT ENZYMIC PREPARATIONS

	Stage I	*Relative rate*	*Stage II*	*Relative rate*	*Stage III*	*Relative rate*	*Stage IV*	*Relative rate*
GL	0.060	1	0.38	1	1.6	1	2.8	1
LG	0.039	0.65	0.24	0.63	1.28	0.70	1.8	0.63
GG	0.016	0.26	0.10	0.27	0.40	0.25	0.65	0 2

Relative rates are given treating the total activity of GL as being equal to 1 and expressing the total activity of LG and GG as a fraction of it.

38° C. The extract was centrifuged and the supernatant was used for enzyme activity tests. Total N = about 3 mg/ml.

Stage III. The preceding solution was brought to 40% saturation with ammonium sulfate and the precipitate discarded; the supernatant was brought to 60% saturation and the precipitate dissolved in borate buffer was used for enzyme activity tests. Total N = about 1.5 mg/ml.

Stage IV. The preparation from stage III was brought to 50% saturation with ammonium sulfate; the precipitate dissolved in borate buffer was brought to 40% saturation and the precipitate discarded; the supernatant was brought to 80% saturation and the precipitate dissolved in the same borate buffer. Total N = about 0.6 mg/ml.

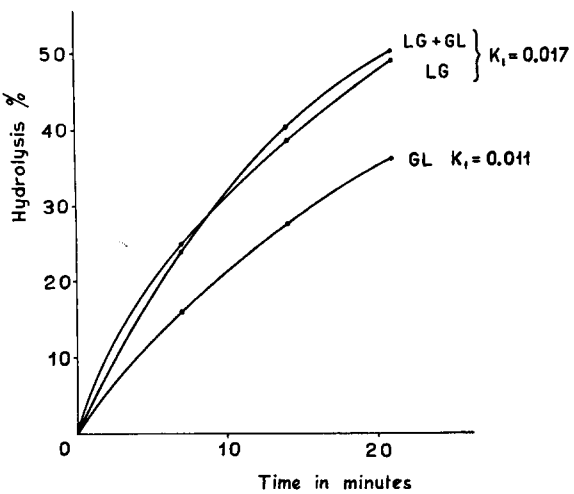

Fig. 1. Hydrolysis of GL, LG and GL + LG by a partially purified preparation (Stage IV) of leucine aminopeptidase preincubated for 15 minutes at 38° C with $MnSO_4$ 0.002 *M*. Total protein N per ml = 0.0052 mg.

The protein N was determined by microkjeldahl after prolonged dialysis to remove excess ammonium sulfate. The amount of hydrolysis was determined according to GRASSMAN AND HEYDE[8]; the substrates were products of Hoffmann La Roche (Basle).

Finally, other experiments confirming that the different enzymic activities are due to the same enzyme are reported in Fig. 1. It is shown that the enzyme, when preincubated with Mn^{++} and added to a solution containing both LG and GL shows a rate of total hydrolysis which is not the sum of the hydrolysis of each peptide as would be expected if two different enzymes were present. Instead, the rate corresponds exactly to that of the peptide which is hydrolysed more rapidly. Identical results are obtained when GL + GG act upon a Co^{++} incubated enzyme mixture.

The results presented allow one to suppose that leucine aminopeptidase either has different active groups whose activity is determined by specific metals and cannot act simultaneously, or has group(s) with different activities when different metals are bound.

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