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Intestinal Dipeptidases

IX. Studies on Dipeptidases of Human Intestinal Mucosa

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Recently a spectrophotometric method for assaying dipeptidase activity has been described.[1] The method has been employed for investigation of various intestinal dipeptidases, including the activities against L-alanyl-L-glutamic acid, L-alanyl-L-proline, glycylglycine, glycyl-L-leucine, and glycyl-L-valine, in different species (for ref. see 2).

Because of interest of covering the dipeptidases performing the hydrolysis of dipeptides containing glutamic acid and proline, issued from studies of gastrointestinal disorders, additional reactions have now been included in the investigation. The present report describes the results obtained from studies of the L-glutamyl-L-proline, L-glutamyl-L-valine, L-valyl-L-glutamic acid, and L-valyl-L-proline dipeptidase activities in the human small intestine.

The dipeptides, L-glutamyl-L-proline, L-glutamyl-L-valine, L-valyl-L-glutamic acid, and L-valyl-L-proline, were all products of YEDA, Rehovoth, Israel. For the assays they were used in aqueous solutions in the following concentrations: 0.008 M L-glutamyl-L-proline; 0.03 M L-glutamyl-L-valine; 0.03 M L-valyl-L-glutamic acid; 0.006 M L-valyl-L-proline. The corresponding amino acids (Mann Research Labs., New York) were used in aqueous solutions in combinations and concentrations as follows: 0.008 M L-glutamic acid and 0.008 M L-proline; 0.03 M L-glutamic acid and 0.03 M L-valine; 0.006 M L-valine and 0.006 M L-proline. The assay procedure was the same as described previously.[1] As enzyme solution served human mucosal homogenates prepared as described elsewhere.[3] The mucosa was scraped off from operation specimens, taken from the distal part of the ileum of four adult humans.[3]

The effect of pH on the different dipeptidase reactions was studied over a pH range from 5.0 to 9.5 by using 0.15 M phosphate and borate buffer solutions. The pH-optima of the four reactions differed slightly from each other (Fig. 1). They were found to be 7.2 for L-glutamyl-L-proline dipeptidase, 6.8 for L-glutamyl-L-valine dipeptidase, 7.9 for L-valyl-L-glutamic acid dipeptidase, and 6.7 for L-valyl-L-proline dipeptidase.

Table 1. Effect of bivalent metal ions (final concentration 5.9×10^{-5} M) on dipeptidase activities of the small intestine of adult human. Optimum pH.

Substrate	Relative rate of hydrolysis [a] in presence of metal ions			
	Co^{2+}	Mn^{2+}	Mg^{2+}	Zn^{2+}
L-Glutamyl-L-proline	0.9	0.9	1.0	1.0
L-Glutamyl-L-valine	0.3	0.5	0.6	0.1
L-Valyl-L-glutamic acid	0.7	1.0	0.9	0.7
L-Valyl-L-proline	1.2	1.2	1.2	1.3

[a] The rate of hydrolysis without metal ions added taken as 1.

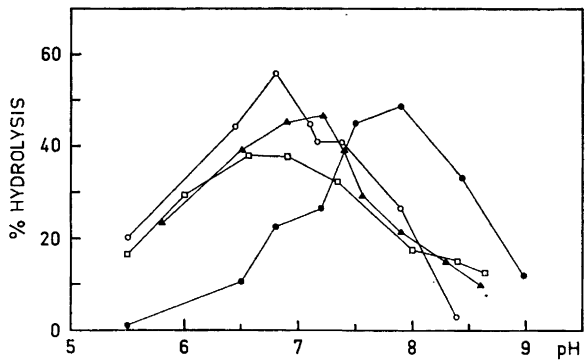

Fig. 1. Effect of pH on the human intestinal dipeptidase activities. ● Mucosal extract containing 3.0 μg of nitrogen added to 1.5 μmoles of L-valyl-L-glutamic acid, 10 min digestion. ○ Mucosal extract containing 2.6 μg of nitrogen added to 1.5 μmoles of L-glutamyl-L-valine, 10 min digestion. □ Mucosal extract containing 2.0 μg of nitrogen added to 0.3 μmole of L-valyl-L-proline, 20 min digestion. ▲ Mucosal extract containing 2.8 μg of nitrogen added to 0.4 μmole of L-glutamyl-L-proline, 30 min digestion.

The influence of Co^{2+}, Mn^{2+}, Mg^{2+}, and Zn^{2+} ions on the four different reactions was also studied. The metal ions were added to the assay mixtures in 100 μM concentrations [1] (final concentration 5.9×10^{-5} M) and the various dipeptidase activities were studied at optimum pH. The results, given in Table 1, revealed that the L-glutamyl-L-valine dipeptidase activity was strongly reduced by all the metal ions investigated, while the L-valyl-L-glutamic acid dipeptidase activity only was reduced by Co^{2+} and Zn^{2+} ions. The L-valyl-L-proline dipeptidase activity showed a slight increase after addition of the metal ions, while they had no influence on the L-glutamyl-L-proline dipeptidase activity.

Determination of the specific activity (units per mg N [4]) against the four different dipeptides in the human intestinal mucosa, gave the following mean values: 2.4 for the L-glutamyl-L-proline dipeptidase activity; 22.5 for the L-glutamyl-L-valine dipeptidase activity; 12.0 for the L-valyl-L-glutamic acid dipeptidase activity, and 1.9 for the L-valyl-L-proline dipeptidase activity.

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