THE ABSORPTION OF PEPTIDES

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SUMMARY

The absorptive pattern of a series of peptides has been investigated with an *in vitro* sac preparation from rat intestine.

Results demonstrate a specificity of hydrolysis and ability to traverse the intestinal wall.

The mechanism is discussed whereby accumulation of intact peptide may occur in the serosal solution.

INTRODUCTION

Although it has long been known that peptides are liberated within the intestinal lumen during the partial enzymic degradation of ingested proteins, little information has accumulated concerning a necessity for complete breakdown to the amino acid level as a requirement for actual absorption. It is generally agreed that most if not all of the breakdown products from dietary proteins are absorbed as amino acids. Nevertheless, the possibility exists that peptides may also penetrate the intestinal mucosa in significant amounts. AGAR, HIRD AND SIDHU[1] have demonstrated that small quantities of glycyl-glycine are transported through an *in vitro* intestinal preparation. In addition a trace of L-leucyl-glycine was also transported. NEWEY AND SMYTH[2] have employed both *in vitro* and *in vivo* methods for a study of the absorption of three glycyl dipeptides. They reported that very little, if any, of the dipeptide was in the blood or in the fluid carried through the intestinal wall. In the presence of residual dipeptide in the intestinal lumen, very small amounts of the peptide at most were found on the other side of the intestinal barrier. In a preliminary study from this laboratory[3], the *in vitro* technique of WILSON AND WISEMAN[4] was employed to study the absorption of alanyl-phenylalanine. The investigation has now been expanded using this convenient technique to investigate a series of small peptides in order to ascertain rates and specificities of hydrolysis and penetration through the isolated rat intestine.

EXPERIMENTAL

The preparation of sacs from everted small intestines of rats was identical to the method described by WILSON AND WISEMAN. The sacs were filled with and incubated in Krebs-Ringer bicarbonate buffer, pH 7.4, containing 200 mg % glucose. Initial peptide concentrations were 0.01 *M*. The sacs were incubated for various time inter-

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vals at 37° in an atmosphere of 95 % oxygen and 5 % CO_2. Following each experiment, the sac was removed from the mucosal solution, washed, blotted on filter paper, and emptied of its contents which represented the serosal solution. The resulting solutions as well as original sample were analyzed by paper chromatographic and quantitative ninhydrin techniques.

RESULTS

A comparison of the *in vitro* absorption of 5 dipeptides is shown in Fig. 1. The graphic representation is designed to illustrate the relative concentrations of peptide and amino acid in the mucosal and serosal solutions, following incubations of 8 and 120 min. The substrate, L-alanyl-L-phenylalanine, was found to be 90 % hydrolyzed in the first 8 min of incubation. The liberated amino acids were found in greater

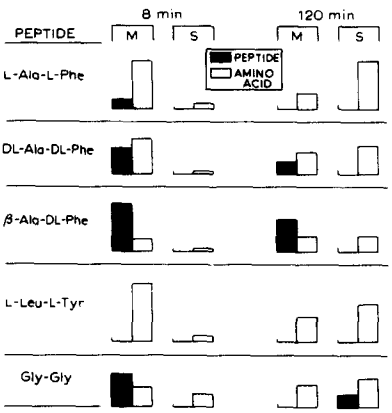

Fig. 1. Relative concentrations of peptides and amino acids after various times of incubation of peptides (0.01 *M*) in an everted intestinal sac: Ala = alanine; Phe = phenylalanine; Leu = leucine; Tyr = tyrosine; Gly = glycine; M = mucosal solution; S = serosal solution.

concentrations on the mucosal side although a significant amount of free amino acids had crossed the intestinal wall. During even shorter periods of incubation, larger amounts of residual peptide were found on the mucosal side although in no case could this peptide be shown on the serosal side by chromatographic analysis. After 30 min or more of incubation, a higher concentration of amino acids was found on the serosal side than on the mucosal side, thus confirming the previously established active absorption of many amino acids[5,6]. By comparison, DL-alanyl-DL-phenylalanine was hydrolyzed at a much slower rate and even at 120 min, residual peptide was evident. An even slower hydrolysis was evident using β-alanyl-DL-phenylalanine as a substrate, whereas L-leucyl-L-tyrosine, under identical conditions, was completely hydrolyzed in the first 8 min of incubation, with the amino acids subsequently absorbed against a concentration gradient. With none of the first 4 substrates were peptides chromatographically detectable on the serosal side of the preparation at any time. When glycyl-glycine was tested in this preparation, a slow hydrolysis was evident in brief periods of incubation. Only a trace amount of glycyl-glycine was found on the serosal side after 8 min, but progressively thereafter larger and larger amounts of the peptide were chromatographically detectable on the serosal side. Even after 120 min a significant amount of peptide remained on the serosal side although it was no longer detectable on the mucosal side. These results confirm the observed absorption of glycyl-glycine through an intestinal preparation as reported by AGAR *et al.*[1].

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In order further to examine the mechanism by which glycyl-glycine absorption occurs, glycine and a series of polyglycines were studied in an identical fashion which is illustrated by Fig. 2. Glycine alone was tested and found to be rapidly absorbed through the preparation against a concentration gradient. Glycyl-glycine, as previously observed, was slowly hydrolyzed on the mucosal side, and progressively larger

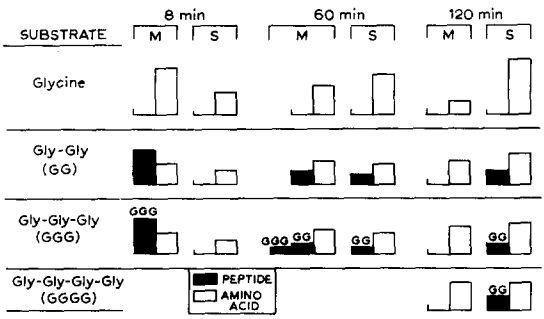

Fig. 2. Relative concentrations of peptides and amino acids after various times of incubation of glycine and glycine polymers (0.01 *M*) in an everted intestinal sac.

amounts of intact peptide appeared on the serosal side of the preparation. Triglycine was studied in an identical fashion, and at 60 min, the mucosal side of the preparation contained, in addition to glycine, two peptides, glycyl-glycine and triglycine, whereas only glycyl-glycine was found as a peptide on the serosal side. No peptide remained on the mucosal side after 120 min, although significant amounts of glycyl-glycine were still evident on the serosal side. The tetraglycine was studied only for 120 min, and it was observed that no peptide larger than glycyl-glycine was found on the serosal side.

In an attempt to gain more information about the absorptive process, studies were carried out in an exact reverse fashion to see if transport could occur from

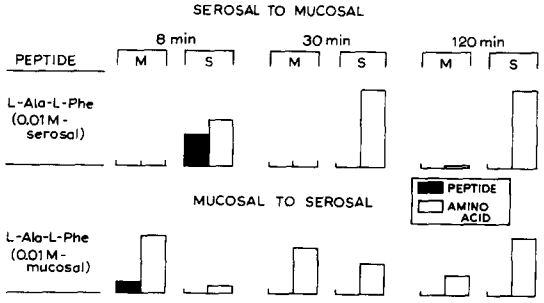

Fig. 3. A comparison of the transport of L-alanyl-L-phenylalanine (0.01 *M*) in the serosal to mucosal direction to the mucosal to serosal direction.

the serosal side to the mucosal side through the everted sacs. L-alanyl-L-phenylalanine, (Fig. 3) as a substrate on the serosal side only, was found to be hydrolyzed slowly, but at no time was a significant amount of the peptide or more than a trace of the component amino acids found to be transported to the mucosal side. For comparison the mucosal to serosal transport is shown for the same substrate under

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identical conditions and indicates a rapid rate of hydrolysis with transport of the component amino acids to the serosal side.

To determine the necessity for intact intestinal cells for the hydrolytic procedure which had been observed with the previous peptides, a number of sacs were incubated with only the buffer–glucose solution on both sides. Following the incubation, the resulting solutions were tested for enzymic activity in the absence of the intestine. It was found that heat-labile peptidase activity was present in both the mucosal and serosal solutions, but a marked specificity was observed. A substrate such as L-leucyl-L-tyrosine was completely hydrolyzed by either solution in 30 min, but in contrast glycyl-glycine was very resistant to cleavage in both solutions. Even after 120 min only minimal hydrolysis was evident in the mucosal solution and even less occurred in the serosal solution.

DISCUSSION

Attempts to evaluate the possibility of peptide absorption have been extremely limited[1,2]. It has now been possible to extend these evaluations with several peptides and to examine under comparable conditions for a number of dipeptides the rates of cleavage and possible transport from the mucosal to the serosal side of an isolated sac preparation from the small intestine of a rat. Of the peptides examined in this system, only glycyl-glycine has been observed to cross the intestinal wall and accumulate on the serosal side in quantities sufficient for detection. The resistance of this peptide to hydrolysis on the mucosal side undoubtedly aids in permitting the magnitude of accumulation which was observed. A slow rate of hydrolysis, however, of a peptide on the mucosal side cannot be the only factor involved in the question as to whether the peptide may penetrate through the wall. As was observed in the cases of other peptides (*e.g.* β-alanyl-DL-phenylalanine), which were relatively slowly hydrolyzed on the mucosal side, no significant accumulation of peptide occurred on the serosal side. When hydrolysis occurs, it has not been possible exactly to ascertain the site of cleavage, *e.g.* mucosal solution, intestinal cells, serosal solution, but it has been shown that considerable peptidase activity resides in both the mucosal and serosal solutions, which bathe the intestinal preparations. The demonstration of peptidase activity on the serosal side adds a possible explanation to the results which had been observed. It is conceivable that a relatively slow rate of penetration of peptide through the intestinal wall might be coupled with sufficient peptidase activity in the serosal solution of the *in vitro* preparation such that an adequate accumulation of peptide on the serosal side might never occur to allow chromatographic detection. Thus the observed penetration of glycyl-glycine through the everted sac results from the combination of a resistance to hydrolysis on the mucosal side of the wall, the ability to pass through the wall intact, and an even greater resistance to enzymic attack on the serosal side.

ACKNOWLEDGEMENTS

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STUDIES ON THE ELECTRON TRANSPORT SYSTEM

XVIII. ISOLATION OF COENZYME Q (Q_{275}) FROM BEEF HEART AND BEEF HEART MITOCHONDRIA

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SUMMARY

Two different methods for purification of coenzyme Q from beef heart mitochondria and from beef heart have been described. Selective extraction following saponification or direct extraction of total lipids have been used for initial extraction. The coenzyme Q in these extracts has then been purified by chromatography on Decalso or silicic acid followed by crystallization from ethanol. The coenzyme Q obtained by these two procedures have melting points which range from 49.3 to 50°, $E^{1\%}_{1\,cm}$ at 275 mμ ranges from 162 to 165. All purified preparations have the same R_F when chromatographed on silicone-treated paper and identical visible, ultraviolet and infrared spectra.

INTRODUCTION

The ease with which coenzyme Q** (Q_{275}) can be obtained in high purity from beef heart or from beef heart mitochondria has facilitated the recognition of this compound as an essential component in the electron transport system[1, 2, 3].

Coenzyme Q is a neutral lipid, insoluble in water, poorly soluble in polar organic solvents, but highly soluble in non-polar solvents, especially hydrocarbons. These properties can be made the basis of efficient purification procedures. The first step in

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** The nomenclature of the coenzyme Q series of compounds is discussed in paper XV of this series.

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