accumulation of serum proteins in the duodenum, in the absence of the digestive power of pancreatic proteinases.

Our thanks are addressed to Dr. CHARLOTTE M. ANDERSON in whose department this work was done and to Dr. PATRICIA G. JOHANSEN for discussion and advice.

One of the authors (B.H. is a fellow of the "Stiftung für biologisch-medizinische Stipendien der schweizerischen Akademie der medizinischen Wissenschaften".

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Received August 9th, 1968

BBA 63350

Peptide substrates for a proteinase of *Clostridium histolyticum*

Together with collagenases[1], a thiol-activated proteinase[2], and peptidases[3], *Clostridium histolyticum* produces in its culture medium a proteinase that is optimally active in the presence of calcium salts and for which no synthetic substrates are known[4]. The only information on its specificity was presented in a comparison of N-terminal amino acids of peptides released from gelatin by a fraction containing collagenase and this proteinase[5]. These data suggested that the proteinase specifically

hydrolyses peptide bonds in which the nitrogen atoms are contributed by amino acids with hydrophobic side chains.

In a survey of substrates customarily used for the mammalian proteases we found that the *Cl. histolyticum* proteinase readily hydrolysed substituted dipeptides of the form R–Gly–X-amide where X may be leucine or phenylalanine. Electrophoretic analysis of the digestion products of the phenylalanine analogue showed that the prime bond broken was that between glycine and phenylalanine. It seems that the N- and C-terminal substituents are necessary since splitting of the Gly–Phe bond in Gly–Phe-amide and Z–Gly–Phe was not detectable under comparable conditions of digestion. Furthermore, it appears that a hydrophobic side chain is required in the X position since Z–Gly–Gly-amide was not hydrolysed.

Crude enzyme containing all the proteases was prepared from *Cl. histolyticum*, strain N.C.T.C. 503, essentially according to the method of MACLENNAN, MANDL AND HOWES[6]. The mixture of proteases was first fractionated by gel filtration under conditions similar to those used by YOSHIDA AND NODA[7]. The enzymes were eluted in the order: peptidases (mol.wt. > 100 000), collagenase (mol.wt. about 100 000), thiol-activated proteinase (mol.wt. about 50 000) and proteinase (mol.wt. about 20 000). The proteinase was assayed using casein and benzoylglycylleucine amide as substrates. The profiles of proteinase activity in the eluate against these substrates peaked at the same point and were similar in shape.

The proteinase fraction obtained by gel filtration was largely free from peptidase and partly free from collagenase and thiol-activated proteinase. The proteinase was separated from the bulk of these remaining proteases by selective adsorption on ECTEOLA-cellulose and elution with a $CaCl_2$ gradient. The activity of the eluate was tested against casein and both the leucine and phenylalanine analogues of the synthetic substrates. The activity profiles were again similar, suggesting that the proteinase fraction contains one enzymic species.

It shares with the neutral proteinases of *Bacillus subtilis*[8], *Bacillus thermoproteolyticus* (thermolysin)[9] and *Pseudomonas aeruginosa*[10] the ability readily to hydrolyse peptide bonds on the amino side of residues with hydrophobic side chains provided the peptide bond is not terminal.

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Received July 17th, 1968