THE SPECIFICITY OF PORCINE TRYPSIN

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During the past several years it has been the custom to use bovine trypsin as the source for tryptic digestion of proteins. This enzyme, although highly specific for arginine and lysine residues, is usually contaminated with chymotrypsin and must be pre-treated with TPCK[2] prior to use in order to avert formation of chymotryptic peptides (Kostka and Carpenter, 1964; Walsh et al., 1962 Heller and Smith, 1965). Furthermore, the autolytic nature of bovine trypsin requires that repeated addition of enzyme be made in order to insure total tryptic digestion. This report deals with the enzymatic specificity of porcine trypsin, a stable proteolytic enzyme which can be obtained essentially chymotrypsin free and which should prove useful as a digestive agent.

Experimental and Discussion

Porcine trypsin[3] (2X) was prepared by the method of Travis and Liener, (1965) while bovine trypsin (2X) and ribonuclease A were commercial products

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[2]Abbreviations: TPCK, L-(1-tosylamido-2-phenyl)-ethyl-chloromethyl ketone; BAEE, N-benzoyl-L-arginine ethyl ester; ATEE, N-acetyl-L-tyrosine ethyl ester.

[3]This material is commercially available from The Enzyme Development Corporation, 64 Wall St., New York, N. Y. 10005.

(Worthington).

Porcine and bovine trypsins, prior to use, were checked for purity by disc electrophoresis (Reisfeld et al., 1962). The former was homogenous while bovine trypsin was found to contain one major and at least four minor components. Porcine trypsin had a 25% higher specific activity against BAEE than did bovine trypsin and contained less than 0.02% chymotryptic activity when assayed with ATEE. The bovine enzyme had a 2% chymotrypsin content.

Oxidized bovine trypsin and oxidized bovine ribonuclease, prepared by the method of Hirs (1956) were used as substrates for determining the specificity of porcine trypsin. In each case, samples of either substrate were homogenized in 0.1M triethylamine-acetate buffer, pH 8.0, and either native bovine trypsin, TPCK-treated bovine trypsin, or native porcine trypsin added, at an enzyme-substrate molar ratio of 1:25. All solutions were then incubated at 37° and tested for release of free amino groups by removal of aliquots at various time intervals followed by development with ninhydrin by the method of Rosen (1957). At the end of 3 hours, digestion had stopped in all cases and enzyme was added to each solution to lower the enzyme-substrate molar ratio to 1:12.5.

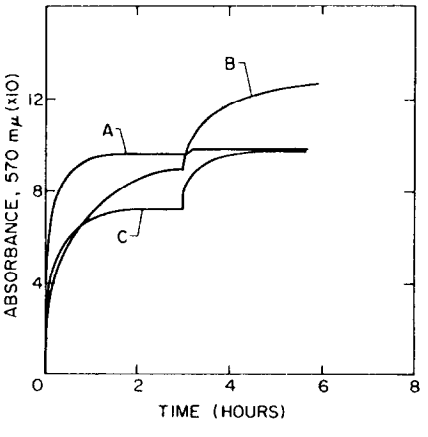

Figure 1 Hydrolysis of oxidized bovine trypsin by porcine trypsin, curve A, bovine trypsin, curve B, and TPCK-treated bovine trypsin, curve C. At 3 hours fresh trypsin was added to each solution.

The results of the digestion of oxidized bovine trypsin are shown in Fig. 1. These results show the obvious autolytic character of bovine trypsin and also the effect of the chymotryptic activity of untreated bovine trypsin since the total amount of free amino groups released was higher in this case.

At the end of 5 hours each of the digests was acidified with acetic acid and any insoluble material removed. The supernatant was subjected to finger-printing using, essentially, the procedure of Katz, Dreyer, and Anfinsen (1959). Chromatography was performed with n-butanol:acetic acid:water (4:1:5) followed by high voltage paper electrophoresis at pH 3.7 for 75 minutes at 2000V. The sheets were then stained with 0.2% ninhydrin in ethanol.

Patterns obtained by digestion of substrates with porcine and TPCK-treated bovine trypsin yielded identical peptide maps. Untreated bovine trypsin resulted in similar peptide patterns and, in addition, varying amounts of contaminating peptides which presumably arose as a result of chymotryptic digestion.

The results of these experiments indicate that porcine trypsin can be used safely for structural studies on proteins since it apparently cleaves the same peptide bonds as the TPCK-treated bovine. In addition, the enzyme has the advantages of being essentially chymotrypsin free as well as stable for several hours at alkaline pH (Vithayathil *et al.*, 1961). It is suggested that this enzyme be used for tryptic digestion of proteins in place of the bovine species.

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