SEQUENCE STUDIES ON BOVINE ∝-CHYMOTRYPSIN[1]

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The amino acid sequence has been determined for bovine chymotrypsinogen A by Keil, et al (1963), Hartley (1964), and Sorm, et al (1965). The sequence for the A chain of oxidized ∝-chymotrypsin has also been determined by Meedom (1958). When these sequences are compared there is one discrepancy evident. This research was undertaken to attempt to resolve this difference noted at residues 7, 8 and 9 from the amino end of chymotrypsinogen A. Our work confirms the results of Hartley. The A chain of ∝ - chymotrypsin is the 13 amino acids at the N terminus of chymotrypsinogen A.

Commercial ∝-chymotrypsin (Calbiochem A-grade, lot #53251) was used for the analysis. Paper chromatography was employed as the primary method of amino acid identification. Chromatograms were run on 24.5 x 24.5 cm paper in a Shandon ascending tank. The solvents used were butanol-acetic acid-water (12:3:5) and phenol-ammonia (200:1). Thin-layer chromatography on silica-gel coated plates and Eastman Chromasheets was also used. Chromatograms were developed with

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ninhydrin dip reagent. DNP-amino-acids were also separated by paper chromatography, the ether soluble derivatives being separated by the solvent pair butanol-ammonia (n-butanol saturated with 0.1% NH_3) and 1.5 M phosphate buffer. Water soluble derivatives were separated using butanol-acetic acid-water (12:3:5). Quantitative amino-acid analysis was done on a Phoenix automatic amino-acid analyzer, using a modification of the Moore and Stein technique.

Primary degradation of the α-chymotrypsin was accomplished by oxidation of the disulfide bridges connecting the three polypeptide chains. Four hundred mg of bovine α-chymotrypsin were reacted with 300 ml performic acid reagent for four hours at 0°C. After evaporation of the performic acid, the oxidized material was taken up in 0.1 M borate. A substantial portion of the material, which failed to dissolve, was identified by N-terminal analysis as the B-chain. The borate soluble material was applied to a column of DEAE-cellulose and the A-chain eluted with water. Fractions collected were monitored by their absorbancy at 220 mμ. Total amino-acid content and end-terminal analysis showed this preparation to be the A-chain, and ultraviolet spectra showed no absorption peak at 280 mμ indicating very little contamination by the B or C chains, although this contamination increased with the number of fractions. The first three peak fractions, representing the purest material, were pooled and used for the sequence analysis.

Approximately 1 mg of this A-chain preparation was digested with 20 μg DIFP-treated carboxypeptidase at 25°C and a kinetic analysis made of the liberated amino-acids. Leucine was detected after 8 hours of digestion, glycine after 24 hours, serine after 120 hours, and an increased amount of leucine after 140 hours, which was 15 hours after the addition of another 20 μg carboxypeptidase. Since the addition of more carboxypeptidase had liberated only one more amino-acid, it was assumed that a proline residue penultimate to the C-terminal was blocking the digestion. The reaction medium was heated to 40°C for 24 hours and the sample taken at the end of this time analyzed quantitatively on the Phoenix amino-acid analyzer. Giving the amount of glycine observed a mole value of 1, the results showed: serine = 0.9, leucine = 1.85, and valine = 0.5. The diffi-

culty encountered in hydrolyzing the bond connecting valine to the remainder of the A-chain indicated that this bond was with proline, giving a sequence of -Pro-Val-Leu-Ser-Gly-leu. This sequence agrees with that of Hartley. This C-terminal sequence combined with the data on the total amino-acid content and N-terminal amino-acid establishes the sequence shown in Table 1, which also shows the sequences found by Keil, et al (1963), Hartley (1964), Sorm, et al (1965) and Meedom (1958).

TABLE 1.

Meedom:
Cys-Gly-Val-Pro-Ala-Ile-Val-Pro-Gln-Leu-Ser-Gly-Leu

Sorm:
Cys-Gly-Val-Pro-Ala-Ile-Glu-Val-Pro-Leu-Ser-Gly-Leu

Hartley:
Cys-Gly-Val-Pro-Ala-Ile-Gln-Pro-Val-Leu-Ser-Gly-Leu

Glauser-Wagner:
Cys(gly,val,pro,ala,ile,gln) Pro-Val-Leu-Ser-Gly-Leu

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