Synthesis of the Basic Trypsin Inhibitor from Bovine Pancreas (Kunitz and Northrop) by Fragment Condensation on a Polymer Support

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Summary A highly active peptide, indistinguishable by disc-electrophoresis, from the natural bovine pancreatic basic trypsin inhibitor has been synthesized by fragment condensation on a polymer support.

THE synthesis of five protected peptide fragments which cover the entire 58 amino-acid residues of bovine pancreatic basic trypsin inhibitor (BTI)[1] has been accomplished (Figure). After assembling these fragments on a polymer support followed by deprotection and purification, we have succeeded in isolating a highly active peptide indistinguishable from natural BTI by disc-electrophoresis.

The protected dodecapeptide (I) was prepared by applying the pentachlorophenyl trichloroacetate procedure[2] to condense three subunits: Z-Arg(NO_2)-Pro-OH, Z(OMe)-Asp-(OBzl)-Phe-Cys(Bzl)-Leu-Glu(OBzl)-Pro-OH and H-Pro-Tyr-Thr-Gly-OH. For the synthesis of the protected hexadecapeptide (II), the modified azide procedure[3] was applied to four subunits: Z(OMe)-Pro-Cys(Bzl)-Lys(Z)-$NHNH_2$, Z(OMe)-Ala-Arg(Tos)-Ile-Ile-Arg(Tos)-$NHNH_2$, Z(OMe)-Tyr-Phe-Tyr-$NHNH_2$ and H-Asn-Ala-Lys(Z)-Ala-Gly-OH. The protected nonapeptide (III) was prepared by the same azide condensation of Z(OMe)-Leu-Cys(Bzl)-Gln-Thr-NH-NH_2 and H-Phe-Val-Tyr-Gly-Gly-OH. Synthesis of the protected nonadecapeptide (IV) was achieved by a modified azide procedure of four subunits: Z(OMe)-Cys(Bzl)-Arg(Tos)-$NHNH_2$, Z(OMe)-Ala-Lys(Z)-Arg(Tos)-$NHNH_2$, Z(OMe)-Asn-Asn-Phe-Lys(Z)-Ser-Ala-$NHNH_2$ and H- Glu-(OBzl)-Asp(OBzl)-Cys(Bzl)-Met-Arg(Tos)-Thr-Cys(Bzl)-Gly-OH. *p*-Methoxybenzyl azidoformate[4] was allowed to react with H-Gly-Ala-OH to prepare Z(OMe)-Gly-Ala-OH (V). Throughout these syntheses trifluoroacetic acid (TFA)[4] served to cleave the Z(OMe) group of necessary intermediates. All fragments and subunits were characterized by elemental and amino-acid analyses.

Z(OMe)-Gly-Ala-OH was esterified onto the bromomethylated copolymer of styrene and 2% divinylbenzene[5] in the presence of dicyclohexylamine.[6] The resulting Z(OMe)-

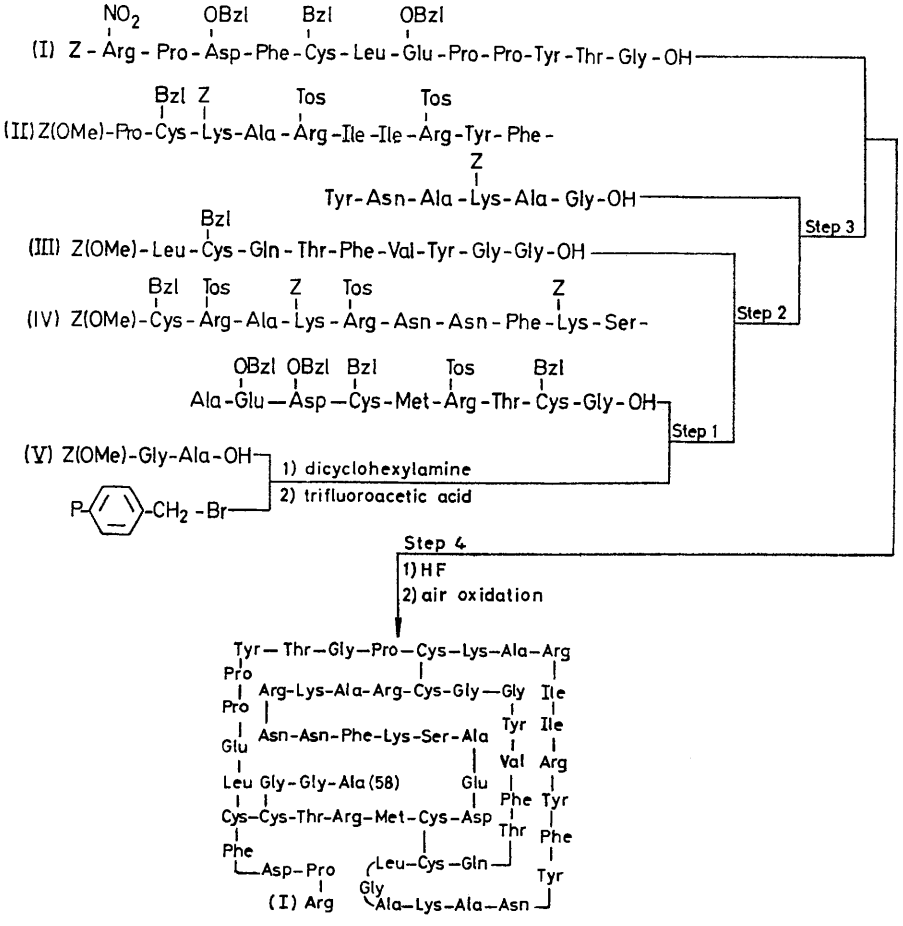

FIGURE. Synthetic scheme of BTI.

Gly-Ala-resin (yield 72%, peptide content 0·1 mmol/g) after removal of the Z(OMe) group by 50% TFA in methylene chloride, was condensed with (IV) by dicyclohexylcarbodi-imide (DCC) in the presence of *N*-hydroxysuccinimide[7] at room temperature for 4 days. This deprotection and condensation sequence was pursued until the desired sequence of BTI was achieved. In each step, the DCC activation took place at the glycine terminal of (IV), (III), (II), and (I) and racemization-free fragment condensation has thus been established. The amount of the carboxy-component used was increased from 1·5 equiv. to higher ratios as the chain length increased. At each step, an unchanged amino-component was masked by treatment of the resin with acetic acid and DCC. Part of the resin was removed and submitted to acid hydrolysis. The apparent coupling yields were 75% in step 1, 82% in step 2, 78% in step 3, and nearly 100% in step 4, based on the recovery of Ser which presents only one residue in BTI.

The peptide resin thus obtained (560 mg) was treated with hydrogen fluoride[8] to cleave the peptide from the resin and to remove all protecting groups from the peptide. Anisole and Met were added as scavengers. The deblocked peptide was purified by Sephadex G-25 using 0·1N-acetic acid as eluent. Each fraction was diluted with 0·1N-sodium acetate and exposed to air at pH 4·5 for 3 days to form three disulphide bridges. The active fractions, determined according to Sorm and his co-workers,[9] were subsequently purified by affinity chromatography through a trypsin–sepharose column[10] and the desired compound was eluted with 0·2M-potassium chloride at pH 2·0. After desalting by Sephadex G-25, followed by lyophilization, the peptide obtained (6 mg, yield 7·5% starting with the blocked peptide-resin) migrated in disc-electrophoresis at pH 2·3 as the natural bovine pancreatic BTI (Sigma, Cryst, Type 1-P). Upon acid hydrolysis, it contained the constituent amino-acids in ratios predicted by theory, except for Ile, which gave only 1 (theory 2) mol. equiv. after 24 h acid hydrolysis as mentioned by Dlouha and his co-workers[1b] during their structural studies. The specific activity of the synthetic peptide was 82%, when assayed according to Goldstein[11] using tosylarginine methyl ester as a substrate.

In 1971, Noda and his co-workers[12] performed the stepwise solid phase synthesis of BTI. The difference in the activity of his product and ours shows that fragment condensation in peptide synthesis is better than simple stepwise solid phase synthesis as also pointed out by Jones[13] because the end product is much easier to separate. Other sequences generally accompany the latter method making separation more difficult.

(*Received, 24th September* 1973; *Com.* 1338.)

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