Synthesis of the Nonacosapeptide Corresponding to the Entire Amino Acid Sequence of Duck Glucagon

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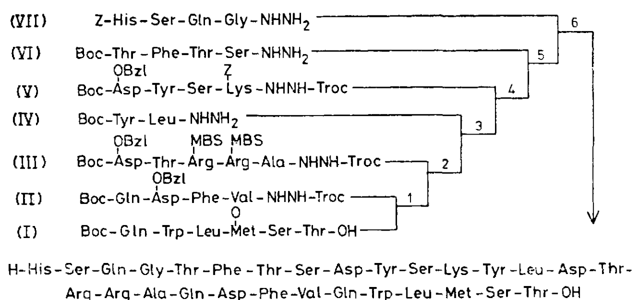
Summary The nonacosapeptide corresponding to the entire amino acid sequence of duck glucagon was synthesized using amino acid derivatives bearing protecting groups removable by methanesulphonic acid; it was obtained in crystalline form.

THE structure of duck glucagon was determined by Sundby *et al.*[1] in 1972. We have synthesized the nonacosapeptide corresponding to the entire amino acid sequence of this avian pancreatic hormone by a method different from those employed for the synthesis of structurally related mammalian glucagon by Wünsch *et al.*[2] and the Chinese research group.[3] We obtained the peptide in crystalline form.

Amino acid derivatives bearing protecting groups removable by methanesulphonic acid (MSA)[4] were employed, *i.e.*, Arg(MBS), Asp(OBzl), and Lys(Z) (MBS = *p*-methoxybenzenesulphonyl, Bzl = benzyl, and Z = benzyloxycarbonyl). In addition, to prevent alkylation of the sulphur atom of Met (at position 27) during the final step, it was reversibly protected as its (±)-sulphoxide by oxidation with sodium perborate. Unless this is done, the methyl group of anisole used as a cation scavenger may be transferred to this sulphur atom.

The acid labile Boc (t-butoxycarbonyl) group served as temporary protection for the α-amino function of the various peptide fragments. Prior to each condensation reaction, the Boc group was cleaved using 4 M ethanesulphonic acid[6] in trifluoroethanol (in step 1) or trifluoroacetic acid[7] (in the later steps), depending upon the solubility of the intermediates, which decreased progressively with increasing chain length. In order to minimize the destruction of the Trp residue during this treatment, anisole containing 0·1% ethanedithiol (EDT) and skatole were used as cation scavengers and the Trp content was assessed by hydrolysis with 4 N MSA.[8] A higher concentration of EDT (*e.g.*, 2%) reduced partially the Met(O) residue to Met.

Seven peptide fragments were selected as building blocks to construct the entire amino acid sequence of duck glucagon using Rudinger's azide procedure.[9] They were synthesized in a stepwise manner by known amide forming reactions. In order to incorporate the Asp(OBzl) residue to suitable peptide hydrazides, three fragments, II, III, and V, were synthesized starting with Troc-$NHNH_2$[10] and the Troc group (βββ-trichloroethoxycarbonyl) was removed from these fragments by Zn, prior to each condensation reaction. For the synthesis of fragment I, the Met residue was oxidized at the Z(OMe)–Leu–Met–Ser–Thr–OH stage with sodium perborate, since Z(OMe)–Met(O)–Ser–Thr–OH is water soluble and difficult to isolate from the inorganic salt. Prior to this oxidation, Z(OMe)–Met–OH was treated with this reagent at room temperature and left overnight, and Z(OMe)–Met–(±)–sulphoxide, free from the sulphone, was obtained quantitatively. When the deprotected product was examined in the long column of an amino acid analyser, equal amounts of the diastereoisomeric sulphoxides were detected.



SCHEME. Synthesis of duck glucagon.

The necessary fragments thus obtained were then assembled successively according to the Scheme. Using excess of acyl components (1·5—2·5 equiv.), each coupling reaction was performed in dimethylformamide (steps 1 and 2) or a mixture of dimethylformamide and *N*-methylpyrrolidone (in the later steps), depending on the solubility of the intermediates.

The resulting protected nonacosapeptide and protected intermediates were purified by washing with 5% citric acid and water followed by repeated precipitation from dimethylformamide with appropriate solvents, such as ethyl acetate, ethanol, or methanol, and their purities were assessed by t.l.c., acid hydrolysis, and elemental analysis.

For deprotection, the protected nonacosapeptide was treated with MSA in the presence of anisole and skatole and kept in an ice-bath for 15 min, and then at room temperature for 45 min. The deprotected peptide was precipitated as a powder with ether, converted into the corresponding acetate with Amberlite CG-4B (acetate form), treated with 0·5 N ammonia, and kept in an ice-bath for 30 min. This latter treatment was applied since it will cause a reversible N→O shift at the Ser and Thr residues, if this shift occurs at all. This side reaction is known in the treatment of Ser and Thr rich peptides by hydrogen fluoride.[11] The product was purified by column chromatography on Sephadex G-25 with 0·5% acetic acid as eluent, and then by gradient elution with ammonium acetate buffer (pH 6·9, 0·06 M) on CM-cellulose. [27-Met(O)]-duck glucagon thus obtained was then reduced by incubation with mercaptoethanol.[12] After column chromatography on Sephadex G-25, the synthetic nonacosapeptide was crystallized from 0·02% sodium chloride solution at pH 8·5 and its homogeneity was assessed by disc electrophoresis at pH 2·3, acid hydrolysis, and aminopeptidase digestion.

When the lipolytic activity on rat adipocytes was examined (by Dr. H. Iwatsuka, Takeda Chem. Ind.), synthetic duck glucagon was found to be biologically equivalent to that of porcine glucagon (Sigma G-4250), but immunologically it exhibited cross-reactivity with anti-porcine glucagon sera (30K, Lot 25)[13] by 46%.

The new deprotecting reagent, MSA, was also used by Fujino *et al.*[14]) in the synthesis of mammalian glucagon, which was obtained in a crystalline form.

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