SOLID-PHASE SYNTHESIS OF A TETRADECAPEPTIDE CORRESPONDING TO THE SEQUENCE 80-93 OF CYTOCHROME B_5

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Recently, the amino acid sequences of the microsomal cytochromes B_5 of man, monkey, pig, chick [1], and rabbit [2] have been established, and the previously-proposed [3] amino acid sequence of calf cytochrome B_5 has been refined [4]. It is suggested that the bond of the porphyrin with the protein containing 93 amino acid residues is affected by the coordination of the iron of the heme with histidine residues. Continuing our investigations on the synthesis of histidine-containing fragments of cytochrome B_5 [5], by the solid-phase method [6] we have synthesized the tetradecapeptide HBr – H-L-His^{80} – L-Pro – L-Asp – L-Asp – L-Arg – L-Ser^{85} – L-Lys – L-Ile – L-Thr – L-Lys – L-Pro^{90} – L-Ser – L-Glu – L-Ser^{93}-OH (I), corresponding to the sequence 80-93 of its protein chain. The synthesis was performed by the method of "activated" (p-nitrophenyl) esters, using as catalyst 1,2,4-triazole [7] for all the amino acids with the exception of N^{α}-Boc-N^{im}-benzylhistidine, Boc-N^{ω}-nitroarginine, Boc-O-benzylserine, Boc-threonine, and Boc-isoleucine (where Boc represents the tert-butoxycarbonyl group), which were caused to react by the carbodiimide method. The peptide was separated from the polymer by the action of dry hydrogen bromide on a suspension of the polymer in trifluoroacetic acid. According to paper chromatography, the product isolated consisted of a mixture of two substances: R_{fI} 0.14 (main fraction) and R_{fII} 0.43 (in small amount). The tetradecapeptide was isolated from this mixture by gel filtration on Sephadex G-15 in water. The fraction containing the main amount of substance was freeze-dried and then subjected to gel filtration on Sephadex G-15 again. The homogeneity of the tetradecapeptide (I) synthesized was confirmed by paper chromatography and electrophoresis in various buffers; its structure was determined by amino-acid analysis and also by the combined electrophoresis and paper chromatography of its acid hydrolysate.

EXPERIMENTAL

Chromatography was performed on Leningrad B ["fast"] paper in the following solvent systems: 1) butan-2-ol–10% ammonia (3:2), 2) tert-butanol–88% formic acid–water (70:15:15), and 3) butan-1-ol–acetic acid–water (4:1:5).

Electrophoresis was performed on Leningrad B paper in 0.2 M acetate (pH 2.7), 0.2 M borate (pH 8.6), and 0.2 M phosphate (pH 7.36) buffers.

N^{im}-Benzyl – L-histidyl – L-prolyl – L-asparagyl – L-asparagyl – N^{ω}-nitro-L-arginyl – L-seryl – L-lysyl – L-isoleucyl – L-threonyl – L-lysyl – L-prolyl – L-seryl – L-glutamyl – L-serine Hydrobromide (I). The peptide was synthesized on a chloromethylated copolymer of styrene with 2% of divinylbenzene (7% of chlorine). N^{α}-Boc-O-benzylserine was added by boiling 1 g of the polymer with 0.58 g of the protected amino acid and 0.27 ml of triethylamine in 20 ml of anhydrous ethanol for 48 h. The amount of Boc-O-benzylserine added to the polymer, determined colorimetrically [8] after acid hydrolysis of the sample in 6 N hydrochloric acid at 110° C for 48 h, was 0.31 mmole/g of polymer. In order to introduce Boc-O-benzylserine, Boc-isoleucine, Boc-N^{ω}-nitroarginine, Boc-threonine, and Boc-N^{im}-benzylhistidine into the peptide chain, N,N'-dicyclohexylcarbodiimide was used as the condensing agent; the other amino

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acids were introduced by the p-nitrophenyl ester method with 1,2,4-triazole as catalyst. For each amino acid the cycle comprised the following steps: the splitting out of the Boc protection by the action of a 1 N solution of hydrogen chloride in acetic acid for the first five amino acids and by a 1 N solution of hydrogen chloride in dioxane for the following amino acids (15 ml, 30 min); neutralization of the hydrochloride with 10% triethylamine in chloroform (15 ml, 15 min); condensation with the subsequent amino acid (5 eq.) under the action of N,N'-dicyclohexylcarbodiimide (5 eq.) in methylene chloride or dimethylformamide (DMFA), or with the p-nitrophenyl ester of the protected amino acid (5 eq.) in the presence of 1,2,4-triazole (6 eq.) in DMFA. The time of condensation for the carbodiimide method was 10 h and for the p-nitrophenyl ester method 24 h. After the performance of 13 cycles of condensation, 0.39 g of the polymer was washed with DMFA, methanol, and ether, and was dried in vacuum over caustic potash for 12 h. The peptide was separated from the polymer by the passage of a dry current of bromine-free hydrogen bromide through a suspension of the polymer in 10 ml of dry trifluoroacetic acid (90 min). The precipitate was separated off and washed with trifluoroacetic acid (3 × 5 ml). The solvent was driven off in vacuum at 25° C. The residue was washed with ether, separated off, and dried in vacuum over caustic potash. Weight 0.125 g. Paper chromatograms of the product in systems 1 and 2 showed two ninhydrin-positive spots with R_{fI} 0.14, R_{fII} 0.08 (main substance) and R_{fI} 0.45 and R_{fII} 0.32 (weak). A solution of 60 mg of the mixture in 1 ml of water was deposited on a column of Sephadex G-15 (23 × 230 mm) equilibrated with water. Elution was performed with water (v 30 ml/h). After 30 ml, the main fraction (25 ml) was collected. It was freeze-dried and rechromatographed on a column of Sephadex G-15. The yield of tetradecapeptide after freeze-drying was 40 mg (63.5% calculated on the Boc-O-benzylserine added to the polymer), $[\alpha]_D^{24} - 79.8°$ (c 1.2; DMFA), R_{fI} 0.14, R_{fII} 0.08, R_{fIII} 0.20.

An amino acid analysis of the acid hydrolysate gave the following ratio of the amino acids: Lys 2.21: N^{im}BzlHis 1.05; Arg 1.0; Asp 1.97; Thr 1.0; Glu 1.15; Pro 2.23; Ile 0.95; Ser 3.18.

SUMMARY

1. The synthesis of the hydrobromide of the tetradecapeptide HBr·H-L-His(Bzl^{im})-L-Pro-L-Asp-L-Asp-L-Arg(NO_2)-L-Ser-L-Lys-L-Ile-L-Thr-L-Lys-L-Pro-L-Ser-L-Glu-L-Ser-OH corresponding to sequence 80-93 of cytochrome B_5 isolated from the microsomes of calf thymus has been synthesized by the solid-phase method.

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