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SOLID PHASE SYNTHESIS AND PURIFICATION OF A PENTAPEPTIDE

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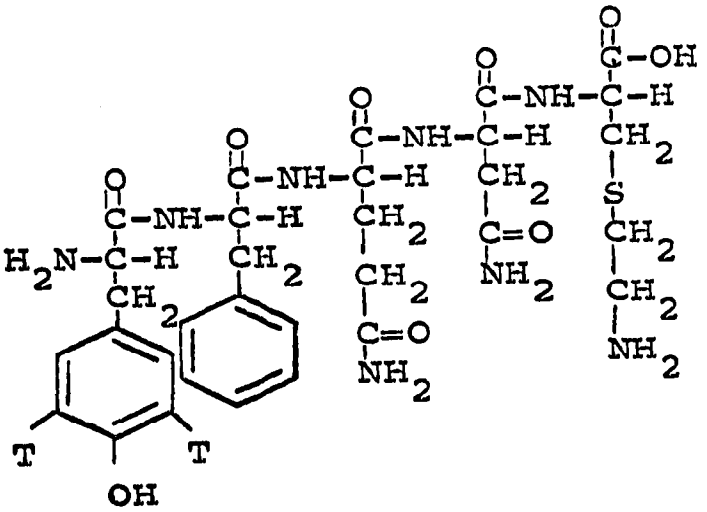
SUMMARY

The synthesis of a radiolabelled derivative of the 2–6 fragment of vasopressin was achieved by solid phase peptide synthesis. This pentapeptide is expected to serve as a model compound for vasopressin precursor studies. The usage of preparative electrophoresis for purification of the model pentapeptide is illustrated.

INTRODUCTION

The recent postulation by SACHS *et al.*[1,2] that the biosynthesis of vasopressin proceeds through a precursor on ribosomes via pathways common to the biosynthesis of other peptide chains has prompted the present investigation. In a collaborative effort with SACHS we have now synthesized a derivative of the 2–6 fragment of vasopressin (3,5-ditritio-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-amino-



ethyl-L-cysteine (I)). Aminoethylation, followed by tryptic hydrolysis of suspected vasopressin precursors of high molecular weight should afford the model synthetic pentapeptide. Compound I may therefore serve as a valuable model for the determination of which, if any, peptides of high molecular weight serve as vasopressin precursors. A labelled pentapeptide was required for proper fingerprinting of possible labelled protein precursors[3].

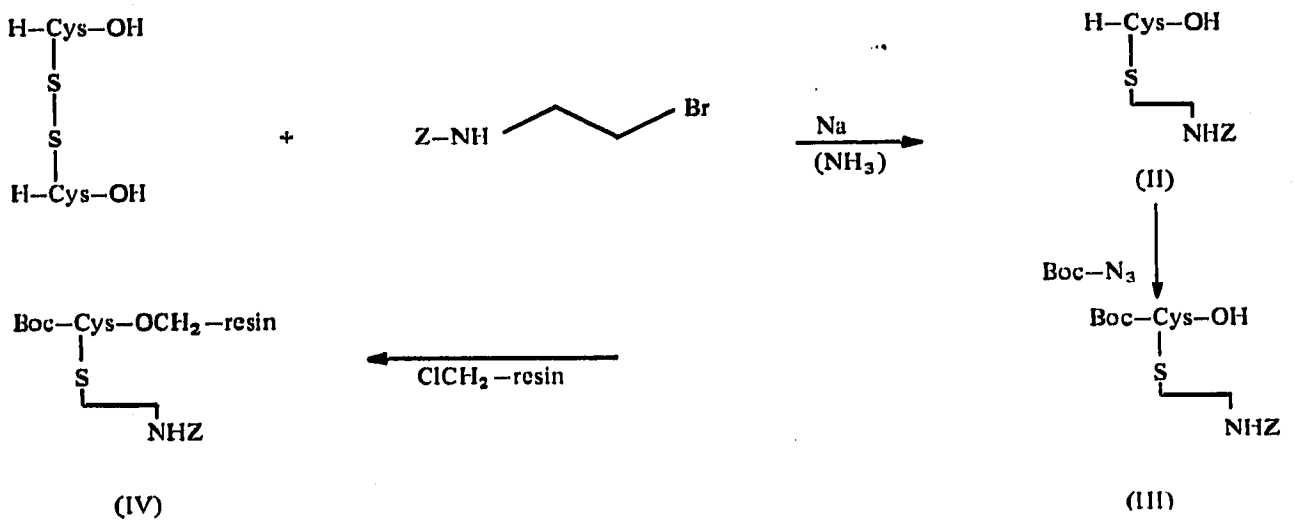


Fig. 1. Attachment of the C-terminal amino acid derivative to the resin.

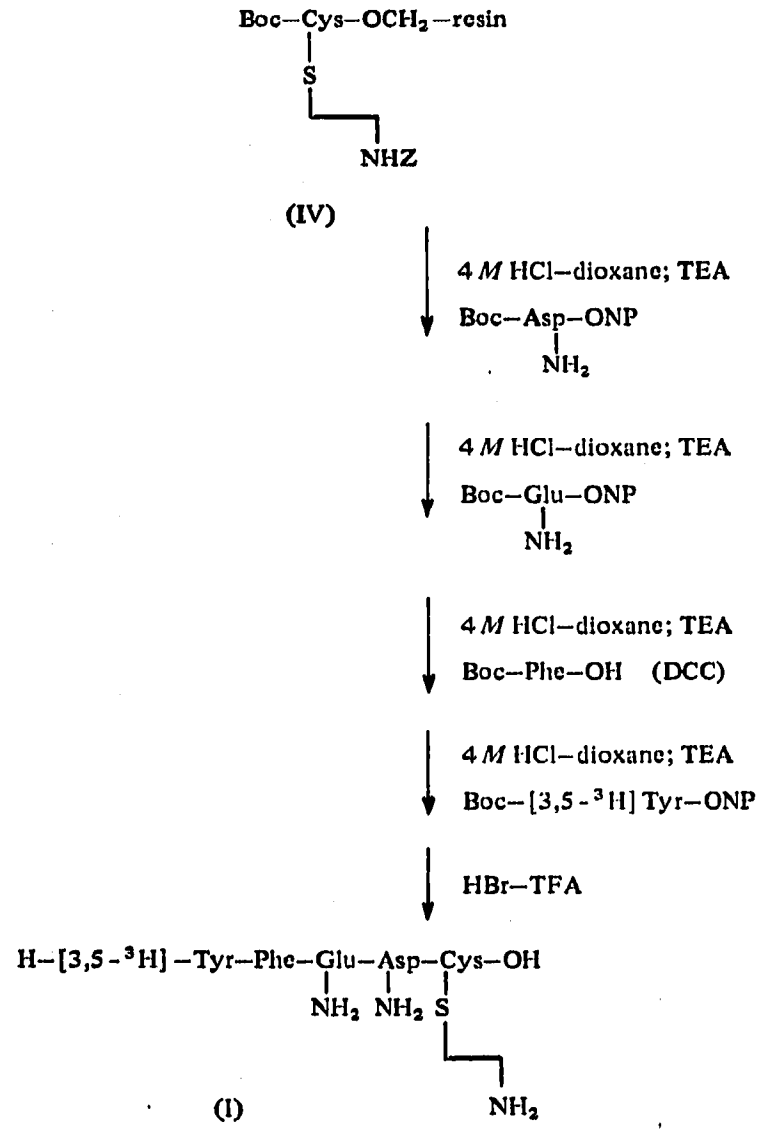


Fig. 2. Solid phase peptide synthesis of 3,5-ditritio-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-aminoethyl-L-cysteine.

SYNTHESIS

The synthesis of the pentapeptide was achieved by the method of solid-phase peptide synthesis[4,5] using α-Boc* amino protection with anchoring of the peptide chain to the resin support by means of a benzyl ester bond[6].

Fig. 1 outlines the synthesis and attachment to the resin of the first amino acid derivative. Reaction of N-benzyloxycarbonyl-2-bromoethylamine with L-cystine in sodium and liquid ammonia gave S-benzyloxycarbonyl-aminoethyl-L-cysteine (II)[7]. Reaction of (II) with *tert.*-butyloxycarbonylazide gave N-*tert.*-butyloxycarbonyl-S-benzyloxycarbonylaminoethyl-L-cysteine (III). This latter protected L-cysteine derivative was attached to chloromethylated polystyrene (2% crosslinked with divinylbenzene) by refluxing for three days in ethanol in the presence of triethylamine. The resultant N-*tert.*-butyloxycarbonyl-S-benzyloxycarbonylaminoethyl-L-cysteinyl resin (IV) was used in solid phase peptide synthesis as outlined in Fig. 2.

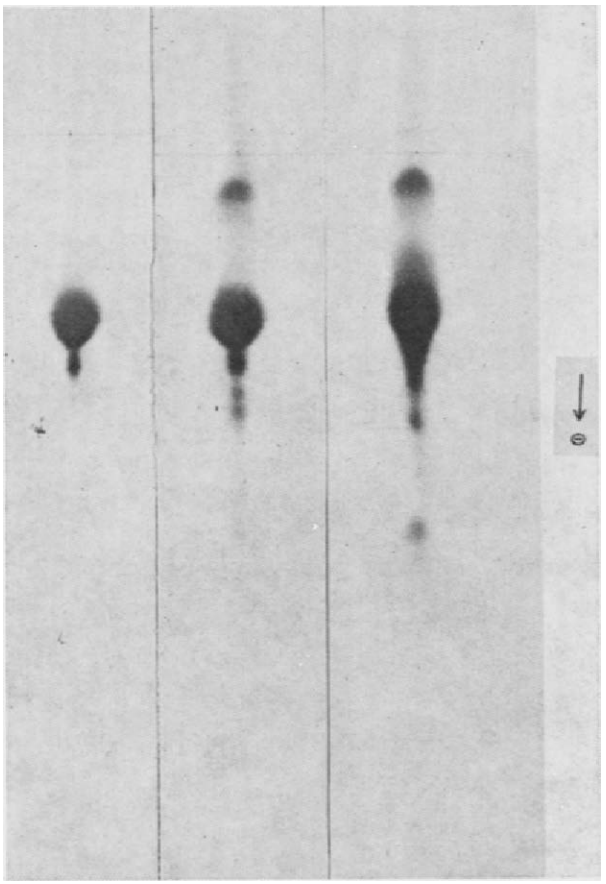


Fig. 3. Electrophoresis of pentapeptide at various stages of purification. Right: crude pentapeptide; center: pentapeptide after gel filtration; left: pentapeptide after preparative electrophoresis. The electropherograms were run in 0.1 *M* pyridine acetate buffer (pH 5) at 87.5 V/cm for 30 min on S & S 2040-B paper and stained with ninhydrin.

* Abbreviations used: Boc = *tert.*-butyloxycarbonyl; Z = benzyloxycarbonyl; DCC = dicyclohexylcarbodiimide; TFA = trifluoroacetic acid; DMF = dimethylformamide; TEA = triethylamine; ONP = *p*-nitrophenyl ester.

N-*tert.*-Butyloxycarbonyl-S-benzyloxycarbonylaminoethyl-L-cysteinyl resin (IV) was (a) deprotected with 4 *M* HCl in dioxane, (b) neutralized with triethylamine in chloroform, and (c) coupled with five equivalents of N-*tert.*-butyloxycarbonyl-L-asparagine-*p*-nitrophenyl ester. The same sequence of deprotection, neutralization and coupling was carried out with five equivalents of N-*tert.*-butyloxycarbonyl-L-glutamine-*p*-nitrophenyl ester. This cycle was repeated with N-*tert.*-butyloxycarbonyl-L-phenylalanine (using dicyclohexylcarbodiimide as the coupling agent). The final cycle was carried out using N-*tert.*-butyloxycarbonyl-3,5-ditritio-L-tyrosine-*p*-nitrophenyl ester. The crude pentapeptide (I) was obtained by bubbling hydrogen bromide into a suspension of the peptide resin in trifluoroacetic acid.

PURIFICATION

The crude pentapeptide (205 mg) was initially purified by gel filtration on Sephadex G-10 using a 2.5 × 85 cm column and eluting with 0.2 *M* acetic acid at a

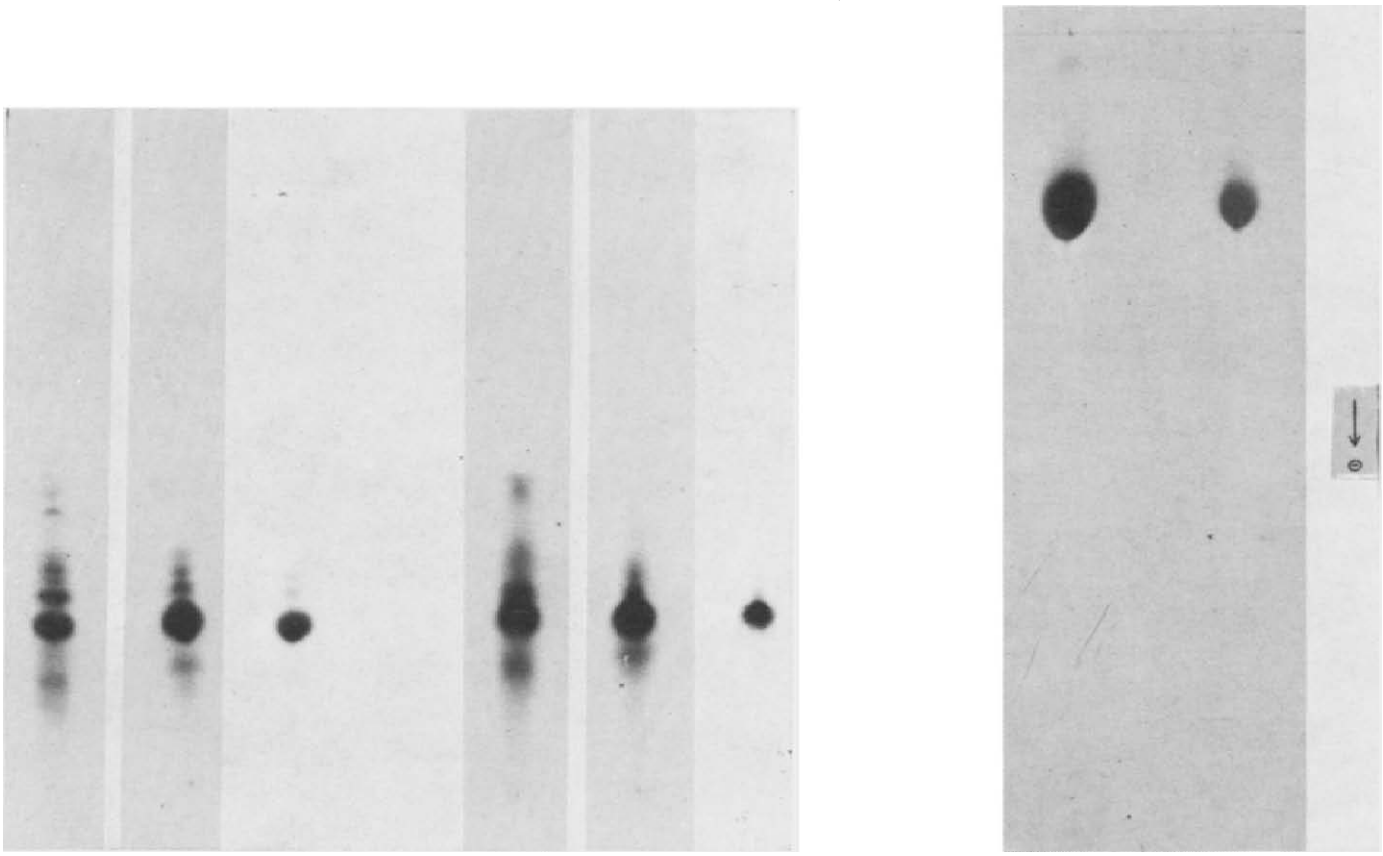


Fig. 4. Thin-layer chromatography of pentapeptide at various stages of purification. The three chromatograms on the left were run in the system butanol–acetic acid–ethyl acetate–water (1:1:1:1) and the three chromatograms on the right were run in the system butanol–acetic acid–pyridine–water (15:3:10:12). In each case the crude pentapeptide is on the left, pentapeptide after gel-filtration in the center, and pentapeptide after preparative electrophoresis on the right. The chromatograms were run on Silica Gel 6F and stained with ninhydrin.

Fig. 5. Electrophoresis of pentapeptide after final purification. A concentration of 1 mg/0.05 cc was used. The sample on the left was spotted with a volume of 4.75 λ. The sample on the right was spotted with a volume of 1.25 λ. The electropherogram was run in 0.1 *M* pyridine acetate buffer (pH 5) at 87.5 V/cm for 30 min on S & S 2040-B paper and stained with ninhydrin.

rate of 45 ml/h. Fractions of 100 drops/tube were collected. Tubes 39–43 were pooled and lyophilized to give 47 mg (20.4% yield based on the cysteine content of IV). Further purification of the pentapeptide was achieved by preparative electrophoresis using the Camag high-voltage electrophoresis system. A 100-mg sample of the pentapeptide (after gel filtration) was applied to 200 × 400 mm Whatman No. 3MM paper and run in pyridine acetate buffer (pH 5) at 3500 V for 30 min and afforded 68 mg of product. Fig. 3 depicts the electropherogram of the peptide at each stage of purification. It can be seen that although some purification was achieved, the product was not yet homogeneous. Similar conclusions were drawn from thin-layer chromatography in two different systems (Fig. 4).

The final purification was achieved by a repetition of the preparative electrophoresis procedure. A portion of the partially purified pentapeptide was applied to 200 × 400 mm S & S 2040-B paper and run in 0.1 *M* pyridine acetate buffer (pH 5) at 3500 V for 30 min. The final product was recovered by extraction of a narrow band and was ascertained to be essentially homogeneous, as shown in Fig. 5.

This paper illustrates the usefulness of preparative electrophoresis as a valuable supplemental tool for the purification of small peptides. Purification of intermediate peptides has also been achieved by this technique[8]. The general methods involving purification of peptides by high-voltage electrophoresis have been previously reviewed[9,10].

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