Application of Polyamide Resins to Polypeptide Synthesis: an Improved Synthesis of β-Endorphin Using Fluorenylmethoxycarbonylamino-acids

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Summary Use of base-labile fluorenylmethoxycarbonyl-amino-acids enables solid phase peptide synthesis of human β-endorphin to be carried out under much milder reaction conditions than formerly with improvement in product purity and yield.

WE recently described[1] a solid phase synthesis of the opiate-like 31-residue polypeptide human β-endorphin (I). This synthesis was carried out using a polydimethylacrylamide solid support together with t-butoxycarbonylamino-acids, benzyl-based side chain protecting groups, and a benzyl ester peptide-resin linkage. This conventional combination of protecting groups and resin linkage necessitated the usual vigorous acidic conditions both during synthesis of the peptide chain (30 × 30 min in 1 N HCl–AcOH) and particularly for detachment from the solid support (60 min in liquid hydrogen fluoride). The damaging effect of these acidic treatments is seen in the formation of major byproducts.[1] We now describe a new synthesis of human β-endorphin in which treatment with acidic reagents is minimised with substantial improvement in product yield and purity.

H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH

(I)

$HOCH_2C_6H_4$-*p*-$OCH_2CO.OC_6H_2Cl_3$-2,4,5

(II)

The new procedure employs base labile *N*-α-fluorenylmethoxycarbonyl (Fmoc) amino-acids[2] in combination with acid-labile t-butyl based side chain protecting groups and *p*-alkoxybenzyl ester resin linkage.[3] Preliminary assessment of this system for polyamide-based solid phase synthesis has been described elsewhere.[4] The present synthesis was commenced by addition of a permanent internal reference-spacer residue (Boc–norleucine) to the amino-functionalised resin[5] followed by the benzyl alcohol linkage reagent (II).† Symmetrical anhydride and

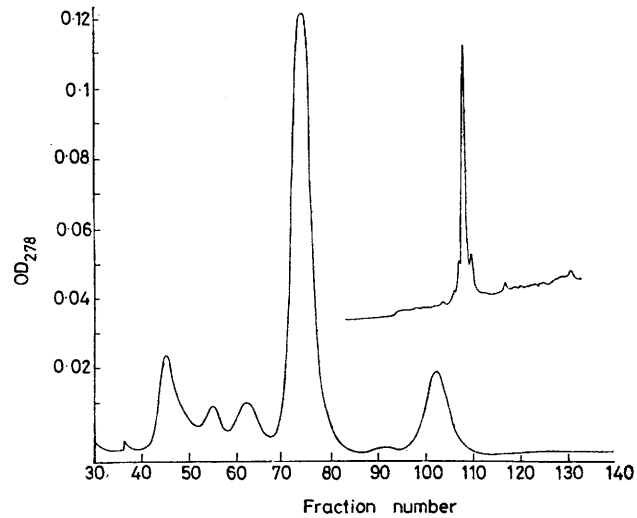

FIGURE. Chromatography of total synthetic product on carboxymethylcellulose CM-52 using 0·015—0·15 M gradient of ammonium acetate, pH 6·0. The elution profile should be compared with that obtained previously using Boc-amino-acids throughout the synthesis (ref. 1). Inset: h.p.l.c. of main peak on μ-Bondapak C_{18}. The h.p.l.c. effluent was monitored at 278 nm; monitoring at 230 nm gave the same profile.

† This activated ester, m.p. 103—4·5 °C, was prepared by chloromethylation of phenoxyacetic acid, alkaline hydrolysis, and conversion of the resulting *p*-hydroxymethylphenoxyacetic acid into its 2,4,5-trichlorophenyl ester with dicyclohexylcarbodi-imide.

activated ester solid phase coupling procedures[5] were used for these preliminary operations. Assembly of the β-endorphin sequence proper commenced with establishment of the benzyl ester linkage to the resin using the symmetrical anhydride of biphenylisopropoxycarbonyl (Bpoc) γ-t-butylglutamic acid in the presence of 4-dimethylaminopyridine.[6] The resin loading was 0·23 mmol g^{-1}. The Bpoc group was cleaved by treatment with 0·09 N HCl–AcOH for 30 min, the resin neutralised, and the following 29 residues‡ added as immediately preformed anhydrides[5] of Fmoc-amino-acids. (Asparagine and glutamine were introduced as *p*-nitrophenyl esters.) Intermediate Fmoc-peptide resins were de-protected with 20% piperidine in dimethylformamide (DMF). Use of this deprotection procedure with DMF as the sole solvent throughout the synthesis permitted a very simple and efficient reaction cycle consisting only of (1) DMF, 5 × 1 min; (2) 20% piperidine in DMF, 3 + 7 min; (3) DMF, 10 × 1 min; (4) Fmoc-amino-acid anhydride (6-fold excess), 60 min; and (5) DMF, 5 × 1 min. This reaction cycle enabled six amino-acid residues to be added per day without difficulty. Resin samples were removed at each step for qualitative ninhydrin tests[7] and at intervals for amino-acid analysis§ and h.p.l.c. examinaiton of intermediate peptides.¶

Treatment of a sample of the peptide resin (60 mg) with anhydrous trifluoroacetic acid for 2 h in the presence of an excess of methionine cleaved 90% (5·14 μmol) of the peptide, of which 5·03 μmol was subjected to a single chromatography on carboxymethylcellulose CM-52 (Figure). The elution conditions were the same as those of the last step of the previous three-step purification procedure.[1] Recovery in the main peak was 2·31 μmol (46%); found: Asp, 2·00 (calc. 2); Thr, 2·84 (3); Ser, 1·73 (2); Glu, 3·14 (3); Pro, 1·14 (1); Gly, 2·96 (3); Ala, 2·00 (2); Val, 0·89 (1); Met, 1·04 (1); Ile, 1·11 (2),** Leu, 2·00 (2); Tyr, 1·96 (2); Phe, 1·92 (2); Lys, 5·35 (5). The product gave a single fluorescamine and ninhydrin-reacting spot on paper electrophoresis at pH 6·5 (R_{Lys} 0·45) and t.l.c. in butano–acetic acid–water–pyridine (90:18:60:72), R_F 0·67. Analytical h.p.l.c. on a μ-Bondapak C_{18} silica column using a gradient of 5—60% acetonitrile in 0·01 M aqueous ammonium acetate (pH 4·5) gave the elution profile shown; purity is estimated at *ca.* 85%. A single symmetrical peak was obtained by h.p.l.c. on the cation exchanger, Partisil-SCX, using a gradient of 0·5 M ammonium acetate, pH 6·0, and 1·0 M ammonium acetate–10% acetonitrile.[8] The overall yield of isolated product was 41%, disregarding chains terminated at step 4 in the synthesis.§

Biological properties of the synthetic β-endorphin will be reported separately.

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‡ The last residue (*O*-t-butyl tyrosine) was conveniently added as its *N*-α-Boc derivative, enabling simultaneous cleavage of all protecting groups and the resin linkage in the final step.

§ Amino-acid incorporation was comparable to that reported previously for the assembly of Boc-amino-acids on polydimethylacrylamide (ref. 1), except for an unexplained 25% irreversible chain termination at step 4. There was no significant detachment of peptide from the resin throughout the entire assembly.

¶ Use of an acid-labile *p*-alkoxybenzyl ester linkage precludes analysis of intermediate peptide-resins by solid phase Edman degradation (ref. 1 and E. Atherton, J. Bridgen, and R. C. Sheppard, *FEBS Letters*, 1976, **64**, 173).

** The -Ile-Ile- sequence in β-endorphin is incompletely hydrolysed under the standard conditions (6 N HCl, 110 °C, 18 h).

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