The Synthesis and Opiate Activity of β-Endorphin

by

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Abstract: The synthesis of the untriakontapeptide β-endorphin is described. The synthetic peptide is shown to possess opiate activity comparable with that of the natural product by the opiate receptor binding assay.

In a recent report (1) we described the isolation and characterization of an untriakontapeptide from camel pituitary glands. Its amino acid sequence was shown to be identical to the COOH-terminal 31 amino acids of ovine β-lipotropin (2, 3): 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-His-Lys-Lys-Gly-Gln-OH. Bioassay of the peptide showed that it has significant opiate activity when compared with normorphine (1, 4). The untriakontapeptide was designated as β-endorphin (1). This paper describes the synthesis of β-endorphin by the solid-phase method (5) and its biological activity.

EXPERIMENTS AND RESULTS

Protected β-Endorphin Benzhydrylamine Resin. Attachment of

Abbreviations: LPH, lipotropin; DIEA, diisopropylethylamine; BAW, 1-butanol-acetic acid-water (4/1/1, $^{v}/_{v}$); BPAW, 1-butanol-pyridine-acetic acid-water (6/6/1.2/4.8, $^{v}/_{v}$); TFA, trifluoroacetic acid.

α-benzyl N^{α}-t-butyloxycarbonyl glutamate (6) to benzhydrylamine resin (Beckman, 2.5 g) was performed by means of its symmetrical anhydride (7) as previously described (8). After retreatment of the resin with the same amount of symmetrical anhydride, it was dried in vacuo over P_2O_5 for 1 hr. After hydrolysis of a sample in propionic acid-12 N HCl (9), amino acid analysis (10) gave 0.34 mmol Glu per g. Another sample was submitted to treatment with HF (11) in the presence of anisole at 0° C for 1 hr. Amino acid analysis of the HF cleaved product gave 0.23 mmol of Gln per g (68% cleavage); the identification of the product as glutamine was also confirmed by thin-layer chromatography in BAW and BPAW solvent systems.

The resin just described was submitted to the schedule for introduction of each of the remaining amino acid residues as employed for the synthesis of ovine β-LPH(42-81) (8) with two exceptions. Two treatments with 5% DIEA in CH_2Cl_2 were used for neutralization, and for the second stage of anhydride couplings trifluoroethanol was added to a concentration of 20% to enhance coupling efficiency (12).

N^{α}-Boc protection was used with the following side-chain protecting groups: Lys, Z(o-Br); His, Boc; Glu, Bzl; Thr, Bzl; Ser, Bzl and Tyr, Z(o-Br). Boc-Asn, Boc-Gln and Boc-Gly were coupled as their p-nitrophenyl ester (13).

β-Endorphin. A sample (0.6 g) of protected β-endorphin resin was submitted to TFA deprotection steps to remove the Boc group[1].

[1] This tactic has been found to be useful for methionine-containing peptides as a means of avoiding the butylation that occurs in liquid HF (14).

The dried resin[2] was then stirred in the presence of 1.8 ml of anisole and 15 ml of liquid HF at 0°C for 1 hr. After the removal of HF, the oily residue was washed with two 15-ml portions of ethyl acetate. The peptide was extracted from the resin with three 15-ml portions of 50%

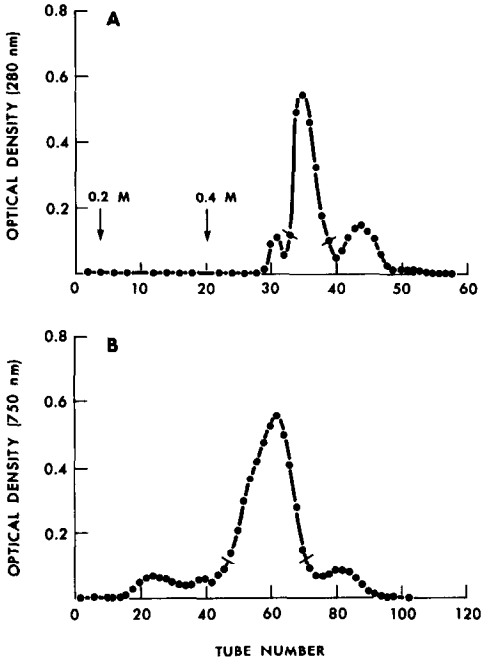

Figure 1 (A) Chromatography of crude β-endorphin (110 mg) on CMC (1 x 55 cm column). Solutions of 0.2 and 0.4 M ammonium acetate, pH 6.5, were introduced as indicated through a 500 ml mixing chamber containing the starting buffer, 0.01 M ammonium acetate, pH 4.5. Fractions of 12.5 ml were collected.

(B) Partition chromatography on Sephadex G-50 of purified synthetic β-endorphin (66 mg) obtained from CMC. It was run in a 2.5 x 44.0 cm column in the solvent system: 1-butanol-pyridine-acetic acid-0.6 M NH_4OAc, 500 : 300 : 1.1 : 1100 [hold-up volume, 63 ml; 4.6 ml/tube] .

[2] Cleavage was slower than in ester attachment since re-treatment of the resin with HF gave additional peptide material to increase the yield of β-endorphin from 20 to 30%.

acetic acid; the combined extracts were evaporated in vacuo to a small volume (3 to 5 ml) and submitted to gel filtration on Sephadex G-10 (2 x 25 cm column) in 0.5 N acetic acid to yield 110 mg of crude β-endorphin. Chromatography of this material on CM-cellulose (Figure 1A) as previously described (15) gave 66 mg. Further purification was effected by partition chromatography on Sephadex G-50 (16) as shown in Figure 1B; isolation of the material represented by the main peak [Folin-Lowry detection (17)] (R_f = 0.21) gave 52 mg of highly purified β-endorphin (20% yield based on the starting resin).

On thin-layer chromatography (BPAW), the synthetic product gave one spot (ninhydrin detection) with an R_f value of 0.35. On paper electrophoresis it gave a single spot at both pH 3.7 and 6.9 with respective R_f values (relative to Lys) of 0.65 and 0.42. Gel electrophoresis (18) of the product at pH 4.5 for 1 hr also gave a single band. A sample was also submitted to electrofocusing in 5% polyacrylamide gel (19); only one band (detection by precipitation with 12% trichloroacetic acid) was observed with an approximate pI of 10. Samples (0.1 mg) of the synthetic and natural β-endorphin were treated separately with 3 μg each of trypsin in 35 μl of Tris buffer (pH 8.5, 0.01 M Mg^{2+}) at 37° C for 5 hr. Paper electrophoresis of the digests at pH 6.7 gave identical patterns as shown in Figure 2. Amino acid analysis of the synthetic product after acid hydrolysis gave: $Lys_{5.0}$, $His_{1.0}$, $Asp_{2.0}$, $Thr_{2.9}$, $Ser_{1.8}$, $Glu_{3.0}$, $Pro_{1.1}$, $Gly_{3.0}$, $Ala_{1.9}$, $Val_{1.0}$, $Met_{0.9}$, $Ile_{1.3}$, $Leu_{2.0}$, $Tyr_{1.0}$, $Phe_{2.0}$. Amino acid analysis after complete enzymic digestion (first with trypsin and chymotrypsin, and then leucine aminopeptidase) gave $Lys_{4.9}$, $His_{0.8}$, $(Thr, Ser, Asn, Gln)_{9.2}$, $Glu_{1.1}$, $Pro_{1.0}$, $Gly_{2.9}$,

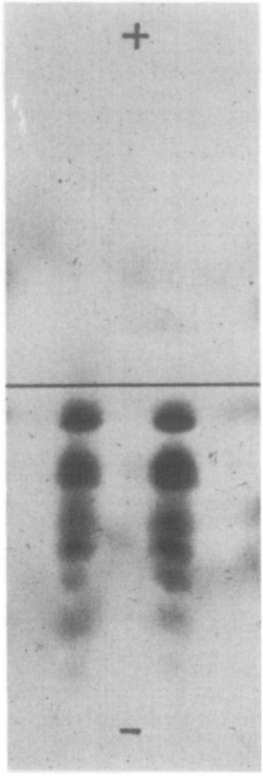

Figure 2 Paper electrophoresis of natural (left) and synthetic (right) β-endorphin after treatment with trypsin. Electrophoresis was run at pH 6.7 for 2 hr at 400 volts. The peptides were revealed by the ninhydrin reagent.

$Ala_{2.0}$, $Val_{1.2}$, $Met_{1.1}$, $Ile_{2.0}$, $Leu_{2.2}$, $Tyr_{1.1}$, $Phe_{2.0}$.

Opiate Activity . The opiate activity was assayed by the receptor binding assay (20) using a membrane fraction from rat brain homogenate. The natural β-endorphin (1) was employed in the assay for comparison. Results, summarized in Table 1, indicate that both natural and synthetic endorphins possess significant and identical opiate activity. Preliminary experiments showed that the synthetic product exhibits analgesic activity as assayed by the D'Amour and Smith (21) rat tail flick test.

Table 1

Opiate Activity of Synthetic β-Endorphin

β-Endorphin	Dose (μM)	Response[a]
Natural	3	42.8 ± 6.9
	7.5	82.0 ± 5.9
Synthetic	3	44.8 ± 6.8
	7.5	78.6 ± 2.9

[a] Percentage of inhibition of stereospecific ^{3}H-morphine binding. Mean ± standard error from 3 determinations.

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