Total Synthesis of Human Parathyroid Hormone (1—84)

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Human parathyroid hormone (hPTH) which is a linear peptide with 84 amino acid residues was synthesized by the segment condensation method in solution applying the maximal-protection approach followed by deprotection with HF.

The present communication reports the first synthesis of hPTH(1—84) by the classical solution procedure. The sequence, determined by Keutmann *et al.*[1] in 1978, is as shown in Figure 1.

The peptide was divided into 13 segments as indicated in Figure 1, and every carboxy terminus was protected as a phenacyl ester except for the C-terminal segment (79—84), which was protected as a benzyl ester. Each segment was synthesized step by step in dimethylformamide (DMF) with Boc-amino acids using trifluoroacetic acid as the deprotecting reagent. To avoid possible modification of the Trp residue, both dimethyl sulphide and ethanedithiol were added when all the Trp-containing segments were treated with the acidolytic reagent. All the side chain functional groups were protected as follows: Arg(Tos), His(Tos), Asp(OBzl), Glu(OBzl), Ser(Bzl), Thr(Bzl), and Lys(ClZ) (2-chlorobenzyloxycarbonyl). Among these, only the tosyl groups attached to His residues were completely removed by treatment with 1-hydroxybenzotriazole after the Boc–His(Tos) had been incorporated into the peptides.[2] Each segment thus obtained can be utilized not only as an amino component after removal of the Boc group, but also as a carboxy component after selective removal of the phenacyl group with Zn powder in acetic acid. These segments were then coupled using 1-ethyl-3-(3-dimethylaminopropyl)-carbodi-imide and 1-hydroxybenzotriazole as reagents, as detailed earlier.[3] To avoid the solubility problem, these segments were coupled one by one from the C-terminal residue to obtain four intermediates, (1—22), (23—38), (39—68), and (69—84), and these larger segments were further coupled from the C-terminal one to produce the full sequence. 2.4 g of the fully protected hPTH(1—84) were obtained by this means. Every intermediate was fairly soluble in DMF or in a mixture of DMF and *N*-methylpyrrolidone. The fully protected product

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                5                  10                  15
Ser–Val–Ser–Glu–Ile–Gln–Leu/Met–His–Asn–Leu–Gly/Lys–His–Leu–
               20                  25                  30
Asn–Ser/Met–Glu–Arg–Val–Glu/Trp–Leu–Arg–Lys–Lys–Leu/Gln–Asp–
               35                  40                  45
Val–His–Asn–Phe–Val–Ala–Leu–Gly/Ala–Pro–Leu–Ala–Pro/Arg–Asp–
               50                  55                  60
Ala–Gly–Ser–Gln–Arg–Pro/Arg–Lys–Lys–Glu–Asp–Asn–Val–Leu/Val–
               65                  70                  75
Glu–Ser–His–Glu–Lys–Ser–Leu–Gly/Glu–Ala–Asp–Lys–Ala/Asp–Val–
               80              84
Asp–Val–Leu/Thr–Lys–Ala–Lys–Ser–Gln
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Figure 1. Structure of human parathyroid hormone.

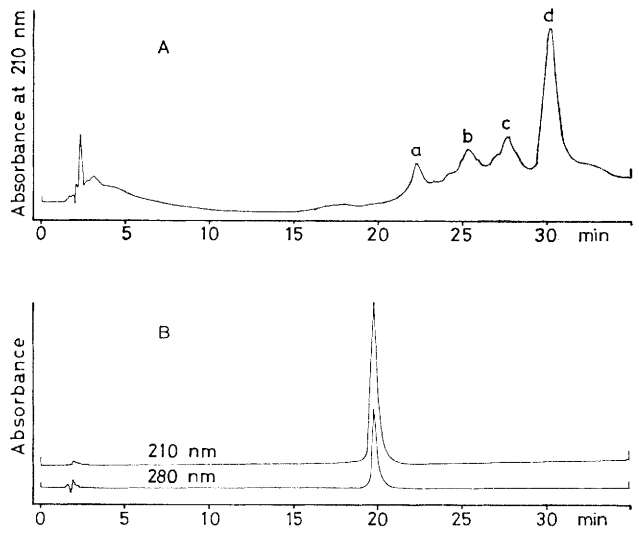

Figure 2. H.p.l.c. of synthetic hPTH(1—84): (A) Major product after chromatography on Sephadex G-50; (B) Synthetic hPTH-(1—84) after final purification. Recorded on Hitachi Liquid Chromatograph Model 638; column: Nucleosil $5C_{18}$ (Macherey–Nagel) 4 mm × 150 mm; eluants: Mixtures of acetonitrile and 10 mM phosphate buffer (pH 2.6) with Na_2SO_4 (50 mM), in which the acetonitrile concentration was increased linearly within 25 min from 26.4% to 35.5% for A and from 25% to 60% for B.

(1 g) was treated with HF at 0 °C for 1 h in the presence of anisole, dimethyl sulphide, and ethanedithiol to obtain the free peptide. The product was purified on CM-cellulose, and the resultant major product was further purified on Sephadex G-50; yield of crude product at this stage was 60 mg. The homogeneity of the product was determined by reversed-phase high performance liquid chromatography (h.p.l.c.) as shown in Figure 2(A). Three extra peaks (a, b, and c) were identified as those for the side products with Met(O) residue(s). The major peak (d) was isolated by h.p.l.c.; after two successive purifications by h.p.l.c., 10 mg of a homogeneous compound was obtained as shown in Figure 2(B), which revealed a single band on SDS gel electrophoresis at pH 6.8. This material did not move from the origin on cellulose t.l.c. with any solvent systems tried so far. During the above purification process, all side products, such as optical isomers, α-amino-succinyl peptides, and side chain-alkylated products, should have been removed completely as has been discussed in our previous paper.[3] An acid hydrolysate of the final product (6 M HCl containing thioglycolic acid, 110 °C, 48 h) showed the following amino acid content: Lys 9.18(9), His 3.72(4), Arg 5.15(5), Asp 10.03(10), Thr 1.00(1), Ser 6.19(7), Glu 10.89(11), Pro 3.12(3), Gly 4.00(4), Ala 7.14(7), Val 8.00(8), Met 1.58(2), Ile 0.94(1), Leu 10.00(10), Phe 0.91(1), and Trp 0.57(1). Peptide mapping of a tryptic digest of this material gave 12 of the expected peaks on h.p.l.c. together with a fused peak for Lys, Ala–Lys, and Ser–Gln. Thus, the product was judged to be highly homogeneous and proved to have the expected amino acid sqeuence for hPTH; $[\alpha]_D^{25}$ −81.2° (*c* 0.3, 1% AcOH). The potency in the *in vitro* assay of rat kidney adenylate cyclase activity[4] was determined to be 394(320—486) IU/mg using WHO bovine PTH(1—84) as the standard, which was reasonably comparable with the reported value of 350(275—425) MRC U/mg for native hPTH.[5]

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