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SYNTHESIS OF [2-SERINE, 8-VALINE]-HUMAN CALCITONIN[1]

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[2-Serine, 8-valine]-human calcitonin, an analog peptide, was synthesized by a liquid phase method, and exhibited a hypocalcemic activity of 80-120 MRC units.

To investigate the effect of variation in the primary structure of calcitonin on its hypocalcemic activity, we synthesized [2-serine, 8-valine]-human calcitonin (I), an analog of the hormone, which was a hybrid peptide between salmon calcitonin 1[2] and human calcitonin[3]. The N-terminal decapeptide of I corresponds to that of the salmon's hormone and its C-terminal tetracosapeptide amide corresponds to that of the human hormone.

H-Cys(1)-Ser(2)-Asn(3)-Leu(4)-Ser(5)-Thr(6)-Cys(7)-Val(8)-Leu(9)-Gly(10)-Thr(11)-Tyr(12)-
-Thr(13)-Gln(14)-Asp(15)-Phe(16)-Asn(17)-Lys(18)-Phe(19)-His(20)-Thr(21)-Phe(22)-Pro(23)-Gln(24)- : I
-Thr(25)-Ala(26)-Ile(27)-Gly(28)-Val(29)-Gly(30)-Ala(31)-Pro(32)-NH_2

Boc-Cys(1)-Ser(2)-Asn(3)-Leu(4)-Ser(5)-Thr(6)-Cys(7)-$NHNH_2$: II
Cbz-Val(8)-Leu(9)-Gly(10)-Thr(11)-Tyr(12)-Thr(13)-Gln(14)-Asp(15)-Phe(16)-$NHNH_2$: III
Cbz-Asn(17)-Lys(Boc)(18)-Phe(19)-$NHNH_2$: IV
Cbz-His(20)-Thr(21)-Phe(22)-$NHNH_2$: V
Cbz-Pro(23)-Gln(24)-Thr(25)-Ala(26)-Ile(27)-Gly(28)-Val(29)-Gly(30)-Ala(31)-Pro(32)-NH_2 : VI

The analog I was built up from five fragments (II-VI). Protection of side functional groups of these fragments was minimized in order to apply various chromatographic techniques on the purification of elongated peptides. The fragments were linked stepwise to the C-terminal fragment by the azide method. Every product was purified after removal of the N-terminal protecting group. By setting the molecular size of the N-terminal peptide fragment used in each coupling reaction sufficiently smaller than that of the produced peptide, gel filtration could be employed effectively for purification of the product. When necessary, partition chromatography on Sephadex[4] was further applied for the purification of deprotected peptides.

The disulfide bridge between two cysteinyl residues (Pos. 1 and Pos. 7) was

built prior to the final condensation lest the longer peptide be damaged during the removal of S-benzyl groups[5]. Tyrosine (Pos. 12) and aspartic acid (Pos. 15) were introduced into the peptide chain as N-carbobenzyloxy-O-benzyl ether and N-carbobenzyloxy-β-benzyl ester respectively. Fragment III was obtained from the corresponding t-butyloxycarbonyl hydrazide by HCl treatment. Cbz-Ala-Pro--NH_2 was prepared from Cbz-Ala-Pro-OH by the mixed anhydride method. Cbz-Ala--Pro-OH could be easily prepared by the coupling of Cbz-Ala-ONP and proline, followed by gel filtration on Sephadex LH-20.

In the final stage of the synthesis, the crude calcitonin analog was deprotected with HCl and then purified by CM-cellulose chromatography. The homogeneity of the product was confirmed on tlc with several kinds of solvent systems. Both amino acid analyses after HCl hydrolysis and aminopeptidase M digestion[6] of the analog peptide gave reasonable results.

The biological activity of the analog was measured in accordance with the literature[7] and a hypocalcemic activity of 80-120 MRC units was observed[8]. This potency is similar to or a little higher than that of human calcitonin but much lower than that of salmon calcitonin. It suggests that the high activity of salmon calcitonin is not ascribable only to its N-terminal sequence.

The details of the synthesis will be reported later.

References and Notes

1. The abbreviations used for amino acids and peptides are in accordance with the rules of the IUPAC-IUB Commission on Biochemical Nomenclature. Other abbreviations used are as follows.
   MRC = Medical Research Council Cbz = benzyloxycarbonyl
   Boc = t-butyloxycarbonyl ONP = p-nitrophenyloxy
2. H. D. Niall, H. T. Keutmann, D. H. Copp and J. T. Potts, Jr., Pro. Natl. Acad. Sci. U. S., 64, 771 (1969).
3. R. Neher, B. Riniker, W. Rittel and H. Zuber, Helv. Chim. Acta, 51, 1900 (1968).
4. D. Yamashiro, Nature, 201, 76 (1964).
5. M. Wilchek, S. Sarid and A. Pachornik, Biochem. Biophys. Acta, 104, 616 (1965).
6. G. Pfleiderer, P. G. Celliers, M. Stanulovic, E. D. Wachsmuth, H. Determann and G. Braunitzer, Biochem. Z., 340, 552 (1964).
   R. J. Delange and E. L. Smith in "The Enzymes", Vol. III, p. 102, P. D. Boyer, Ed., Academic Press, New York and London (1971).
7. C. W. Cooper, P. F. Hirsch, S. U. Toverud and P. L. Munson, Endocrinology, 81, 610 (1967).
8. We are indebted to the Research Laboratories of Toyo Jozo Co., Ltd., for biological assay of the product.

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