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SYNTHESIS OF A HENTETRACONTAPEPTIDE AMIDE CORRESPONDING TO THE ENTIRE AMINO ACID SEQUENCE OF HUMAN CORTICOTROPIN RELEASING FACTOR (hCRF)[1)]

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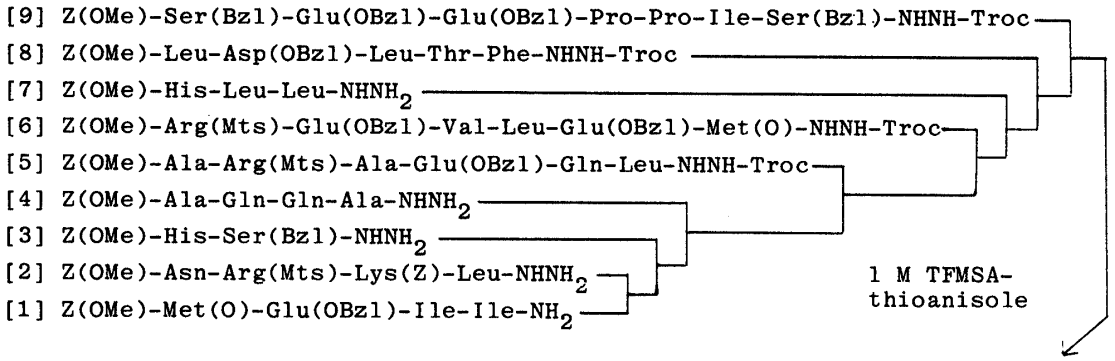
Human corticotropin releasing factor (hCRF), a hentetracontapeptide amide identical with rat CRF, was synthesized in a conventional manner by assembling nine peptide fragments followed by deprotection with 1 M trifluoromethanesulfonic acid-thioanisole in trifluoroacetic acid.

KEYWORDS ——— human corticotropin releasing factor (hCRF) synthesis; rat CRF; thioanisole-mediated deprotection; trifluoromethanesulfonic acid deprotection; Curtius azide rearrangement; immunoreactive corticotropin release

In 1983, the structure of human corticotropin releasing factor (hCRF) was elucidated by Shibahara et al.[2)] using cDNA sequence analysis. Incidentally, this hypothalamic principle was found to be identical with the releasing factor in rats.[3)]

We have synthesized the hentetracontapeptide amide corresponding to hCRF by a method different from those employed for the synthesis of ovine CRF.[4)] Between these two CRFs, substitution of amino acid residues is noted at seven positions; i.e., Gln(2), Thr(22), Lys(23), Asp(25), Leu(38), Asp(39), and Ala(41) of ovine CRF are replaced by Glu(2), Ala(22), Arg(23), Glu(25), Met(38), Glu(39), and Ile(41) respectively in hCRF. The method we employed is essentially the same as described in our previous synthesis of human growth hormone releasing factor.[5)] However, special care had to be taken to build up the entire peptide backbone of hCRF.

Fig. Synthetic Scheme for the Human Corticotropin-Releasing Factor



H-Ser-Glu-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-Ala-Arg-Ala-Glu-Gln-Leu-Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-Leu-Met-Glu-Ile-Ile-NH_2

In a combination of the TFA labile Z(OMe) group for N^{α}-protection, amino acid derivatives bearing protecting groups removable by TFMSA-TFA[6] were employed: Glu(OBzl), Asp(OBzl), Ser(Bzl), Arg(Mts)[7] and Lys(Z). Nine peptide fragments were selected as building blocks to construct the entire peptide backbone of hCRF. Each fragment was synthesized by the known amide-forming reactions. Of these, peptide hydrazides containing the Asp(OBzl) or the Glu(OBzl) residue were synthesized using substituted hydrazine, Troc-$NHNH_2$,[8] the protecting group of which can be removed by Zn[9] or Cd[10] in acetic acid.

The succesive azide condensations from fragment (1) to (7) proceeded satisfactory. However the subsequent azide condensations of fragment (8) and (9) had to be performed at a lower temperature (-18°C) than usual (4°C); otherwise, in both cases, the respective product gave a low recovery of the C-terminal amino acid of the employed acyl component in acid hydrolysis, due to the Curtius rearrangement followed by urea formation.[11] Protected intermediates and protected hCRF were purified by either precipitation from DMF or DMSO with appropriate solvents, such as MeOH or AcOEt, or by gel-filtration on Sephadex LH-60 using DMF-DMSO (7:3) as an eluant. Throughout this synthesis, Asp was taken as a diagnostic amino acid in acid hydrolysis. By comparing the recovery of Asp with that of newly incorporated amino acids, satisfactory incorporation of each fragment was ascertained.

In the final step of the synthesis, protected hCRF was treated with 1 M TFMSA-thioanisole/TFA in the presence of m-cresol (0°C, 90 min) and this thioanisole-mediated deprotection[12] was repeated twice more to ensure complete deprotection. The deprotected peptide was treated with dil. ammonia (pH 8.0, 0°C, 30 min) to reverse any possible N→O shift.[13] The Met(O) residue was then reduced by incubation with dithiothreitol (37°C, 24h). The crude product was purified by gel- filtration on Sephadex G-25, followed by ion-exchange chromatography on CM-Biogel A using gradient elution with 0.2 M $AcONH_4$ buffer (pH 5.0) containing 3 M urea. After being desalted by adsorption chromatography on

Diaion HP-20, the product was finally purified by HPLC on Vydac $5C_{18}$ (4.6 x 250 mm column) using gradient elution with acetonitrile (35 to 45% in 1h) in 0.2% TFA at a flow rate of 0.7 ml per min. (retention time, 39 min). The purified product, $[\alpha]_D^{16}$ - 84.1° (c=0.12 in 1N AcOH), exhibited a sharp single spot on TLC (Rf 0.41 in n-BuOH-AcOH-pyridine-H_2O=4:1:1:2, Rf 0.34 in n-BuOH-AcOH-pyridine-H_2O=30:20:6:24) and a single band in disk isoelectrophoresis (pH 3-10). Its purity was further confirmed by 6N HCl hydrolysis [Asp 2.00(2), Thr 1.00(1), Ser 2.89(3), Glu 9.18(9), Pro 2.28(2), Ala 4.36(4), Val 0.98(1), Met 1.71(2), Ile 2.17(3), Leu 6.97(7), Phe 0.99(1), Lys 1.08(1), His 1.81(2), Arg 2.83(3), recovery of Asp 77.6%] and papain + leucine amino peptidase digestion [Asp 1.00(1), Thr 0.98(1), Ser 2.90(3), Glu 6.27(6), Pro 1.81(2), Ala 4.22(4), Val 1.00(1), Met 1.86(2), Ile 3.13 (3), Leu 7.31(7), Phe 0.96(1), Lys 0.98(1), His 2.10(2), Arg 3.12(3), Asn(1) and Gln(3) were not determined, recovery of Asp 72.4%].

When tested by in vivo assay according to Rivier et al.,[14] our synthetic hCRF (1-10 μg) significantly stimulated secretion of immunoreactive corticotropin in rats.

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REFERENCES AND NOTES

1) Amino acids, peptides and their derivatives are of the L-configuration. The following abbreviations are used: Z=benzyloxycarbonyl, Z(OMe)=p-methoxybenzyloxycarbonyl, Mts=mesitylenesulfonyl, Bzl=benzyl, Troc=2,2,2,-trichloroethyloxycarbonyl, TFA=trifluoroacetic acid, TFMSA=trifluoromethanesulfonic acid.

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