β-FERROCENYLALANYL PEPTIDES

I. SYNTHESIS OF [Fer⁴, Leu⁵] ENKEPHALIN

E. CUINGNET, C. SERGHERAERT, A. TARTAR *

Laboratoire de Chimie Générale, Faculté de Pharmacie, 59045 Lille Cedex (France)

and M. DAUTREVAUX

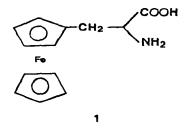*Laboratoire de Biochimie Structurale, Faculté de Médecine, 59045 Lille Cedex (France)*

Summary

The synthesis of [Fer⁴, Leu⁵] enkephalin, a ferrocene-containing peptide, is described.

Introduction

In numerous peptides the phenylalanine side chain appears to be critical for activity. Since the aromatic character of this residue must usually be preserved, the possibilities of replacing it are limited. Two well established approaches for modifying this residue are (a) the introduction of substituents at various positions on the ring, and (b) the use of heterocyclic aromatics. A quite different approach was used however by O. Leukart [1] who replaced phenylalanine by carboranylalanine (Car) in several peptides. An alternative approach to this problem involves replacing of the aromatic moiety of phenylalanine by an organometallic residue such as ferrocene.

As pointed out by Hanzlick [2], who investigated the biological effects of

1

DL-β-ferrocenylalanine (1) (Fer), because this organometallic aromatic system has an elongated cylindrical shape rather than the planar ring of conventional

aromatics, it may provide an interesting tool for studying drug-receptor interaction in a new dimension, i.e. perpendicular to the ring system. We describe below the synthesis of an analogue of [Leu^5]-enkephalin (2) [3], in which Phe^4 is replaced by Fer: [Fer^4, Leu^5]-enkephalin (3):

Tyr-Gly-Gly-Phe-Leu-OH   (2)

Tyr-Gly-Gly-Fer-Leu-OH   (3)

The insertion of Fer into [Leu^5]-enkephalin causes certain changes of physical and chemical properties of this peptide which require modification of the commonly used methods.

Synthesis

[Fer^4, Leu^5]-enkephalin (3) was synthesized by the solid phase technique [4] using the chloromethylated Merrifield resin. The starting resin (Boc-Leu resin, 0.5 mequiv Boc-Leu/g) was prepared by esterification with the Cesium salt of Boc-Leu according to the method of Gisin [5].

The couplings for the remainder of the synthesis of [Fer^4, Leu^5]-enkephalin were carried out on a Beckman 990 B automatic synthesizer according to the sequence given in Table 1.

The α amino functions were protected by the tert-butyloxycarbonyl (Boc) group. To minimize side reactions during HF cleavage, Tyr was incorporated as the 2,6-dichlorobenzyl ether [6].

Deprotection was accomplished with 40% trifluoroacetic acid (TFA) in methylene chloride with neutralization of the resulting THF salt by 5% diisopropyl ethyl amine (DIEA) to give the free amino group.

Each amino acid, except Boc-Fer, was incorporated using a 2.5 fold excess. Couplings were mediated by dicyclohexylcarbodiimide (DCC) 1 equiv, hydroxy-

TABLE 1

SEQUENCE OF COUPLINGS IN THE SYNTHESIS OF [Fer^4, Leu^5]-ENKEPHALIN

step	reagent	volume (ml)	duration (min)	No of times
1	CH_2Cl_2	30	2	2
2	40% TFA CH_2Cl_2	28	2	1
3	40% THF CH_2Cl_2	28	30	1
4	CH_2Cl_2	28	2	3
5	2-propanol	30	2	2
6	CH_2Cl_2	30	2	3
7	50% DIEA CH_2Cl_2	30	2	3
8	CH_2Cl_2	30	4	4
9 [a,b]	Amino acid-CH_2Cl_2	15	2	1
10 [b]	HOBt-DMF-CH_2Cl_2 (1—5)	7	2	1
11	DCC-CH_2Cl_2	7	120	1
12	CH_2Cl_2	30	2	3
13	2-propanol	30	2	2
14 [b]	CH_2Cl_2	30	2	4

[a] Amino acid derivatives were in 2.5 excess, except for Boc-Fer which was coupled twice in equimolecular quantity. [b] The vessel was not drained after these steps. [c] Steps 9—14 were repeated when a second coupling was performed.

benzotriazole (HOBt), 1 equivalent being added to prevent side reactions [7]. The completness of couplings was checked by the ninhydrin colour test procedure of Kaiser et al. [8].

Because of the special properties of the organometallic moiety of Fer, the following modifications were made to the usual procedures:

Synthesis of Boc-Fer, dicyclohexylammonium (DCHA) salt (4)

The DL-β-ferrocenylalanine * α amino function was protected by the Boc group. The synthesis of Boc-Fer was first attempted using 2-tert-butyloxycarbonyloxy-imino-2-phenylacetonitrile (Boc-ON) according to the method of Itoh et al. [10], but yields were low, ranging from 10 to 15%. Ditertiobutyldicarbonate [11] proved to be more satisfactory, and Boc-Fer was obtained in this case in yields ranging from 75 to 80%.

In each case, we observed that yields were considerably improved by adding ascorbic acid as a reducing agent during extraction of Boc-Fer from acidic medium. This is due to the oxidation of the ferrocene of Boc-Fer (5) to the ferricenium ion under these conditions: this oxidized form, Boc-Fer$^+$ (6), which is characterized by its deep green colour is stable, but its ionic nature prevents extraction by organic solvents. Addition of ascorbic acid quantitatively converts 6 into the hydrophobic 5, which can then be easily extracted.

Deprotection

Oxidation of Fer to the ferricenium form, Fer$^+$ was also observed during the deprotection step in the acidic medium used to remove the Boc group. During this operation the resin became deep green. This colour disappears during neutralization with DIEA 5%. Examination of the filtrates indicated that no destruction of the ferrocene moiety occurs during this operations.

HF treatment

The peptidyl resin was cleaved using anhydrous HF (10 ml per gram of resin, 1 hour, 0°C, 1 ml anisole being added as a scavenger). Fer appears to be stable during this treatment but oxidation to the ferricenium form is complete. Elemental analysis of the residual resin indicated only 0.40% Fe remaining which is consistent with a 85% yield for this step.

The CH_3COOH 10% washes of the resin were lyophilized and 735 mg of crude product were obtained.

Purification

The peptide, in its oxidized form: [Fer^{+4}, Leu^5]-enkephalin (VII) appeared to be stable only in quite acidic medium **, but its high solubility in aqueous solutions is a considerable advantage (due to the high lipophilicity of ferrocene, the reduced form (III) is only slightly soluble in water).

This prompted us at first to use the oxidized form during purification: the crude peptide was chromatographed on a CM 52 cation exchange resin eluted

---

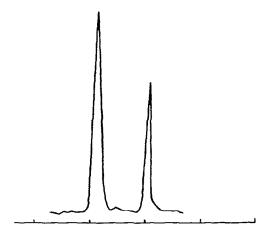* Synthesized by the method of Osgesby and Pauson [9].
** Even in the lyophilized form, VII is not stable and a slow decomposition occurs.

with CH_3COOH (1 M), CH_3COONH_4 (0.1 M). One major peak and a minor peak were observed; amino acid analysis indicated that the major peak had a correct composition (Tyr: 1.05; Gly: 2.1; Leu: 0.94), while in the minor peak tyrosine was low (Tyr: 0.16; Gly: 2; Leu: 0.98).

The fractions corresponding to the main peak were lyophilized but the peptide was partially destroyed during lyophilisation from the buffer solution. The poor stability of the oxidized peptide prompted us to use the reduced peptide during the purification step. In this case, after the HF treatment ascorbic acid was added until the green colour disappeared, and the resulting solution was lyophilized.

[Fer^4, Leu^5]-enkephalin is poorly soluble in water and was purified by chromatography on LH20 in methanol. A major peak was obtained which had the correct composition: Tyr: 0.92; Gly: 2.01; Leu: 1.12; Fer (based on Fe determination): 0.96. HPLC analysis of this material (Scheme 1) gave two

Scheme 1.

resolved peaks of equal importance corresponding to the two diastereoisomers of 4. The HPLC analysis was performed on a Water's μ Bondapark C_{18} reverse phase column (0.39 × 30 cm) with monitoring at 254 nm. The mobile phase used had the following composition: CH_3OH, 60%; H_2O, 35%; 1 M ammonium acetate buffer (pH = 4), 5%.

Conclusion

We have shown that it is possible to incorporate an organometallic amino acid in a peptide. Extension of this work to other peptides and their use as structural probes for receptors is in progress in our laboratory.

Acknowledgements

References

1 O. Leukart, E. Escher, D. Regoli and R. Schwyzer, Helv. Chim. Acta, 62 (1979) 546.
2 R.P. Hanzlik, P. Soine and W.H. Soine, J. Med. Chem., 22 (1979) 424.
3 J. Hughes, T.W. Smith, H.W. Kosterlitz, L.A. Fothergill, B.A. Morgan and R.H. Morris, Nature (London), 258 (1975) 577.
4 R.B. Merrifield, J. Amer. Chem. Soc., 85 (1963) 2149.
5 B.F. Gisin, Helv. Chim. Acta, 56 (1973) 1476.
6 B.W. Erickson and R.B. Merrifield, J. Amer. Chem. Soc., 95 (1973) 3750.
7 A. Arendt and A.M. Kolodziejczkyk, Tetrahedron Lett., 40 (1978) 3867.
8 E. Kaiser, R.L. Colescott, C.D. Bossinger and P.I. Cook, Anal. BioChem., 34 (1970) 595.
9 J.M. Osgerby, and P.L. Pauson, J. Chem. Soc., (1958) 656.
10 M. Itoh, D. Hagiwara T. Kamiya, Tetrahedron Lett., 49 (1975) 4393.
11 L. Moroder, A. Hallett, E. Wunsch, O. Keller and G. Wersin, Hoppe-Seyler's Z. Physiol. Chem., 357 (1976) 1651.