Vol. 85, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 29, 1978 Pages 780-782

D-Ala^2, Δ^ZPhe^4-Methionine Enkephalin Amide, A Dehydropeptide Hormone

by

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Received October 6, 1978

Summary The enkephalin analog, D-Ala^2, Δ^ZPhe^4 amide, has been prepared and shown to be five times as active as D-Ala^2-methionine enkephalin amide in vitro and the dehydrophenylalanine moiety conferred complete stability to chymotrypsin on the peptide.

Since the discovery of the opiate peptides, Met^5 and Leu^5 enkephalins (1), numerous analogs of these compounds have been prepared and tested. Along with our recent work on the synthesis[1] of dehydropeptides (DHP), we are interested in determining the effect[2] of the presence of a dehydro amino acid moiety on the bio-

Tyr-Gly-Gly-Phe-X-OH

1 X = Met or Leu

activity and enzyme stability of peptide hormones. For this reason, we have prepared D-Ala^2, Δ^ZPhe^4-methionine enkephalin amide[3] (2) which we hoped would show the enhanced opoid activity[4] of D-Ala^2-Met enkephalinamide while the

Tyr-D-Ala-Gly-Δ^ZPhe-Met-NH_2

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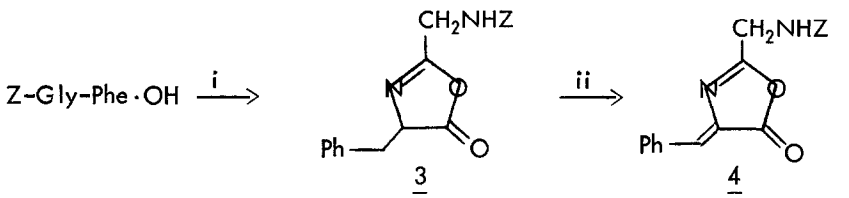
dehydrophenylalanine moiety might enhance its stability toward in vivo enzymolysis of the peptide.

Using our recently reported method for introduction of the unsaturated site[1], we prepared the unsaturated azlactone (4) by the dicyanodichlorobenzoquinone (DDQ) oxidation of the dipeptide azlactone and allowed it to react with methionine amide

0006-291X/78/0852-0780$01.00/0

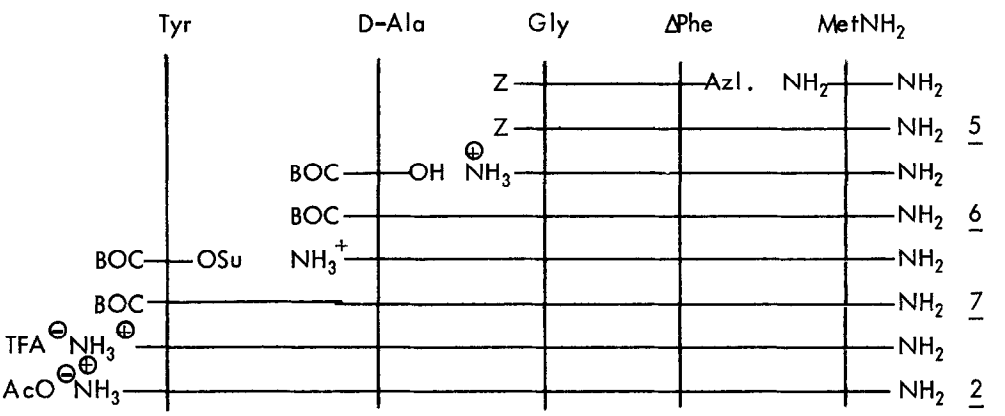
(Scheme I) at room temperature (48 hr.) in dimethylformamide solution. After deblocking with HBr/HOAc, the resulting tripeptide amide[5] (5) was coupled via the mixed anhydride method with BOC-D-alanine to give the blocked tetrapeptide[5] (6). An activated ester of tyrosine was allowed to react with deblocked (trifluoroacetic



Z = $PhCH_2OCO$ i = DCC/THF/5°; ii, DDQ DCC = N,N-dicyclohexylcarbodiimide

acid/CH_2Cl_2) 6 to give the blocked enkephalin[5] 7. Final deblocking of 7 with trifluoroacetic acid, conversion to the acetate salt and purification on Biogel P2 gave the amorphous dehydro hormone, 2, $[\alpha]_D = +31.6^\circ$ (c 1, H_2O). This product showed a single spot in three tlc systems and gave the expected amino acid analysis, Tyr:Ala:Gly:Met, 1.00:0.90:1.00:1.02.

Scheme I



When the Δ^ZPhe[4]-hormone (2) was treated with chymotrypsin (peptide: enzyme, 100:1, 37°) in tris buffer at pH 8.5 for thirty hours, no hydrolysis was observed.[6] This is consistent with our previous observations[1c] on Pro-Δ^ZPhe-His-Leu[1b] and with an early report on the special stability of unsaturated peptides.[7] The

unsaturated peptide showed an ED_{50} of 3.3×10^{-9} in the stimulated guinea pig ileum assay[8,9] whereas the saturated compound was less effective (ED_{50} 1.7×10^{-8}M) by a factor of ca. 5. These results indicate that whatever the changes in molecular shape demanded by the rigid unsaturated site in the phenylalanine moiety, these changes facilitated interaction with the ileum receptor sites, but inhibited interaction with the chymotrypsin active site. We might expect the introduction of a double bond to increase the lipophilicity of the molecule, and this is indeed shown by its solubility and chromatographic behavior. Future speculation about the bioactive conformation of enkephalin must take into account the Z-configuration (Ph and C=O, trans) of the ΔPhe residue and its effect on the overall shape of the molecule.

References

1) Paper IV. Dehydropeptides; (a) Paper I, S. Konno and C. H. Stammer, Synthesis, 598 (1978); (b) Paper II, S. Konno and C. H. Stammer, Int. Journ. Peptide and Protein Res., 1978, in press; (c) Paper III, M. L. English and C. H. Stammer, Biochem. Biophys. Res. Comm., 83, 1464 (1978).

2) ΔPhe^4-Methionine enkephalin has been recently reported, but no biological data was given; see S. G. Link, Ph.D. Dissertation, University of Michigan, 1977, pp. 81-89.

3) Δ^ZPhe = 2,3-dehydrophenylalanine having the Z-configuration.

4) C. Pert., Science, 194, 330-331 (1976).

5) All new compounds showed single spots in three tlc systems and correct elemental and amino acid analyses.

6) Tlc in two solvent systems and visualization of spots by ninhydrin and uv absorption.

7) M. Bergman and H. Schleich, Zeit. Physiol. Chem., 205, 65-73 (1932).

8) We thank Drs. James M. Stewart and Richard E. Chipkin of the University of Colorado Medical College, Denver, Colorado, for this data.

9) S. Ehrenpreis, Methods in Narcotic Research, S. Ehrenpreis and A. Neidle, Eds., M.Dekker, New York, 1975, p. 111.