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The Enzyme Stability of Dehydropeptides[1]

by

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Summary: A pentapeptide, Z-Gly-Gly-Phe-Phe-Ala·OH (1b) and the corresponding unsaturated pentapeptide, Z-Gly-Gly-Phe-Δ^{Z}Phe-Ala·OH (1a), have been synthesized. The saturated compound (1b) was rapidly hydrolyzed by both chymotrypsin and thermolysin to the expected products, but the dehydropeptide was completely unhydrolyzed by either enzyme even after thirty hours. A new method of peptide stabilization to enzymolysis is made available.

In 1932 Bergmann[2] described the resistance of Gly-Δ^{Z}Phe[3] to enzyme preparations of both animal and plant origin; i.e., amino peptidases, carboxy peptidases and tryptic proteinases as well as pepsin. These workers did, however, find that both sheep and swine kidney extracts were capable of hydrolyzing the dehydropeptide after spoilage (Fäulnis) of the enzyme preparations had occurred. Greenstein[4] and coworkers separated the dehydropeptidase activity discovered by Bergmann into two enzyme preparations, dehydropeptidase I and II. Later he showed that dehydropeptidase I was an amino acid acylase and dehydropeptidase II was an aminopeptidase and that neither enzyme was specific for dehydropeptides but acted on saturated substrates also. Since that time, to our knowledge, no dehydropeptidases have been described and we can assume that there are no endogenous enzymes of this kind in animal systems.

Based on the Bergmann report, we were lead to propose that the incorporation of an α,β-unsaturated amino acid residue into a peptide sequence might confer enzyme stability on the peptide. It seemed quite possible, also, that the intrinsic bioactivity of the peptide might not be seriously impaired. This paper reports the synthesis of a dehydropeptide substrate designed to investigate the question of dehydropeptide stability to a couple of powerful endopeptidases.

Both 1a, the dehydropeptide, and 1b, the corresponding saturated peptide were synthesized. The dehydro dipeptide azlactone 4, m.p. 168-169°, $[\alpha]_D$ -65°

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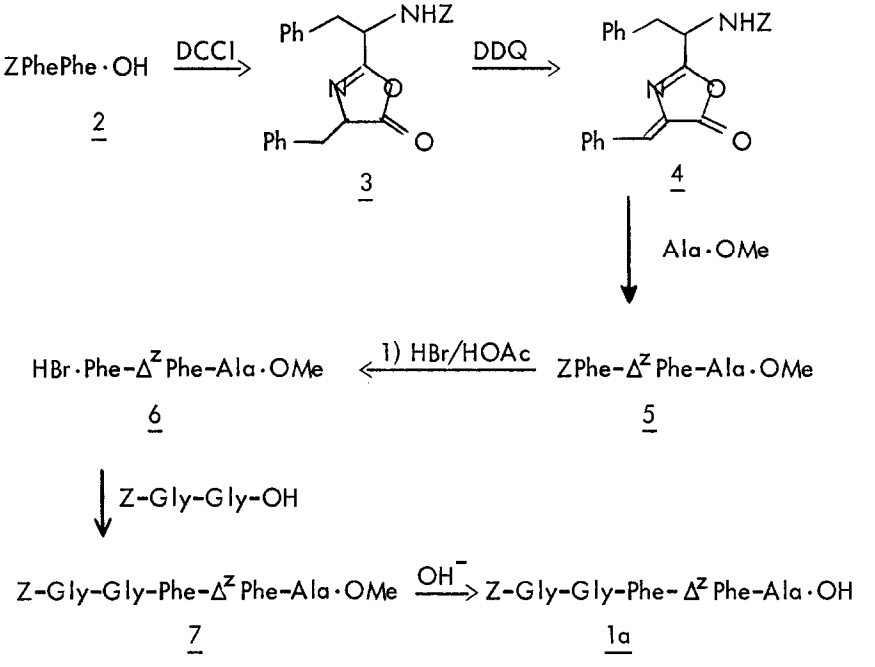
(c 1,DMF) was prepared by DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) dehydrogenation[1a] of the saturated azlactone[5] (3) (Scheme I) in 43% yield.

Z-Gly-Gly-Phe-X-Ala

1a X = Δ^Z Phe

1b X = Phe

Scheme I



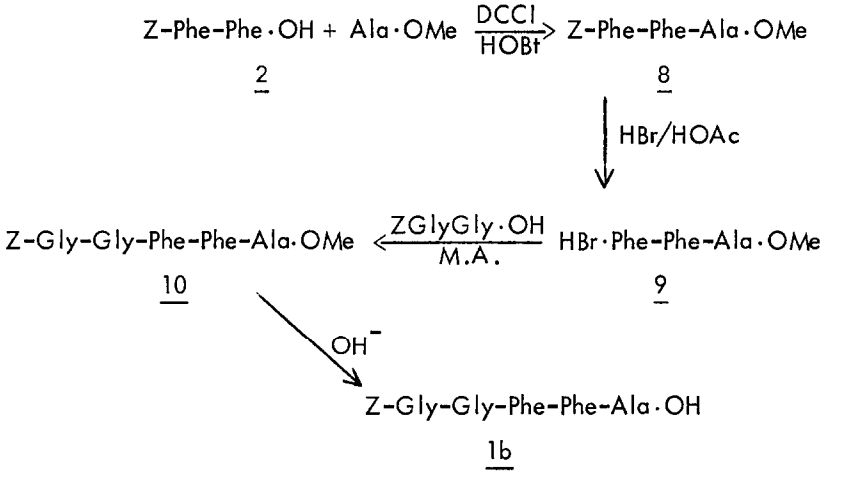
Treatment of 4 with alanine methyl ester in DMF solution at 30° for 24 hr gave the dehydrotripeptide[5] in 68% yield. As previously discussed[1b], it is possible that the N-terminal phenylalanine moiety of 4 might be partially racemized during the dehydrogenation reaction. The fact that the tripeptide 5 showed a clean doublet in its nmr spectrum corresponding to the alanine methyl group is good evidence that 5 is not a mixture of L,L- and D,L-diastereomers and, consequently, that no racemization had occurred.

After deblocking 5 with HBr/HOAc, the hydrobromide 6 was coupled with the Z-Gly-Gly·OH[6] by the standard isobutylchloroformate mixed anhydride procedure.

The crude dehydropentapeptide 7 was saponified to form the amorphous 1a, $[\alpha]_D^{27}$ -35.5° (c 1,DMF) which had an acceptable amino acid analysis,[7] Gly:Phe:Ala, 1.87:1.11:1.02 and was homogeneous in three tlc systems.

The saturated peptide, 1b, was prepared according to standard methods as shown in Scheme II. The new compounds, 8 and 9 were characterized[5] as before

Scheme II



and the amorphous final product, (1b), $[\alpha]_D^{27}$ -20.3° (c 1,DMF), had an amino acid analysis of Gly:Phe:Ala, 1.96:2.14:0.95, and showed homogeneity in three tlc systems.

These two peptides, 1a and 1b, were treated separately with chymotrypsin and with thermolysin[8] (EC 3.44 group), a bacterial proteinase. Chymotrypsin is well known to have specific endopeptidase activity for the carboxyl site of aromatic amino acid residues while thermolysin shows endopeptidase specificity for the amino sites for hydrophobic amino acid residues.[9] When treated with each of these enzymes (peptide: enzyme, 100:1; tris/pH 8.5; 37°) the saturated peptide, 1b, was hydrolyzed rapidly to form ninhydrin positive spots corresponding to phenylalanine and alanine as visualized by ninhydrin and uv light (tlc on silica plates using three different solvent systems). The dehydropeptide, 1a, was completely unhydrolyzed by either enzyme under the same conditions even after thirty hours.

This result indicates that enzymolysis, by either of these enzymes, at both the carboxyl and amino sites of the dehydrophenylalanine moiety was prevented by the presence of the double bond. These results suggest that the incorporation of a dehydro

amino acid moiety into a bioactive peptide may be an attractive way to increase peptide stability in living systems. Since other recent work from this laboratory indicates that the incorporation of dehydrophenylalanine into a bioactive peptide does not compromise the activity of the peptide sequence,[1a,c] a new method for the stabilization of bioactive peptides has become available.

References

1) Paper III: Dehydropeptides; (a) Paper IV, J. Chem. Soc., M. L. English and C. H. Stammer, Chem. Comm., 1978, in press; (b) S. Konno and C. H. Stammer, Synthesis, 598 (1978); (c) S. Konno and C. H. Stammer, Int. Jour. of Peptide and Protein Res., 1978, in press.

2) M. Bergmann and H. Schleich, Zeit. Physiol. Chem., 205, 65 (1932).

3) The Δ means that the dehydrophenylalanine moiety has a double bond between the α- and β-carbon atoms and the superscript z indicates that the dehydro moiety has the Z-configuration (Ph and C=O trans) about the unsaturated site.

4) (a) Methods in Enzymology, Vol. II, ed. by S. P. Colowick and N. O. Kaplan, Academic Press, New York, N.Y., 1955, pp. 109-119; (b) S. M. Birnbaum, L. Levintow, R. B. Kingsley and J. P. Greenstein, J. Biol. Chem., 194, 455 (1952); (c) D. S.Robinson, S. M. Burnbaum, and J. P. Greenstein, J. Biol. Chem., 202, 27 (1953); (d) J. P. Greenstein, Adv. in Enzymology, Vol. 8, Interscience, New York, N.Y., 1948, pp. 117-169.

5) All new compounds had the correct elemental analysis and were characterized by infrared and nmr spectroscopy.

6) A.F.Beecham, Austr. J. Chem., 16, 160 (1963).

7) Hydrolysis of ΔPhe peptides gives NH_3 and phenylpyruvic acid which do not appear in the amino acid analysis.

8) H. Matsubara, A. Singer, R. Sasaki and T. H. Jukes, Biochem. Biophys. Res. Comm., 21, 242 (1965).

9) K. Morihara and M. Ebuta, J. Biochem., 61, 149 (1967).