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CONFORMATIONAL ANALYSIS OF SOME CYCLIC PEPTIDES USING DIFFERENTIAL N-METHYLATION

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Partial N-methylation of peptides with CH_3I-Ag_2O in DMF constitutes a new potential method of conformational analysis. Reactivity of peptide NH's with the methylating reagent reflects their degree of exposure to the solvent. The method was applied to the samples of micromolar quantities of a Gramicidin S derivative and two destruxins.

In the conformational analysis of peptides one of the most important steps is to reveal the nature of each peptide NH; i.e., whether it is exposed to solvent or shielded from solvent either sterically or through H-bonds. Most commonly and successfully used methods are PMR studies[1] of H-D exchange and temperature and solvent dependence of the chemical shifts. In this communication a new and unique method for analyzing peptide NH's, namely "differential N-methylation", is described.

Gramicidin S (I) is a cyclic decapeptide whose conformation is established[2] and has four intramolecular H-bonds between two pairs of Val and Leu residues as shown in Fig. 1. As the first example we studied the diphthalyl derivative of I, which has no NH on Orn side chains. Diphthalyl-I (1 μmol), CH_3I (2 mmol), and Ag_2O (0.13 mmol) in DMF (1 ml) were stirred at room temperature, 0.3 ml aliquots removed from the reaction mixture after 1, 5, and 25 hr, and poured into MeOH. After removing insoluble silver salts the partially methylated peptide was hydrolyzed and analyzed by an amino acid autoanalyzer. The result shown in Fig. 2[3] indicates marked difference of reactivity among the NH's; i.e., NH's of Orn and D-Phe were methylated considerably while Val and Leu residues were almost intact, indicating

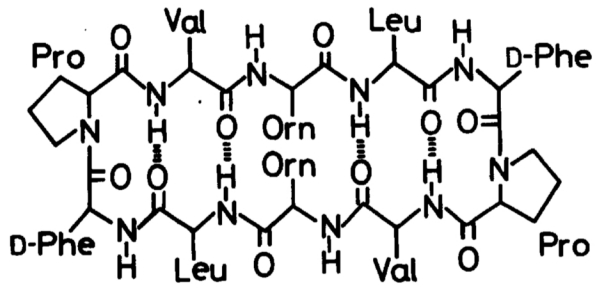

Fig. 1. Conformation of Gramicidin S (I).

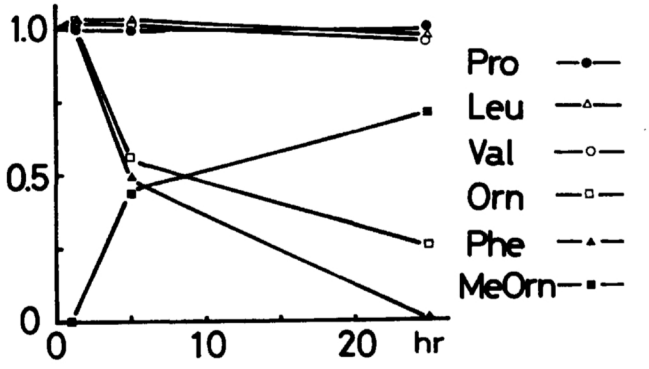

Fig. 2. Differential N-Methylation of Diphthalyl-gramicidin S. The amounts of remaining amino acids and of N^{α}-methylornithine are shown. Proline is taken as standard.

that diphthalylgramicidin S in DMF solution also assumes similar conformation to that of I. The amount of N^{α}-methylornithine formed was also measured and was consistent with the decrease of Orn.

The method was then applied to destruxin B (II), a cyclodepsipeptide, and its biosynthetic precursor, protodestruxin (III). The structure of II is shown in Fig. 2 and III has the same primary structure except that two N-methyl groups of II are replaced by hydrogens. Elaborate PMR study by Naganawa et al.[4] revealed that II assumes a rigid conformation in DMSO-d_6 containing two intramolecular H-bonds as shown in Fig. 3, while III was shown to have a mobile conformation. Consistent with PMR study the NH's of II were methylated more slowly than those of III as shown in Fig. 4. It is interesting that among the NH's of III those of Val and Ala, which are biosynthetic methylation sites, were also methylated more easily than those of β-Ala and Ile by this chemical method. An explanation for this result is that the precursor III, though less rigid than II, assumes a similar conformation as shown in Fig. 3. An alternative explanation is that among the randomly methylated products of III the molecules which were methylated at Ala or at Ala and Val assume a rigid H-bonded conformation[4] resulting in a little slower rate of N-methylation of remaining β-Ala and Ile. The ester function in the depsipeptides is assumed to be intact under the N-methylating conditions because acetylglycine ethyl ester under similar conditions gave exclusively acetylsarcosine ethyl ester.

Though general application of the differential N-methylation method to non-cyclic peptides would encounter some complexity caused by methylation of N- and C-terminal and side chain functional groups, this method possesses following two advantages; i) extremely small amounts of the samples are required (~1 μmol) and ii) unequivocal assignments of H-bonded NH's to specific amino acids.

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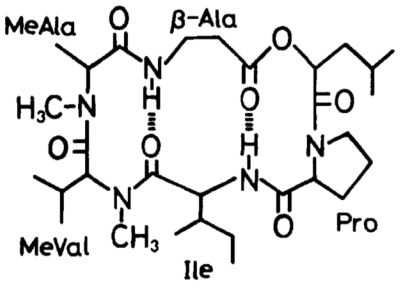

Fig. 3. Conformation of Destruxin B (II).

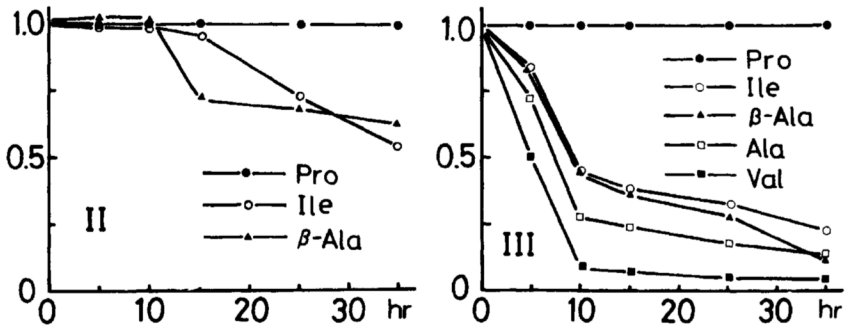

Fig. 4. Differential N-Methylation of Destruxin B (II) and Protodestruxin (III). II or III (2 μmol), CH_3I (4 mmol), and Ag_2O (1 mmol) in DMF (2 ml).

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