CHEMISTRY LETTERS, pp. 703-704, 1980.

A NEW METHOD FOR CONFORMATIONAL ANALYSIS OF PEPTIDES BY HYDROGEN-CHLORINE REPLACEMENT REACTION

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Replacement reaction of peptide NH with chlorine was applied to the peptide antibiotics, gramicidin S and tuberactinamine N. The rate of chlorination followed by NMR spectra demonstrates that the hydrogen-bonded peptide NH in the molecule is much more susceptible to chlorination than the solvent-exposed peptide NH.

Several ^{1}H-NMR techniques have been widely used for the detection of hydrogen-bonded peptide NH's in order to study the conformation of biologically active peptides in solution.[1] These detective methods are based on the followings: (a) the rate of H-D exchange of peptide NH's, (b) the temperature and solvent dependence of peptide NH chemical shifts, and (c) the broadening of peptide NH resonances by addition of a free radical. We report here a new method named "hydrogen-chlorine (H-Cl) replacement" reaction, which was initiated by adding a peptide to a solution containing Cl_2 or adding *t*-butylhypochlorite (*t*-BuOCl) to a solution of a peptide. Protons of the peptide NH susceptible to chlorination were determined from the spectra obtained on a JEOL-JNM-MH 100 spectrometer.

Gramicidin S (I) is a cyclic decapeptide having four intramolecular hydrogen bondings between two pairs of Val and Leu residues (Fig. 1). Table 1 shows that the results of H-D exchange experiment of I are consistent with that reported previously.[2] However, in the H-Cl replacement reaction with Cl_2 or *t*-BuOCl, both NH protons of Val and Leu are more rapidly replaced by chlorine than those of Orn[3] and Phe. Obviously, the peptide NH's involved in intramolecular hydrogen bondings are much more susceptible to chlorination than the solvent-exposed peptide NH's.

In order to examine further the usefulness of this method, the H-Cl replacement of tuberactinamine N (II) (0.083 M) with Cl_2 (10 eq) in Me_2SO-d_6 was carried out. The remaining peptide NH(%) of the position 5 was determined to be 56% after the chlorination for 5 min and 24% for 30 min, whereas 80 to 90% of the remaining NH's were still observed for the positions 1, 2, 3, and 4 after 30 min. These results are consistent with the results of detailed NMR study reported by Wakamiya and

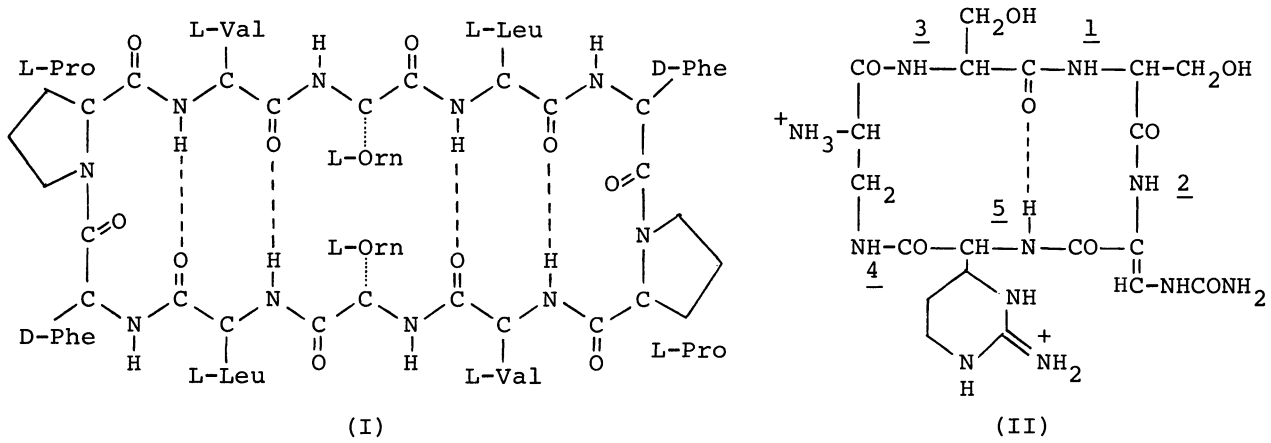

Fig. 1. Conformations of gramicidin S (I) and tuberactinamine N (II).

Table 1. Approximate rates (T/2) of H-D exchange and H-Cl replacement reaction in peptide NH protons of gramicidin S (0.05 M)

	Val	Leu	Orn	Phe
D_2O (10 eq) in Me_2SO-d_6	>24 h	21 h	18 min	8 min
CD_3OD (20 eq) in Me_2SO-d_6	90 min	70 min	13 min	2 min
Cl_2 (20 eq) in Me_2SO-d_6	7 min	10 min	100 min	120 min
t-BuOCl (60 eq) in CH_3OH-CF_3CH_2OH (1:1, v/v)	65 min	110 min	>4 h	>4 h

Shiba[4] on the conformational analysis of II, which showed the presence of an intramolecular hydrogen bonding at the position 5 in the cyclic part (Fig. 1). Hence, the H-Cl replacement is shown to be applicable to readily detect the peptide NH moieties with the hydrogen bonding in the molecule. Further application of this method to conformational analysis of peptides will be presented elsewhere.

The authors express their appreciation to Professor T. Shiba and Dr. T. Wakamiya, Osaka University, for the gift of tuberactinamine N.

References and Note

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2) A. Stern, W. A. Gibbons, and L. C. Craig, *Proc. Natl. Acad. Sci. U.S.*, 61, 734 (1968).

3) In a preliminary experiment, the protonated δ-amino group of N^{α}-acetyl-L-Orn-OEt·HCl was not susceptible to chlorination in Me_2SO-d_6 after 30 min.

4) T. Wakamiya and T. Shiba, *Bull. Chem. Soc. Jpn.*, 48, 2502 (1975).

(Received March 21, 1980)