NMR STUDIES OF H-D EXCHANGE OF α-CH GROUP OF AMINO ACID RESIDUES IN PEPTIDES

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Summary: The advantages applying NMR techniques to the study of racemization is illustrated and discussed. Using NMR it is possible to measure the rate of racemization of each individual amino acid residue in a peptide. It was found that the rate of racemization depends on the nature of the side chain of the amino acid residue, and on the location of the amino acid in the peptide chain.

Introduction

In the course of NMR measurements of peptides containing glycine residues in basic D_2O solutions, we have observed selective decreases in the area of the spectral lines of some of the methylene groups which develop with time. We did not observe, however, any spectral changes when we repeated the same experiments with basic H_2O solutions. This result clearly indicates the existence of hydrogen-deuterium exchange of the protons of the methylene groups of some of the glycine residues of the measured peptide molecules.

In this paper, we report the result of the H-D exchange of the protons of the α-CH groups of several amino acid residues located in different places in the peptide molecules. The importance and application of this H-D exchange reaction are discussed.

Materials and Instruments

The peptides have been synthesized by the dicyclohexylcarbodi-imide me-

thods, (1) or obtained commercially. Varian HA 100 spectrometers have been used for the NMR measurements, and the pH have been determined by a Radiometer pH meter 26. No corrections for pD have been done.

Methods of Measurements

It is convenient to measure, in several cases, rate and degree of an exchange between hydrogen and deuterium by the NMR technique (2). The H-D exchange can be followed by measuring (i) the decrease of the area of the H^1 spectral lines of the exchanging groups; (ii) the changes in the H^1 spectral line shape of an adjacent group which is spin-spin coupled with the exchanging protons. The change in the line shape of an adjacent group due to the H-D exchange arises from the difference in the spin of H ($1=\frac{1}{2}$) and D ($1 = 1$). Hence, the multiplicity of the spectrum of the adjacent group should be different depending whether this group is coupled to hydrogen or deuterium. However, protons coupled to an adjacent deuterium will usually show a single line due to the small magnitude of the spin spin coupling.

The effect of the H-D exchange of the α-CH group of a serine residue on the spectral line shape of the adjacent group, the β-methylene, is illustrated in Fig. 1. The spectral line of the methylene group is a doublet due to the spin-spin interaction with the proton of the α-CH group. Replacing the proton of the latter group with deuterium causes the CH_2 spectral line to appear as a singlet instead of the doublet. We thus observe a decrease in the doublet and an increase in the singlet with time.

It is usually more convenient to measure the H-D exchange according to the second method, since the signals of the β-methyl or methylene group of an amino acid residue are usually stronger and less complicated, especially after the introduction of deuterium in the α-position, than that of the α-CH.

For the measurement of the exchange of the methylene of glycine residues, we are forced to employ the first method, i.e. to measure the H-D exchange reaction from the decreases in the signals area. It is worthwhile, however, to emphasize that the exchange reaction, in this case, is associated also with

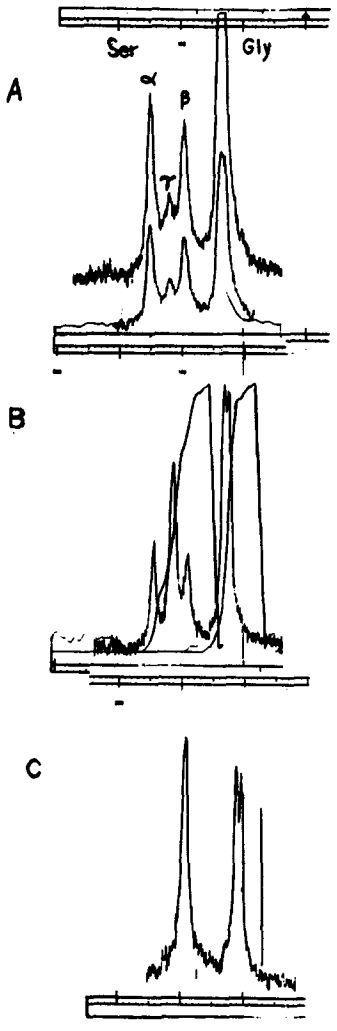

Fig. 1 NMR spectra of the methylene group of Ac-Ser-Gly dissolved in D_2O (pH 13.5) after: A) 0.66h, B) 3.5h and C) 22.0h of the dissolution of the peptide. The doublet consisting of the spectral lines α and β are due to the methylene group of the serine residue ND. $CH(CO) . CH_2OD$. The singlet, the spectral line denoted by γ, is due to the serine residue $ND.CD(CO.CH_2OD$. Integration of the spectral lines is given in B. (Note that the spectral line of the methylene group of the glycine residue does not change).

changes in the line shape. This is due to the fact that introduction of one deuterium atom in the methylene group causes the spectrum of the remaining hydrogen atom to appear as a triplet due to the spin spin interaction between the two nuclei, H and D, which are in the same methylene group. The H^1 spectral

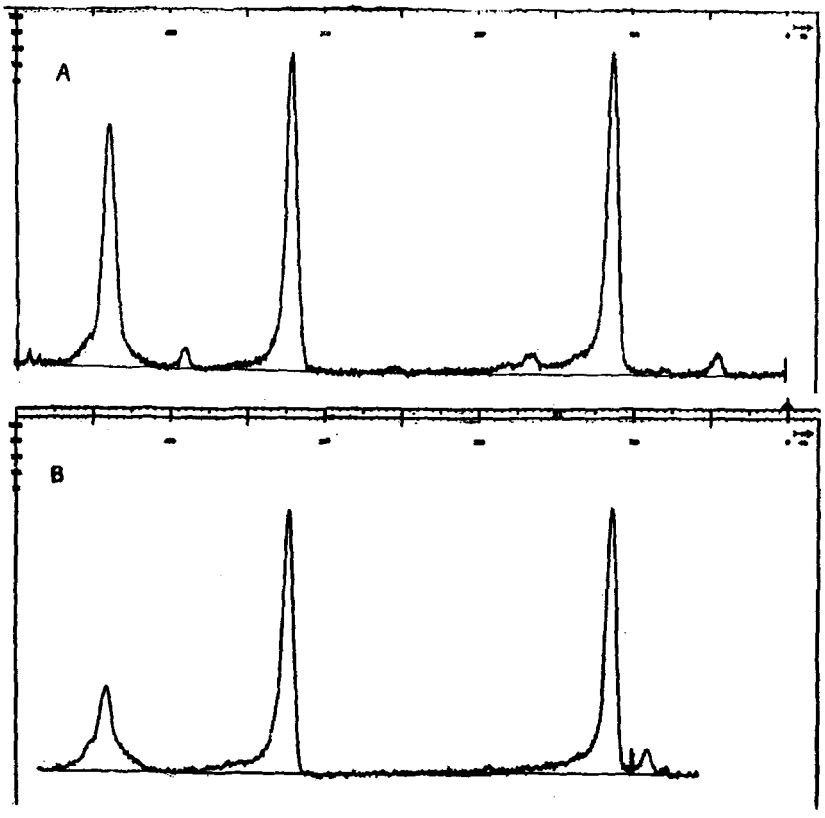

Fig. 2 NMR spectra of the methylene groups of Gly-Gly-Gly dissolved in D_2O (pH 13.3) after: A) 0.83h, B) 5.75h of the dissolution of the peptide. The lines in order of increasing field, from left is right, are due to the methylene groups of the middle glycine residue, the C- and N- terminal residues, respectively. (Note that the spectral intensity of the methylene groups of the middle glycine residue decreases and also changes from a singlet into a broad triplet.)

line is, however, broad due to the deuterium quadropole relaxation (illustrated in Figure 2).

The spectral lines have been assigned from observing the magnitudes and directions of the shifts of the spectral lines with changes of the pH of the solutions (3).

Experimental Results

In order to determine which of the amino acid residues in a peptide molecule undergo the H-D exchange (under the conditions we have generally used:

pH 11-13.5 and room temperature). We examined changes in the spectrum of Gly, Gly-Gly and Gly-Gly-Gly dissolved in basic D_2O solution (pH 13.1). After 21 hours, we did not observe any H-D exchange in the methylene group of Gly and Gly-Gly. In contrast, we observed an appreciable decrease in the area of the signal of the methylene group of the middle glycine residue of triglycine, even after 5 hours, (Figure 1). It should be emphasized, however, that no changes in the spectral lines of the methylene groups of the two terminal glycine residue of triglycine have been detected. The conclusion that we may derive from the above experiments is that the H-D exchange of the hydrogens of the methylene group of glycine, and most probably the exchange of any other hydrogen of the α-C-H group of different amino acid residues, under the mild condition that we have used, occur only when these groups are bonded to two peptide groups.

We also measured various other peptides and found that the exchange occurs only in specific groups while the hydrogens of the other groups remain unaffected. The peptides are: Ac-Gly-Gly, Gly-Gly-Gly-Gly, Gly-Gly-Gly-Gly-Gly Gly-Gly amide, Ac-Ala-Gly, Ac-Ser-Gly, and the groups in which protons exchanges occur are underlined (only the H of the α-CH groups of the two last peptide exchange). This result verifies our previous conclusion about the condition necessary for exchange to occur.

The effect of the pH on the rate of the H-D exchange has been examined by comparing the rates of exchange of the middle glycine residue of triglycine at different pHs. It was found that an increase of the pH from 13.1 to 13.3 results in an increase of more than 10% in the rate.

The dependence of the rate of the H-D exchange on the location of the amino acid residue in the peptide molecule with respect to functional group is illustrated by the following results: (i) The rates of the H-D exchange of the two exchanging methylene groups of tetraglycine are different, being faster for the glycine residue which is further removed from the carboxylic group. (ii) The hydrogens of the methylene group of the blocked glycine residue of

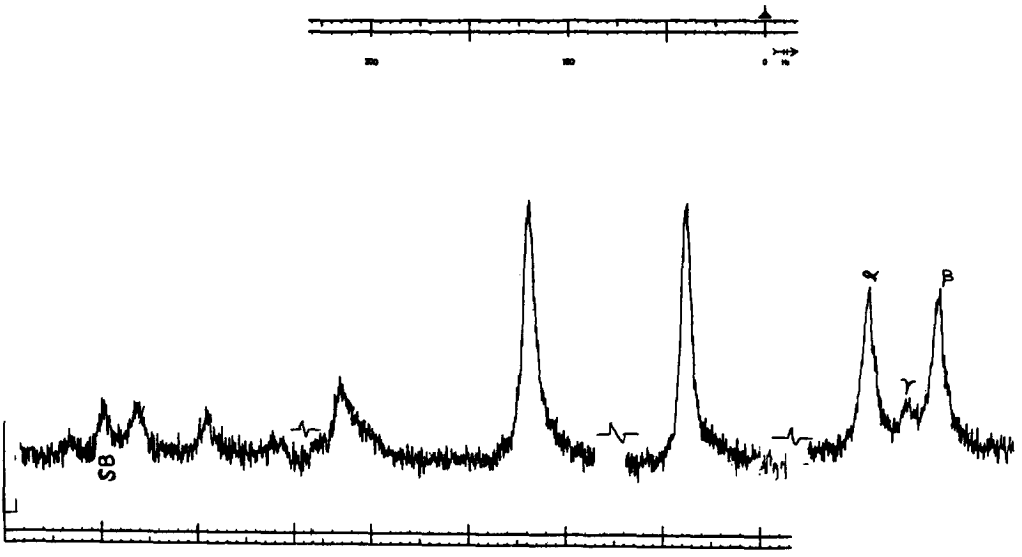

Fig. 3 NMR spectrum of Gly-Ala-Gly-Gly dissolved in D_2O (pH 13.5) after 2.33h. The lines in order of increasing field are: a quartet due to the α-CH group of the Ala residue. The methylene group of the middle glycine residue, the methylene groups of the C and N terminal residue, respectively. The lines in upper field are due to CH_3 group of the alanine residue. While the doublet (lines α and β) is due to the alanine residue $ND.CH(CO).CH_3$, the singlet γ is due to the alanine residue $ND.CD(CO).CH_3$.

$CF_3CONHCH_2CONHCH_2CONHCH_2COO^-$ exchange by far more readily than the middle glycine residue.

The rates of the H-D exchange differ among the various amino acid residues. The difference can be very big, thus comparison between the rates of the H-D exchange of Ac-Ser-Gly and of Ac-Ala-Gly shows that the rate of the former is one order of magnitude faster than that found in Ac-Ala-Gly. Another example which illustrates a large difference in the H-D rate of exchange is given in Figure 3. This figure shows the spectrum of Gly-Ala-Gly-Gly dissolved in basic D_2O solution (pH 13.5). It can be seen from this figure that after 2.2 hours of dissolution, the degree of H-D exchange of the hydrogens of the methylene of

the middle glycine residue is almost half, whereas less than 5% of the α-CH of the alanyl residue have exchanged.

Discussion

The H-D exchange that we have observed is the well-known racemization reaction occurring to optically active amino acids, peptides and proteins. From recent literature, it appears that the main interest in racemization reaction for the above substances, is a preparative one, (4) i.e., to look for a method for preparing amino acid derivatives or peptides without losing their optical activities. In contrast, very few kinetics studies on racemization of peptides (5) have been published. These studies which are important by themselves, may ultimately lead to synthetic applications.

The use of NMR technique for the kinetic studies of racemization of peptides containing different amino acid residues, provide a tool to measure, very simply, the rate of racemization of each of the amino acid residues separately, as compared to the usual methods, such as optical rotation, which measures the overall racemization. Furthermore, the use of the NMR method, enables us to measure "racemization" of non optically active peptides.

It is worthwhile emphasizing that the results published here indicate that it is possible, in several cases, to introduce preferentially isotope (Deuterium or Tritium) into the methylene group of glycine residues in peptides without causing racemization to the peptide molecule.

A detailed study on the H-D exchange and on the racemization of the various amino acid residues in different peptides is in progress.

References

1. T.C. Sheehan and G.P. Hess, J.Am.Chem.Soc. 77, 1067 (1955).
2. See e.g., A. Lapidat, J. Reuben, and D. Samwel, J.Chem.Educ. 41,570(1964).
3. M. Sheinblatt, J.Am.Chem.Soc. 88, 2845 (1966).
4. See e.g., a) M.Goodman and L.Levine, J.Am.Chem.Soc. 84, 2918 (1964).
   b) G.T.Young, J.Chem.Soc. 3701 (1964).
5. A review by A. Neuberger in "Advances in Protein Chemistry. IV", p. 267, 1948, Academic Press.