CONFORMATIONAL ANALYSIS OF AMINO ACIDS AND PEPTIDES USING SPECIFIC ISOTOPE SUBSTITUTION. I. CONFORMATION OF L-PHENYLALANYLGLYCINE.

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Summary: L-Phenylalanyl-(R)-[^{2}H]glycine, L-valyl-(R)-[^{2}H]glycine, and L-phenylalanyl [^{15}N]glycine were prepared. Assignments for pro-R and pro-S proton NMR signals of the glycine residue were done and coupling constants between proton and ^{15}N were obtained. Based on the data an attempt to explain the origin of the nonequivalence of the glycine methylene protons was made, and a conformational model for L-phenylalanylglycine is proposed.

Introduction: The glycine methylene protons in most of aminoacylglycines in the zwitter ionic form exhibit an AB quartet in their proton NMR spectra. For example, the glycine methylene protons of leucylglycine and the middle glycine residue of leucylglycylglycine show this chemical shift nonequivalence in their zwitter ionic form (1), although they give a single line in acidic and basic solutions. Morlino and Martin (2) suggested that the signal at higher field should be assigned to the pro-S proton by comparison with the spectra of L,L- and L,D-phenylalanylvaline. Nakamura and Jardetzky (3) studied eleven aminoacylglycines and found that the chemical shift nonequivalence increased as the side chain became larger. They attributed the nonequivalence to an anisotropic

shielding effect of the peptide plain when in the conformation considered most favorable based on steric effects. However, these conclusions are all somewhat uncertain mainly because they were neither deduced from the absolute assignment of nonequivalent glycine proton signals nor from a definite conformational model with another independent experimental evidence (4).

In the present work, we were able to assign unambiguously pro-S and pro-R signals of the glycine in L-phenylalanylglycine and L-valylglycine using (R)-[^{2}H]glycine. On this basis an attempt to explain the origin of the nonequivalence of the glycine methylene protons is made, and a conformational model is proposed.

Materials and Method: The stereospecifically monodeuterated glycine was prepared by a modification of the procedure of Besmer and Arigoni (5). Lyophilized cells of Erwinia herbicola AJ 2196 was used as the source of aminotransferase. Glycine (0.5 g) and pyridoxal 5-phosphate (1 mg) were dissolved in 10 ml deuterium oxide and the pD (meter reading) was adjusted to 7.9. The lyophilized cells (1 g) were suspended in the solution and incubated at 37°C. The extent of deuteration of glycine was checked by deuterium decoupled proton NMR and the incubation was stopped after three hours when only the signal due to CHD (obsd. $[\alpha]_{227}$ +75°, lit. (5) $[\alpha]_{227}$ +68°) that only the pro-R hydrogen atom had been exchanged with deuterium. [^{15}N]Glycine was purchased from Hikari Co. Ltd.. Dipeptides were synthesized by a previously published method (6). Proton NMR spectra were measured with a Varian XL-100 FT NMR spectrometer.

Result: Figure 1a and 1b show the proton NMR spectra of the zwitter ionic forms of L-phenylalanylglycine and L-phenylalanyl-(R)-[^{2}H] glycine, respectively. In Figure 1b, the observed singlet signal

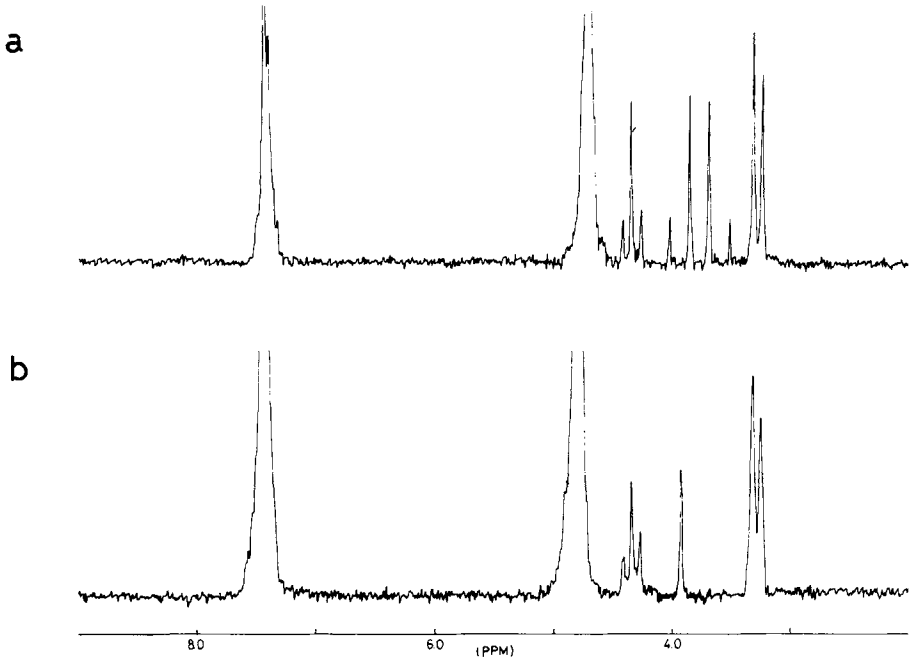

Figure 1. 100 MHz proton NMR spectra of (a) L-phenylalanylglycine and (b) L-phenylalanyl-(R)-[^{2}H]glycine (deuterium irradiated) at pD 5.4 in D_2O at room temperature.

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for the glycine methylene proton can be assigned to the pro-S hydrogen because the pro-R hydrogen has been replaced by a deuterium atom. The lower field resonance of the glycine methylene protons in Figure 1a was therefore assigned to the pro-S proton and the high field resonance to the pro-R proton. The chemical shifts of each proton resonance at different pH's are shown in Figure 2. It is noticeable that for both L-phenylalanylglycine and L-valylglycine the pro-R proton signal appears in higher field. The β-protons of the phenylalanyl residue in L-phenylalanylglycine were assigned as pro-S and pro-R protons (Figure 2) using the assignment for L-phenylalanine itself, as determined by a specific deuteration experiment (9).

L-Phenylalanylglycine in H_2O showed the NH signal with two coupling constants, $J_{H\text{-}N\text{-}C\text{-}H_R}$ = 5.3 Hz and $J_{H\text{-}N\text{-}C\text{-}H_S}$ = 6.4 Hz, at

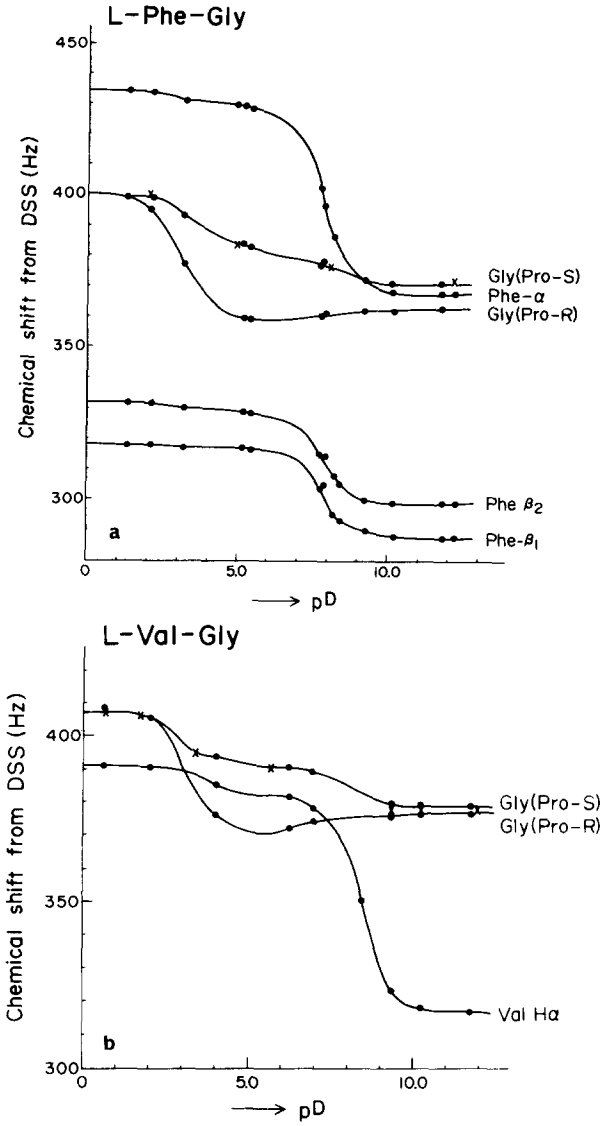

Figure 2. The pD titration curve of chemical shifts of (a) L-phenylalanylglycine and (b) L-valylglycine in D_2O at room temperature. In both figures (x) indicate the values obtained with the specifically deuterated dipeptides.

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pH = 5.2. A direct coupling constants between the proton and the ^{15}N of the peptide group of L-phenylalanyl [^{15}N]glycine was observed to be 93.1 Hz at pH = 5.3. However, the vicinal coupling between

the peptide ^{15}N and H_α of the phenylalanyl residue was too small to be observed.

Discussion: The configuration of the (R)-[^{2}H]glycine was unambiguously determined by the measurement of optical rotation. As demonstrated, therefore, the nonequivalent glycine methylene protons could be assigned without ambiguities. The assignment is the reverse of that proposed by Morlino and Martin (2). Figure 2 shows that the largest chemical shift nonequivalence is found at neutral pH where a zwitter ionic form is predominant. It is therefore clear that the charge interaction between the ammonium and carboxylate groups could play an important role in stabilizing some particular conformations of the zwitter ionic form of aminoacylglycine type dipeptide in aqueous solutions.

The peptide bond of L-phenylalanylglycine is assumed to be trans because of the magnitude of the coupling constants between ^{15}N and the directly bonded proton (7). The very small vicinal coupling constant between the α-proton of the phenylalanyl residue and the ^{15}N implies that the dihedral angle between the ^{15}N-C bond and the C_α-H bond should be about 90° (7). Of the two possible conformations, that which has the carbonyl oxygen and the ammonium group in a close proximity, would be favored by electrostatic interaction. The conformation about the N-C_α (Gly) bond can be derived from the vicinal H-N-C_α-H coupling constants, $^3J_{H-H_R}$ = 5.3 Hz and $^3J_{H-H_S}$ = 6.4 Hz, using the equation presented by Ramachandran et al (8). The two sets of angles estimated by this way are θ_R = 25°, θ_S = 140° and θ_R = 120°, θ_S = 0°. We might choose the latter set of angles as the preferred mean conformation, since this allows favorable electrostatic interactions between the ammonium and carboxylate groups. As a result of the considerations made above,

the most likely mean conformation for L-phenylalanylglycine can be described as shown in Figure 3. This model satisfies all the observed coupling constants. The chemical shift nonequivalence of the glycine methylene can thus be explained as follows: the pro-R hydrogen is shielded stronger than the pro-S hydrogen by the peptide carbonyl group, and therefore the signal of the former shifts to a higher field than that of the latter. The probability of this type of conformation seems to increase as the bulkiness of the side chain in the aminoacyl residue of the aminoacylglycines increases. This is consistent with the results of Nakamura et al (3).

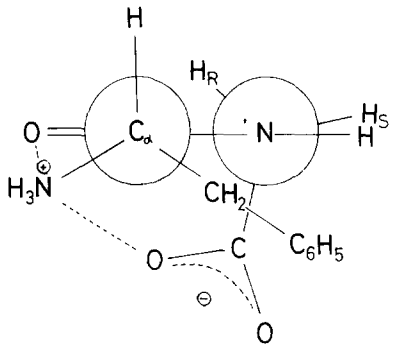

Figure 3. Newman projection of the proposed mean conformation of L-phenylalanylglycine.

Finally for the dipeptide, the populations of the rotational isomers around the C_α-C_β (Phe) bond can be considered by comparing with results for phenylalanine. The results, shown in Figure 4, have been obtained by stereoselective deuteration of the β-methylene of phenylalanine (9). Two relevant features of the results are, firstly that the population of III decreases in phenylalanylglycine possibly due to the steric hindrance in this conformer, secondly that I is still the most stable conformation in phenylalanylglycine. This

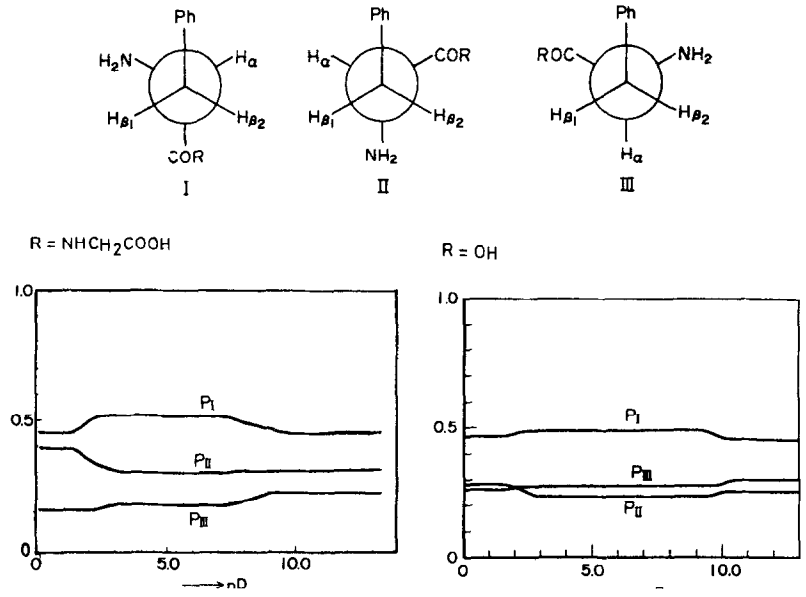

Figure 4. Relative population of conformational isomers about the C_α-C_β (Phe) bond in phenylalanylglycine and phenylalanine calculated from the observed vicinal coupling constants, $J\alpha\beta_1$ and $J\alpha\beta_2$ by assuming J_t = 13.56 Hz and J_g = 2.6 Hz (9, 10).

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fact, together with the result of valylglycine, indicate that the contribution of the aromatic ring current to a chemical shift difference between the two glycine methylene protons should not be major.

The usefulness of site- and stereo-specific incorporation of stable isotopes such as deuterium, nitrogen-15, or carbon-13 into amino acids for the NMR spectroscopic study of conformation of peptides or proteins in solutions is well documented. Further studies on the solution conformations of amino acids and peptides using stable isotopes will be presented in a subsequent publication.

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