Differences in NMR Spectra between Some α - and γ -Glutamyl Dipeptides

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In previous papers we reported that, in NMR spectra in D₂O solution, glutamyl α -H of α -glutamyl dipeptides resonate at lower field and those of γ -glutamyl dipeptides resonate at higher field than δ 3.9¹⁾ and that the sequence of a dipeptide containing glutamic acid could be determined by measuring NMR spectra of the dipeptide in acidic, aqueous and basic solutions.²⁾

This paper deals with the differences in NMR spectra between some α - and γ -glutamyl dipeptides.

The synthesis of glutamyl dipeptides and preparation of sample solutions were described in earlier papers.^{1,2)} NMR spectra were measured with a Hitachi model R-22 (90 MHz). Chemical shifts were expressed relative to DSS as an internal standard.

As shown in Table I, glycyl methylene peak of α -glutamyl-glycine splitted into an AB pattern in D₂O solution. Two methyl groups of α -glutamyl- α -aminoisobutyric acid and those of α -glutamylvaline were also unequivalent in D₂O solution. They became equivalent in acidic solution. On the other hand, glycyl methylene of γ -glutamylglycine, two methyl groups of γ -glutamyl- α -aminoisobutyric acid and those of γ -glutamylvaline were equivalent in both of aqueous and acidic solutions. Two methyl groups of α - and γ -glutamylleucine were unequivalent both in aqueous and acidic solutions.

Unequivalences of glycyl methylene have been observed in a many kind of dipeptides containing glycine

as C-terminal constituent,^{3~6)} Nakamura *et al.* conclude that the observed unequivalence reflects the restriction of rotation in molecules, connected with an increase of the life-time of all rotamers in which the positive (NH₃⁺ of N-terminal amino acid) and negative (COO⁻ of C-terminal glycine) charges appear *cis* to each other.⁴⁾ In acidic solution glycyl COO⁻ is protonated and an interaction with NH₃⁺ group disappears, so glycyl methylene become equivalent. In the case of γ -glutamylglycine, it is considered that glutamyl residue is present as α -zwitter ion and glycyl carboxyl group is not ionized in aqueous solution,²⁾ so glycyl carboxyl group dose not restrict the rotation in molecule and glycyl methylene appears as singlet. Unequivalences of two methyl groups of α -glutamyl- α -aminoisobutyric acid and those of α -glutamylvaline in aqueous solution and their changes to equivalences in acidic solution and absence of such an unequivalences in the case of γ -glutamyl peptides in both of aqueous and acidic solutions are also interpreted by Nakamura's theory.

The differences in chemical shifts of N-monosubstituted amides depend on *cis-trans* isomerism of amide bond.⁷⁾ But it is unlikely that unequivalences in dipeptides cited above are due to influence of *cis-trans* isomerism of the amide bond in dipeptides,⁸⁾ for the following experimental data are not explained by *cis-trans* isomerism; (1) Glycyl methylene of α -glutamylglycine give four lines of AB pattern. (2) Peak areas of unequivalent two methyl groups of α -glutamyl- α -aminoisobutyric acid and those of α -glutamylvaline are equal (The most part of N-monosubstituted amide are present as *trans* form even in the case of N-monosubstituted formamide which is the smallest acyl amide.⁷⁾). (3) In the case of γ -glutamyl dipeptides, such an unequivalences are not recognized.

Unequivalences of two methyl groups of α - and γ -glutamylleucine in both of aqueous and acidic solutions are not interpreted solely by the interaction of positive and negative charges. Restriction of rotation in molecule by bulky side chain may also participate.

TABLE I. CHEMICAL SHIFTS OF METHYLENE GROUP OF GLYCYL RESIDUE AND METHYL GROUPS OF α -AMINOISOBUTYRIC RESIDUE, VALYL RESIDUE AND LEUCYL RESIDUE (Hz from DSS as an Internal Reference. 90 MHz)

	In DCl solution†	In D ₂ O solution
α -L-Glutamylglycine	365(s)	352(d, <i>J</i> =18Hz), 342(d, <i>J</i> =18Hz)
γ -L-Glutamylglycine	359(s)	354(s)
α -L-Glutamyl- α -aminoisobutyric acid	131(s)	126(s), 125(s)
γ -L-Glutamyl- α -aminoisobutyric acid	128(s)	130(s)
α -L-Glutamyl-L-valine	86(d, <i>J</i> =7Hz)	82(d, <i>J</i> =7Hz), 80(d, <i>J</i> =7Hz)
γ -L-Glutamyl-L-valine	84(d, <i>J</i> =7Hz)	81(d, <i>J</i> =7Hz)
α -L-Glutamyl-L-leucine	82(d, <i>J</i> =6Hz), 78(d, <i>J</i> =6Hz)	80(d, <i>J</i> =6Hz), 77(d, <i>J</i> =6Hz)
γ -L-Glutamyl-L-leucine	81(d, <i>J</i> =6Hz), 77(d, <i>J</i> =6Hz)	80(d, <i>J</i> =6Hz), 77(d, <i>J</i> =6Hz)

† Concentration; 0.1 mmole/0.1 N DCl, 1 ml.

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