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Application of tritium high resolution NMR spectroscopy to analysis of tritium-labelled amino acids and peptides

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Summary. A method has been developed for the qualitative and quantitative analysis of complex isotopic mixtures of tritium-labelled amino acids and peptides by using high resolution ^3H NMR spectroscopy at 266.8 MHz. Determined were tritium distribution in alanine, glycine, tryptophan and 4-hydroxyproline amino acids, as well as in glycine and valine residues of peptides. Approaches have been worked out for the determination of spin coupling constants and isotope chemical shifts for the strongly coupled non-equivalent atoms of the methylene groups.

Keywords: Amino acids – Tritium NMR – Tritium-labelled amino acids-Tritium-labelled peptides

Introduction

Nuclear magnetic resonance spectroscopy is widely used to obtain different structural information about organic compounds, including the distribution of magnetic hydrogen isotopes in labelled molecules [1, 2]. Deuterium distribution in amino acids was measured earlier by observing the weakening of ^1H NMR signals [3, 4]. Although high resolution tritium NMR spectroscopy has been used routinely for almost 25 years, investigations of biological problems were developed not long ago [5, 6]. Also, it is possible now to apply modern ^3H NMR techniques to analyses complex isotopic samples by means of ^3H J-resolved, ^3H—^3H COSY, double quantum filtered and several types of ^3H—^1H correlation spectroscopy. But in these experiments only samples containing very high radioactivity, for example, up to 80.7 GBq, are examined [7]. Many organic and biological compounds are unstable with such high activity.

We developed relatively simple methods by means of one-dimensional ^3H NMR spectroscopy to analyse complex isotopic mixtures of tritium-labelled amino acids and peptides. Remarkably, most experiments have been carried out

without using proton-decoupling techniques. This allowed us to measure homo- and heteronuclear spin coupling constants as well as isotope chemical shifts with good accuracy.

Materials and methods

Preparation of tritium-labelled samples

The samples of amino acids and peptides were obtained by early developed [3, 4, 8, 9] means of high-temperature solid-phase catalytic isotopic exchange (HSCIE) at 100–180°C temperature range and possessed different values of specific radioactivity. The labelled compounds were isolated by chromatography on Aminex 150Q sulphocationic exchanger in the H^+ form and ligand exchange chromatography on carboxylic cationic exchanger Amberlite CG 50 (III) saturated with copper ions (II) [10].

Measurement

NMR spectroscopy of deuterated water solutions was carried out on a Bruker AC 250 spectrometer (^1H at 250.13 MHz, ^3H at 266.8 MHz), using a $^1H/^3H$ 5-mm dual probe. Varian 4-mm sample tubes with radioactive solutions were placed in standard 5-mm sample tubes. Chemical shifts of proton were measured relative to DSS (2,2-dimethyl-2-silapentane-5-sulfonic acid sodium salt) as internal standard. Chemical shifts of tritium signals in fully labelled components were assumed to correspond to the values of proton.

Results and discussion

Results are shown in Figs. 1–8 and in Table 1. The following symbols are used to designate spectral parameters: $^2J_{TH}$ (J_{AX} or J_{BX}) – geminal spin coupling constants (SCC) between ^3H and ^1H; $^2J_{TT}$ (J_{AB}) – geminal SCC between nonequivalent methylene tritium nuclei; $^3J_{TH}$, $^3J_{TT}$ – vicinal SCC; $^2\Delta T$, $^3\Delta T$ – two-bond and three-bond tritium isotope chemical shifts (ICS) from protons, respectively; ΔT_A, ΔT_B – two-bond ICS for nonequivalent tritium nuclei; $V_{A(\alpha)}$, $V_{B(\beta)}$, $V_A(X)$, $V_B(X)$ – chemical shifts (CS) for nuclei A (or α), B (or β), $A(X)$, $B(X)$; V_0 – the center of a spectrum.

Recently we developed a new method called "STM" (Superposition of Theoretical Multiplets) for quantitative analysis of [2,3-^3H] alanine multicomponent isotopic mixtures [11, 12]. This method presented as a variation of Bruker's "Panic" software is shown in Fig. 1. The number of mole of each component of the three-component alanine isotopic mixture as well as SCC and ICS were selected until the summed theoretical spectrum was identical with the experimental one. Fortuitously, a close coincidence of multiplets centers led to the equation $0.5 * {}^2J_{TH} \cong {}^2\Delta T$ (in Hz) at the resonance frequency of 266.8 MHz. With the help of STM we determined the composition of different alanine eight-component isotopic mixtures [12, 13]. This result has good correlation with the one calculated from the mathematical model [10]. Previously in a short report [14] the results of NMR study of tritium-labelled tryptophan and 4-hydroxyproline were given. Now we present in detail the ^3H NMR analyses of tritium distribution in these complex isotopic mixtures as well as new results of tritium-labelled peptides obtained by means of HSCIE under different condi-

Table 1. SCC* (Hz) and ICS** (ppb) for tritium-labelled tryptophan, 4-hydroxyproline and val-gly-gly

Comp.	Groups	$^2J_{TH}$	$^2J_{TT}$	$^3J_{TH}$	$^3J_{TT}$	$^2\Delta T$
[G-^{3}H]Trp	$C_{(3)}T_\alpha$	−16.64	−17.20	5.20	5.40	27[a]
	$C_{(3)}T_\beta$	−16.64	−17.20	8.40	8.90	26[b]
	$C_{(2)}T$			8.50[c] / 10.5[d]	9.10[c]	4.5[e]
[G-^{3}H]Hyp	$C_{(5)}T_\alpha$	−11.30	−14.0	1.0		19
	$C_{(5)}T_\beta$	−11.30	−14.0	3.9		21
1-Gly	CT_A	−17.73	−18.75			25
	CT_B	−17.73	−18.75			24
[G-^{3}H]VGG[f]						
2-Gly	CT_A	−18.40	−19.80[g]			26[h]
	CT_B	−18.40	−19.80			24[h]

* Error is ±0.25 Hz; ** Error is ±1 ppb; [a] $^3\Delta T = 11$ ppb; [b] $^3\Delta T = 8$ ppb; [c] J^{cis}; [d] J^{trans}; [e] $^3\Delta T\,(H_\alpha)$; [f] obtained at 180°C; [g] Error is ±0.5 Hz; [h] Error is ±2 ppb

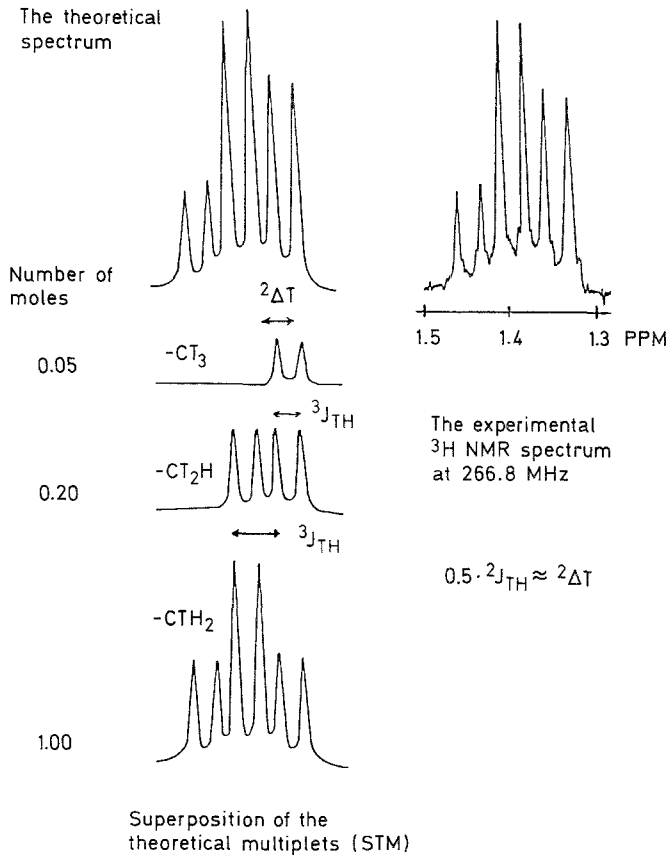


Fig. 1. Theoretical and experimental proton-coupled 266.8-MHz ^{3}H spectra (in D_2O) of three-component [3-^{3}H]alanine isotopic mixture

^{3}H NMR spectroscopy of [G-^{3}H] Trp

Position	Chem. shifts ppm	Activity quota. %	Degree of subst. %
2	3.95	12	41
3	3.35	23	47
2′	7.32	17	57
4′	7.75	6	20
5′	7.22	11	37
6′	7.28	11	37
7′	7.55	14	47

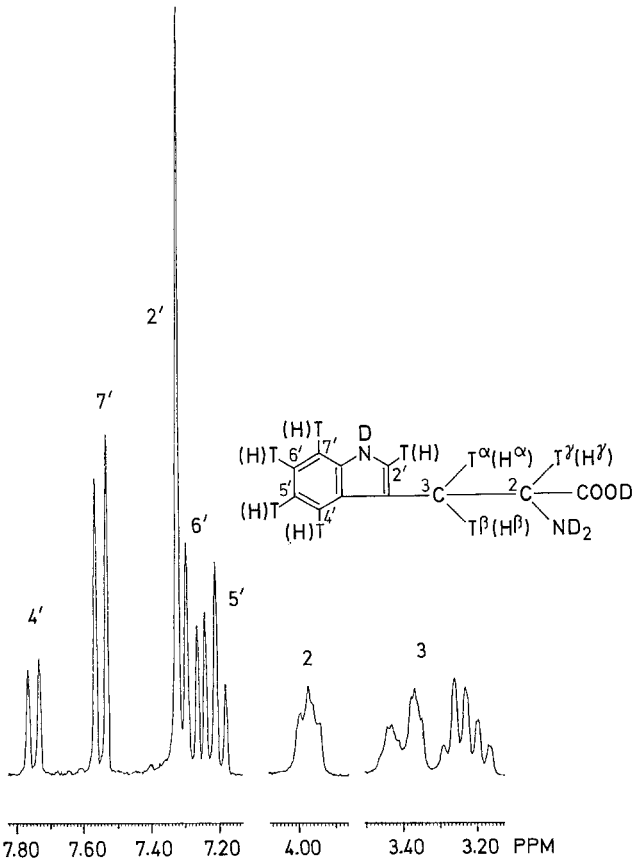


Fig. 2. Proton-coupled 266.8-MHz ^{3}H spectrum (in D_2O) of [G-^{3}H]tryptophan

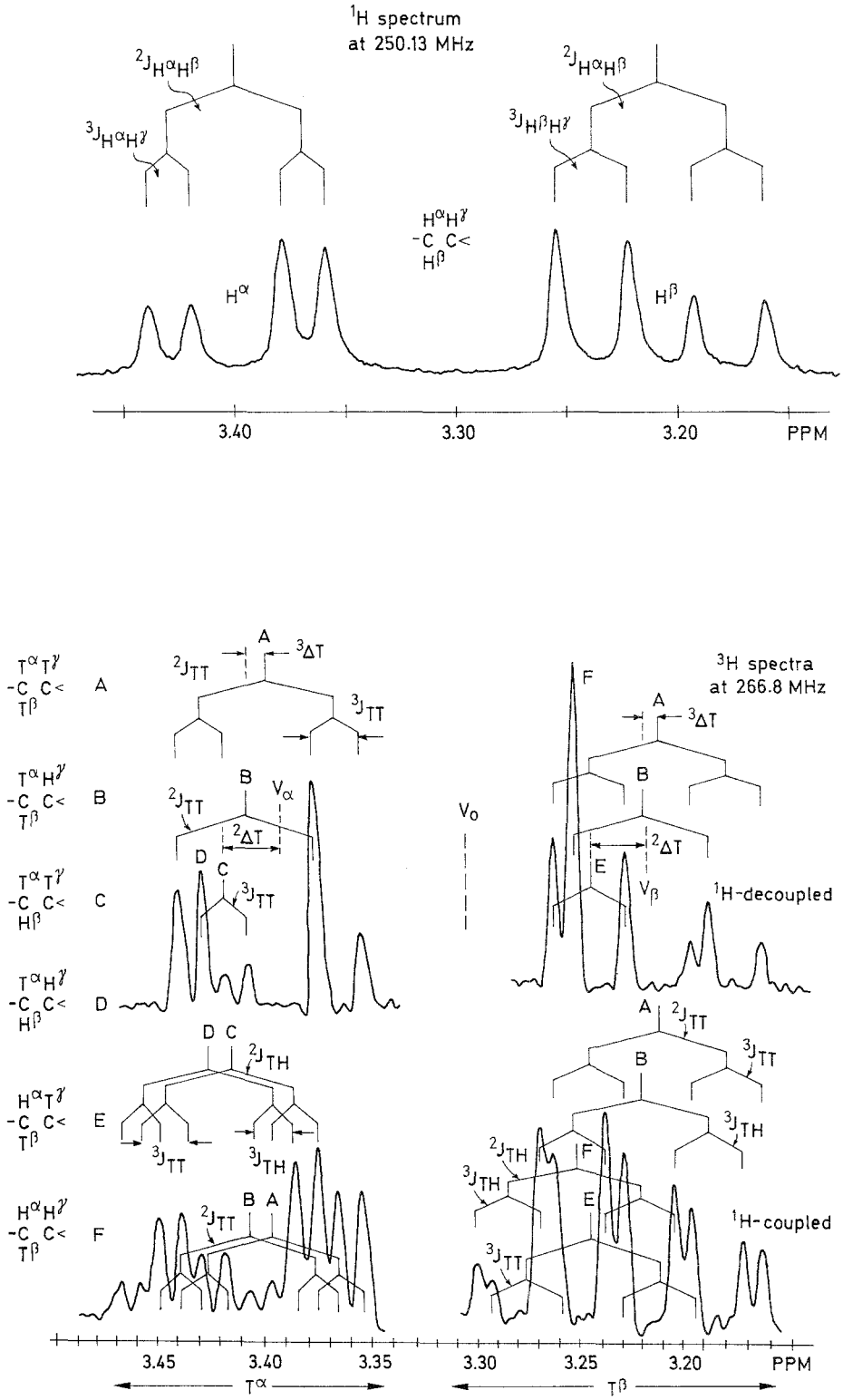


Fig. 3. NMR spectra of the methylene section of tryptophan (in D_2O). Above: model 250.13-MHz ^1H spectrum. Below: Gaussian ^1H-decoupled and ^1H-coupled 266.8-MHz ^3H spectra of [G-^3H]tryptophan six-component isotopic mixture

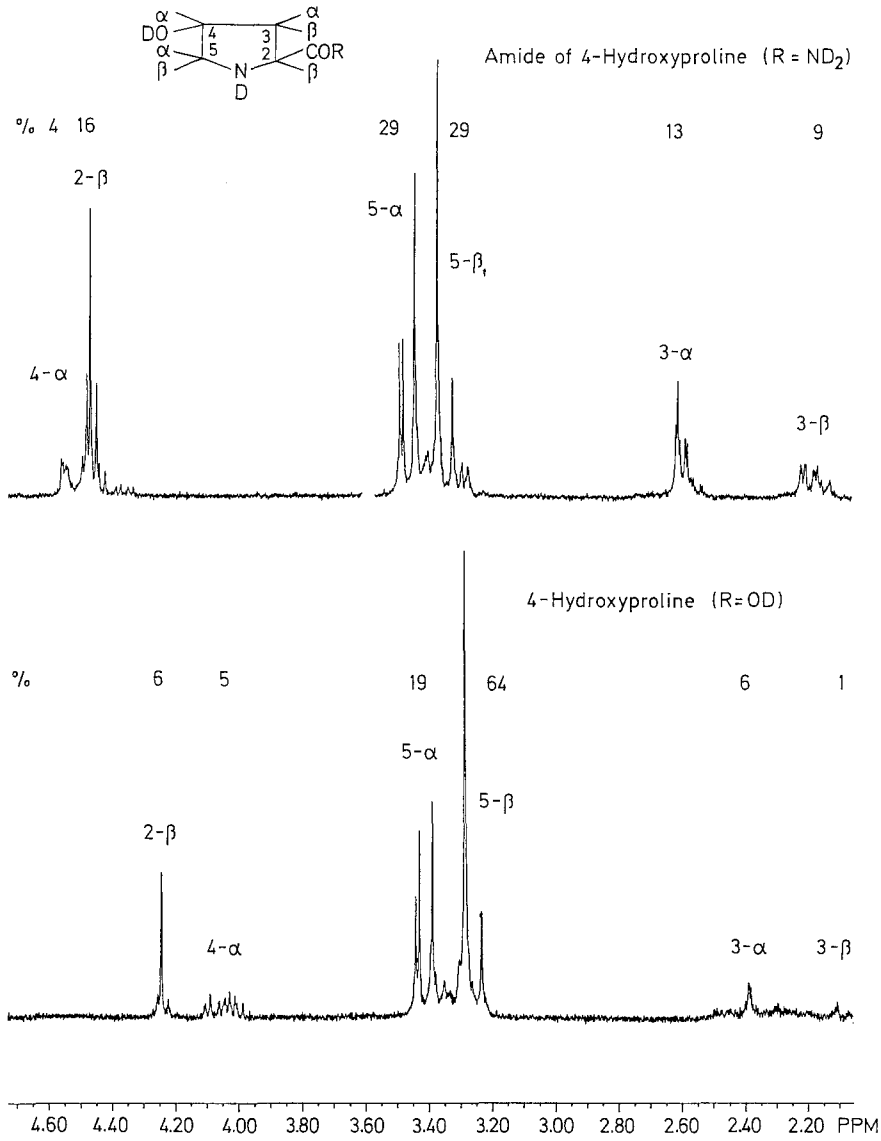
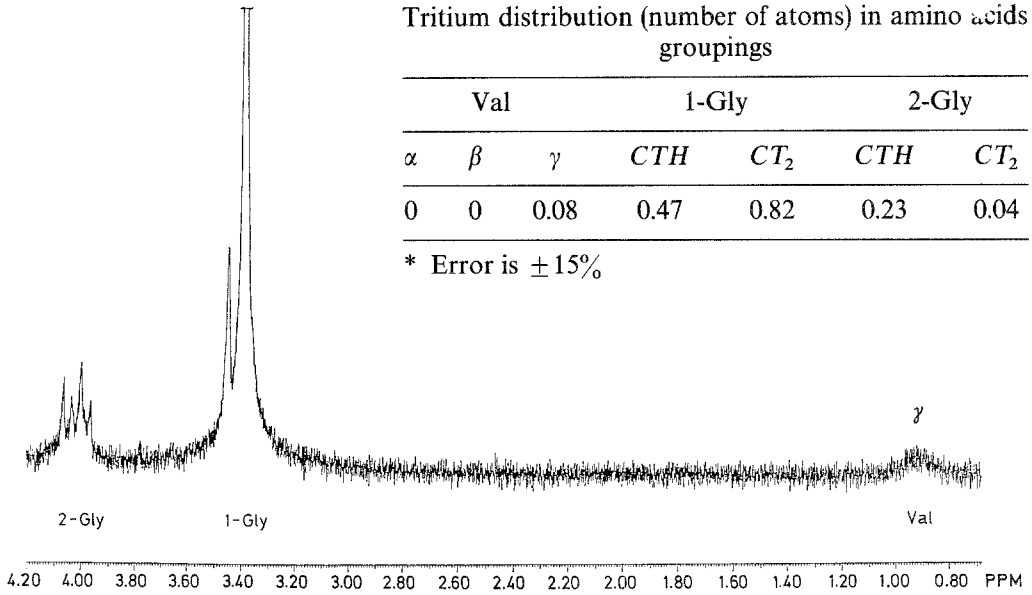


Fig. 4. Proton-decoupled 266.8-MHz ^3H spectra (in D_2O/CD_3OD) of [G-^{3}H]4-hydroxyproline (below) and its amide (above)

tions. In Fig. 2 is shown the spectrum of highly labelled tryptophan. The tritium distribution in the molecule is presented. The Gaussian signals of the non-equivalent methylene tritium nuclei are presented in Fig. 3. It is possible here to analyse qualitatively the composition of the six-component isotopic mixture as well as to determine SCC and ICS (see Table 1) by using the classical formulas [15]: $V_{\alpha\beta} = V_\alpha - V_\beta = [(V_* + J_{\alpha\beta})^2 - J_{\alpha\beta}^2]^{1/2}$; $V_\alpha = V_0 + 0.5V_{\alpha\beta}$; $V_\beta = V_0 - 0.5V_{\alpha\beta}$, where V_* is the difference of CS between 2nd and 3th lines of $T_\alpha T_\beta$ *AB*-system. By our assumption tritium distribution by the HSCIE reaction depends on distribution of electronic density in amino acid molecules. This was confirmed by study of proton-decoupled spectra of highly labelled 4-hydroxyproline and its amide (Fig. 4). A similar problem arises in the study of

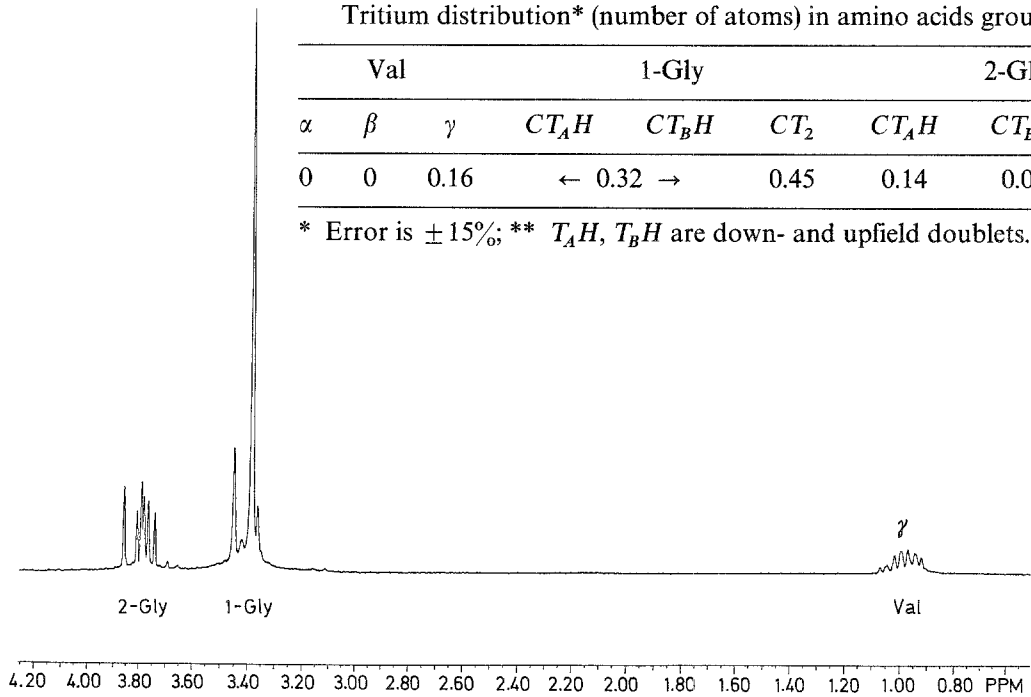


Tritium distribution (number of atoms) in amino acids groupings

Val			1-Gly		2-Gly	
α	β	γ	CTH	CT_2	CTH	CT_2
0	0	0.08	0.47	0.82	0.23	0.04

* Error is ±15%

Fig. 5. Proton-coupled 266.8-MHz ^3H spectrum (in D_2O/NaOD) of [G-^{3}H]gly-gly-val obtained at 150°C



Tritium distribution* (number of atoms) in amino acids groupings**

Val			1-Gly			2-Gly		
α	β	γ	CT_AH	CT_BH	CT_2	CT_AH	CT_BH	CT_2
0	0	0.16	← 0.32 →		0.45	0.14	0.09	0.14

* Error is ±15%; ** T_AH, T_BH are down- and upfield doublets.

Fig. 6. Proton-coupled 266.8-MHz ^3H spectrum (in D_2O/NaOD) of [G-^{3}H]gly-val-gly obtained at 150°C

Tritium distribution* (number of atoms) in amino acids groupings**

Val			1-Gly			2-Gly		
α	β	γ	CT_AH	CT_BH	CT_2	CT_AH	CT_BH	CT_2
0.26	0.08	1.40	0.21	0.10	0.12	0.11	0.09	0.05

* Error is $\pm 15\%$; ** T_AH, T_BH are down- and upfield doublets.

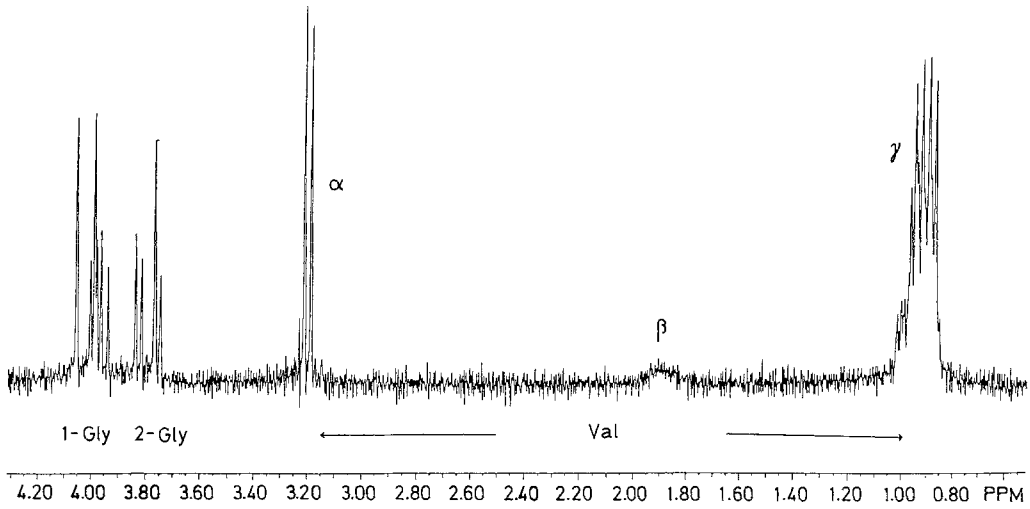


Fig. 7. Proton-coupled 266.8-MHz ^3H spectrum (in D_2O/NaOD) of [G-^3H]val-gly-gly obtained at 150°C

isotopic exchange in peptides with different arrangements of amino acid residues. In Fig. 5–7 are shown the spectra of the tritium-labelled peptides which contain two glycine and one valine groupings placed in various positions. In each spectrum tritium distribution is indicated. The exchange ability of valine atoms increase with a change of their position from the C-end to the N-end. The same correlation is presented for glycine groupings. All these peptides were obtained at 150°C. In Fig. 8 is shown the spectrum of the peptide obtained at 180°C. In this case the label is distributed more uniformly than in the previous sample. Also here is demonstrated the means of determination of spectral parameters for 1-glycine isotopomers. All measured and calculated spectral data are given in Table 1.

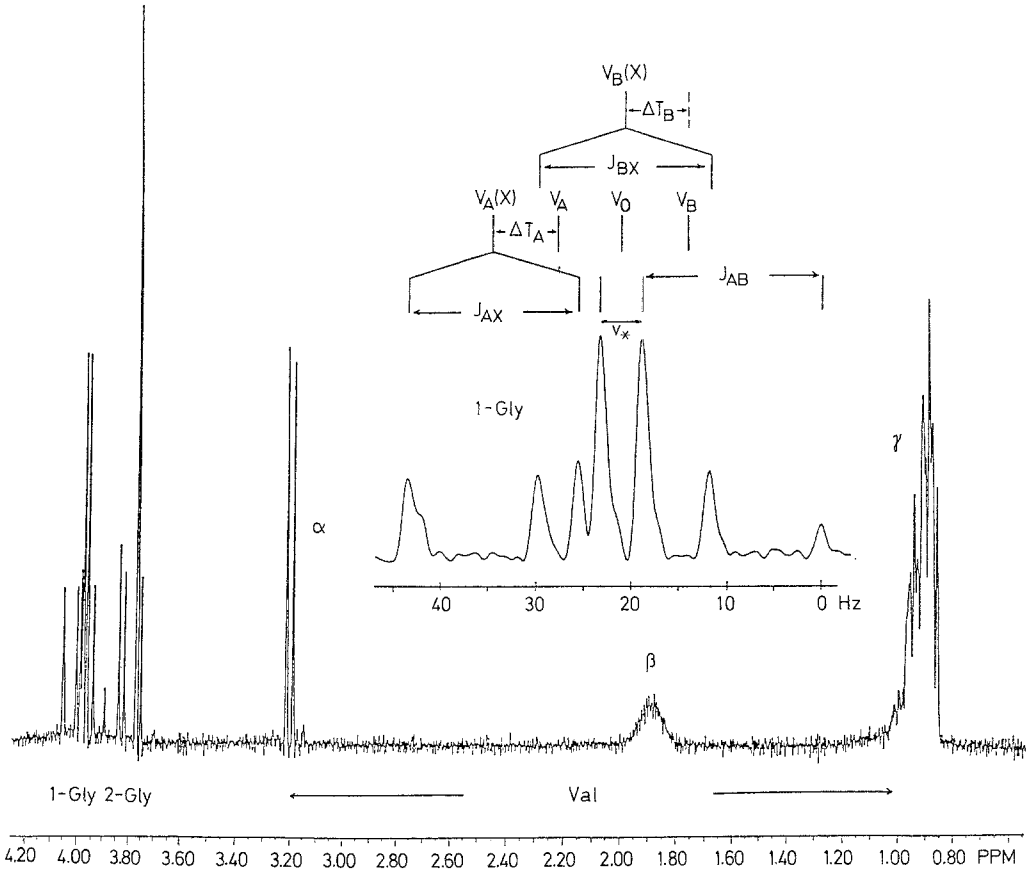


Fig. 8. Proton-coupled 266.8-MHz ^3H spectrum (in D_2O/NaOD) of [G-^3H]val-gly-gly obtained at 180°C

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