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INTERNAL MOTIONS STUDIES OF AN ANGIOTENSIN PENTAPEPTIDE USING PROTON RELAXATION PARAMETERS

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SUMMARY

We report mono and bi-selective excitation proton relaxation studies of internal motion of three side chains in a peptide fragment of angiotensin of sequence Tyr[1].Ile[2].His[3].Pro[4].Phe[5]. The motion of this peptide does not satisfy the extreme narrowing conditions and the general approach to the interpretation of proton relaxation rates given here can be used in larger peptides and proteins.

The introduction of selective proton spin lattice relaxation rate measurements (3,6) and nuclear Overhauser difference spectroscopy (5,6) have greatly increased the knowledge of proton relaxation mechanisms, stereochemistry and dynamics of natural products and biopolymers (4,6,7,8). The motion of proton-proton vectors of amino acid side chains (3,7) have been determined from the cross relaxation parameter, σ_{ij}, for cases where the interproton distance, r_{ij}, was defined and independent of conformation or motion. Examples of such vectors are tyrosine-Hδ-Hε (3) of isoleucine Hγ1-Hγ2 (7), proline Hδ1-Hδ2 (6), and in rigid peptides, the NH-C^{α}H dihedral distance (6). The correlation times of the former three vectors in the pentapeptide fragment of angiotensin were determined using a proton spin-lattice relaxation analysis which is valid outside the $\omega_o^2\tau_c^2 \ll 1$ limit.

MATERIALS AND METHODS

Samples were prepared by dissolving Tyr[1]-Ile[2]-His[3]-Pro[4]-Phe[5] (1) in 100% D_2O to obtain 10 mM solution at pD=2. Particular care was taken to exclude paramagnetic impurities such as oxygen and metal ions from the samples. Partially relaxed spectra were recorded using a Bruker WH270 spectrometer interfaced with a Nicolet 1180 computer. Relaxation rates were evaluated using a $(180°\text{-}\tau\text{-}90°\text{-}T)_n$ pulse sequence in which the 180° pulse was generated, in the selective mode, by the decoupler channel with a typical 10 msec. duration and a power of 0.5V. In biselective relaxation

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rate measurements the decoupler frequency was modulated by a Hewlett-Packard 3300A function generator. To evaluate the degree of selectivity in each experiment and to avoid errors in measurement of the heights of the peaks in the M_τ region close to zero intensity, computer controlled M_∞-M_τ automatic plotting was used (Fig. 1). Temperature was controlled at ±1°C by the Bruker unit.

RESULTS AND DISCUSSION

As theory states (10), for a proton, i, undergoing dipole-dipole interactions with other protons, j, mono-selective, R_0^i (SE), nonselective, R^i (NS), spin-lattice relaxation rates, and cross-relaxation parameters, σ_{ij} are related by the equation:

$$F^i = R^i(NS)/R_0^i\ (SE) = 1 + \sum_{i \neq j} \sigma_{ij}/R^i \qquad (1)$$

where the experimental R_0^i (SE) is equal to R^i, in the initial rate approximation (10). The relaxation rate obtained in a biselective spin-lattice experiment, R_0^i (i,j), was shown (4) to be

$$R_0^i(i,j) = R^i + \sigma_{ij}, \qquad (2)$$

where

$$\sigma_{ij} = \gamma^4\hbar^2[r_{ij}^{-6}][f(\tau_c^{ij})] \qquad (3)$$

Within the extreme narrowing conditions, $\omega_o^2\tau_c^2 \ll 1$ and $f(\tau_c^{ij}) = \tau_c^{ij}/2$; furthermore $F^i = 1.5$ if the intermolecular dipole-dipole IDD mechanism dominates the proton spin-lattice pathway (9). In this case, relaxation rate measurements in the monoselective and selective modes provide information about relaxation mechanisms and motions occurring in the molecule.

For peptides, at high spectrometer frequencies (ω_o) outside the extreme narrowing conditions, F^i's are expected to be lower than 1.5 even if only the IDD mechanism contributes to the proton relaxation process. A further complication arises because in the region $\omega_o^2\tau_c^2 \leq 1$, any σ_{ij} is consistent with two correlation times, as shown in Fig. 2.

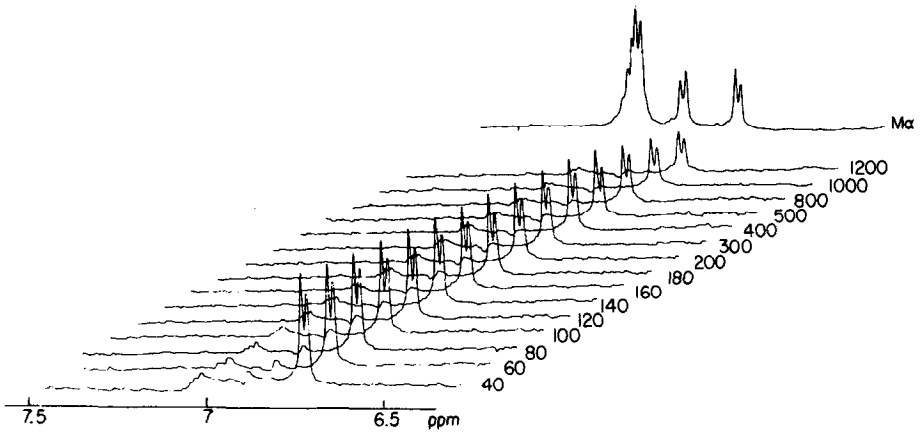

Figure 1. The spectrum of angiotensin pentapeptide recorded with a monoselective (180°-τ-90°-τ) pulse sequence centered at TyrHε doublet; only spectra for 40<τ<1200 milliseconds are shown; the Moo spectrum contains, (low to high field); the PheHδ, Hε and H , TyrHδ, and TyrHε multiplets.

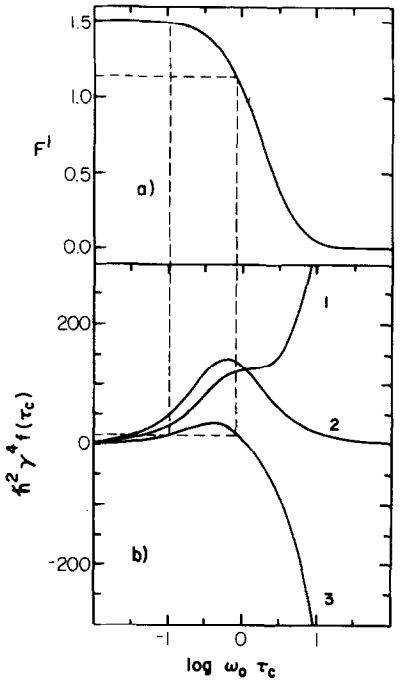

Figure 2. (a) Graph of F = $R^i(NS) / R^i(i)$ versus log $\omega_o \tau_c$ (b) Graph of $R^1(i)$ (1), $R^1(ij)$ (2) and σij (3) for a two spin system; (1), (2) and (3) are readily generalized for proton i, involved in multiple interactions with several protons, j ≠ i. A given positive σ value can have two values of log $\omega_o \tau_c$; each of the latter has a corresponding F^1 value in graph (a). The correct log $\omega_o \tau_c$ value must be consistent with the experimental F^1 and σ value.

Table I

Relaxation parameters obtained for the pentapeptide $Tyr^1Ile^2His^3Pro^4Phe^5$.

	Tyr^1		Ile^2		Pro^4	
	Hδ	Hε	Hδ11	Hδ12	Hδ1	Hδ2
R^i(NS)	1.59	0.99	4.60	4.63	4.50	4.48
	(1.62)	(0.98)	(4.58)	(4.55)	(4.41)	(4.43)
R^i(SE)	1.50	0.95	3.70	3.67	4.32	4.35
	(1.39)	(0.85)	(3.52)	(3.45)	(3.94)	(4.03)
F^i	1.06	1.05	1.25	1.26	1.04	1.03
	(1.16)	(1.15)	(1.30)	(1.32)	(1.12)	(1.10)
$F^i_{cal.}$	1.04	1.04	1.45	1.45	1.01	1.01
	(1.20)	(1.20)	(1.46)	(1.46)	(1.08)	(1.08)
δ(ppm)	7.00	6.68	1.42	1.11	3.85	3.57

Values in parentheses were obtained at 20°C; the others at 10°C. The estimated error in the relaxation rate measurements was found equal to 1%.

The spectrum of (1) contains first order multiplets for the Tyr^1 aromatic protons, the Ile^2 $H\gamma_1$'s and Pro^4 Hδ's. Thus, the proton spin-lattice relaxation study of the pentapeptide presents a vehicle for extending the method of obtaining correlation times of defined interproton vectors of amino acid side-chains to higher molecular weight biopolymers. As summarized in Tables 1 and 2, the F^i ratios for (1) were lower than 1.5 and each of the positive σ_{ij}'s, using the known Hi-Hj distances, yielded two τ_c^{ij}'s. Because the latter are consistent with two different calculated F^i ratios, fig. 1, only the correlation time corresponding to the experimental F^i is the real correlation time. This agreement between experimental and calculated F^i values provides a method for checking the accuracy of the measurements and proves that the IDD mechanism dominates the relaxation pathway for these protons. The disagreement between the observed and calculated $F^{H\gamma 1}$ of the isoleucyl residue is attributed to the fact that $\sigma_{\gamma 11-\gamma 12}$ is related only to the motion of the Hγ11-Hγ12 vector, while the

Table II

Cross-relaxation parameters and correlation times for the pentapeptide $Tyr^1Ile^2His^3Pro^4Phe^5$.

Residue	H_i-H_j	σ_{ij}	τ_{ij}
Tyr^1	Hδ-Hε	0.03	6.0×10^{-10}
		(0.13)	(4.0×10^{-10})
Ile^2	Hδ11-Hδ12	0.58	1.3×10^{-10}
		(0.84)	(1.2×10^{-10})
Pro^4	Hδ1-Hδ2	0.06	6.4×10^{-10}
		(0.27)	(5.7×10^{-10})

Values in parentheses were obtained at 20°C, the others at 10°C.

$F^{H\gamma 11}$ and $F^{H\gamma 12}$ reflect also the slower motions of the vectors contributing to the overall spin-lattice relaxation of these two protons.

The temperature dependence of F^i values and σ parameters confirms the reliability of our data and its interpretation. For example, decreasing the temperature increases the correlation times. Examination of Table 1 and Fig. 2 reveals that the theoretical trend at different temperatures is the same as the experimental trend in the τ_c region we propose.

It has been shown from $^{13}CT_1$ measurements of angiotensin II that the correlation times for the delta ^{13}CH vectors of proline and the delta and epsilon ^{13}CH vectors of tyrosine are similar and close to the molecular correlation time while the shorter NT_1 found for the $^{13}C\gamma 1$ indicates a relevant internal reorientation along the Cβ-Cγ1 axis. This interpretation holds for these same residues in (1):the τ_c value for Ile must reflect that its motion differs from that of the backbone.

These conclusions were confirmed by the F^i ratios for the alpha protons of (1) in Table 3. The F^i values of the latter protons were found very close to those obtained for the Hγ's in Pro^4 and aromatic protons of Tyr^1, showing that similar correlation times modulate the relaxation process of

Table III

The proton relaxation rates of the backbone alpha protons of residue 1, 2 and 4 of the angiotensin pentapeptide

	R^i(NS)	R^i(SE)	F^i
$Tyr^1H\alpha$	2.21	2.13	1.04
$Ile^2H\alpha$	1.91	1.76	1.08
$Pro^4H\alpha$	1.66	1.63	1.01

The correlation time obtained from the F^i values calculated from figure 2(a) was equal to $(6.5 \pm 0.3) \times 10^{-10}$ sec. A 1% experimental error on the relaxation rate and the consequent inaccuracy on the F^i's is taken into account in the propsosed τ_c value.

backbone and side-chain protons in the tyrosyl and prolyl residues. Correlation times for chain motion have hitherto been obtained by ^{13}C or ^{2}H relaxation rates but proton relaxation rates have been used only on side chains of single amino acids whose motions are exceedingly fast. We have reported here the extension of proton relaxation methods to the study of several side chains in a single peptide whose correlation times are not within the extreme narrowing range. Extension to higher molecular weight peptides and other biopolymers is now readily envisaged.

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