Proton Magnetic Resonance Spectra of Amino-acids and Peptides relevant to Wool Structure. Part VI.[1] Relative Residence Times of Peptides of Histidine, Tryptophan, and Valine

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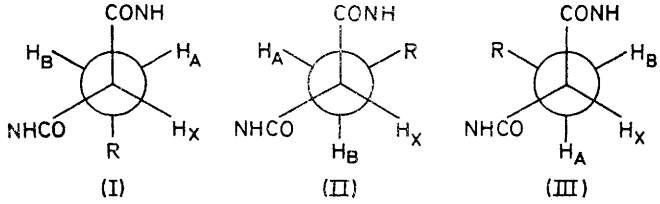
100 and 220 MHz ^{1}H n.m.r. spectra have been recorded, over the temperature range 291—358 K, for acidic and basic D_2O solutions of nine dipeptides and three tripeptides containing histidyl, tryptophyl, and valyl groups. Analysis of the spectra enabled the populations of the side-chain (C_α–C_β bond) rotamers to be calculated from the vicinal coupling constants. For the histidyl and tryptophyl peptides in acid and base, all rotamers are appreciably populated. In the range studied, the rotamer populations of the histidyl residues are almost unaffected by increase of temperature, while for Gly-Tyr and Gly-Trp-Gly there is a slight tendency for the preponderance of rotamer (I) to lessen. In the valyl peptides examined, the population of the rotamer with the vicinal hydrogens *trans* was greatest for acidic solutions of peptides with the valyl residue carbon terminal.

THE analysis of complex ^{1}H n.m.r. protein spectra such as those from oxidized and reduced fractions of wool [2-5] is assisted by the availability of chemical shifts and coupling constants for the constituent amino-acids. Such data are also valuable for investigating the conformations of di- [6] and tri-peptides [7] and the influence of changes in solvent acidity and temperature on these conformations.[8]



For the side-chain C_α–C_β bond of amino-acid fragments, the α-methine and β-methylene hydrogens often constitute an ABC/ABX spin system; extraction of the vicinal coupling constants J_{BC} and J_{AC} enables the populations, *a*—*c*, of the rotational isomers (I)—(III), about the C_α–C_β bond to be determined *via* the Pachler procedure.[9]

EXPERIMENTAL

Materials.—Di- and tri-peptide samples (Tables 1—3) were purchased from Sigma Chemical Company and the t-butyl alcohol reference from B.D.H. Solvents were deuterium oxide (99.7% isotopic purity) from Prochem, deuterium chloride (20% solution in D_2O) from Koch–Light, and sodium deuterioxide (30% solution in D_2O) from CIBA.

Spectra.—^{1}H N.m.r. spectra of 4—5% w/w aqueous peptide solutions were recorded on a JEOL MH-100 or Varian HR-220 spectrometer in the internal-lock mode, with t-butyl alcohol (2%) as internal standard. Solution acidities, pD, were measured as described previously.[2,10] The JEOL probe temperature was measured from the separation of ethanediol resonances together with the Varian calibration graph; typical spectrometer conditions were: sweep widths 108—1 080 Hz, sweep times 250 or 500 s (low-to-high field scans), frequency response 5—20 Hz and radiofrequency (r.f.) attenuator 10—20 db. The probe temperature of the Varian spectrometer was measured with a Comark elec-

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TABLE 1

Temperature dependence of ^{1}H n.m.r. parameters and rotamer populations of histidyl residues in peptides

Peptide	pD	T/K	Chemical shifts (δ) [a] δ_B	δ_A	δ_X	$\delta_A - \delta_B$	Coupling constants J/Hz J_{AB}	J_{BX}	J_{AX}	Fractional rotamer populations a	b	c
His-Gly [b]	1.0	294		2.21 [d]	3.15			13.5 [e]		0.75		0.25
Gly-His	1.2 [b]	333	2.03	2.15	3.60	0.116	−15.6	7.9	5.7	0.48	0.28	0.24
	0.8 [c]	353	2.08	2.20	3.69	0.124	−16.0	8.1	5.9	0.50	0.30	0.20
Gly-His-Gly [b]	0.5	295	1.99	2.08	3.58	0.089	−15.5	7.8	6.2	0.47	0.33	0.20
		334	1.99	2.09	3.88	0.099	−15.6	7.6	6.2	0.45	0.33	0.22
His-Phe [c]	13.1	302	1.49	1.57	2.31	0.075	−14.9	7.5	5.2	0.45	0.24	0.31
		317	1.49	1.59	2.33	0.102	−15.0	7.4	5.5	0.44	0.26	0.30
		354	1.52	1.67	2.38	0.156	−15.3	7.7	5.7	0.46	0.28	0.26

[a] Downfield from internal t-butyl alcohol. [b] Recorded at 220 MHz. [c] Recorded at 100 MHz. [d] $\frac{1}{2}(\delta_B + \delta_A)$. [e] $J_{AX} + J_{BX}$.

TABLE 2

Temperature dependence of ^{1}H n.m.r. parameters and rotamer populations of tryptophyl residues in peptides

Peptide	pD	T/K	Chemical shifts (δ) [a] δ_B	δ_A	δ_X	$\delta_A - \delta_B$	Coupling constants J/Hz J_{AB}	J_{BX}	J_{AX}	Fractional rotamer populations a	b	c
Trp-Tyr [b]	0.8	302		2.05 [d]	2.98			14.0 [e]		0.80		0.20
		325		2.12	3.05			14.4		0.84		0.16
Gly-Trp [b]	1.3	291	1.96	2.08	3.52	0.125	−14.7	7.8	5.6	0.47	0.27	0.26
Trp-Tyr [b]	12.3	302	1.80	1.89	2.40	0.093	−14.1	5.4	5.8	0.25	0.29	0.46
		326		1.87	2.51			11.8		0.60		0.40
		358		1.88	2.49			12.7		0.68		0.32
Gly-Trp [b]	12.8	301	1.92	2.12	3.31	0.202	−14.5	7.8	4.8	0.47	0.20	0.33
		335	1.96	2.16	3.38	0.200	−15.0	7.5	5.1	0.45	0.23	0.32
		353	1.96	2.16	3.37	0.199	−15.0	7.6	5.2	0.45	0.24	0.31
Gly-Trp-Gly [c]	11.5	297	1.89	2.04	3.43	0.148	−14.6	8.2	5.7	0.51	0.28	0.21
		324	1.94	2.08	3.47	0.139	−14.7	8.0	5.8	0.49	0.29	0.22
		348	1.96	2.09	3.48	0.134	−14.8	7.8	5.8	0.47	0.29	0.24

[a] Downfield from internal t-butyl alcohol. [b] Recorded at 100 MHz. [c] Recorded at 220 MHz. [d] $\frac{1}{2}(\delta_A + \delta_B)$. [e] $J_{AX} + J_{BX}$.

TABLE 3

Temperature dependence of ^{1}H n.m.r. parameters and rotamer populations of valyl residues in peptides

	pD	T/K	Chemical shifts (p.p.m.) C_α–H [a]	$\Delta\delta CH_3$	Coupling constants J/Hz $C_\alpha H - C_\beta H$	Fractional rotamer populations a [e]	$b + c$ [e]
Val-Tyr [b]	0.8	301	2.54	0.021	5.6	0.27	0.73
		327	2.64	0.016	5.7	0.28	0.72
		353	2.69	0.012	5.6	0.27	0.73
Tyr-Val [b]	0.8	301	2.94	0.008	6.4	0.35	0.65
		327	2.97		6.4	0.35	0.65
Val-Tyr-Val [c]	0.8	297					
N-Terminal			2.59		5.6	0.27	0.73
C-Terminal			2.87		6.6	0.36	0.64
Val-Phe [b]	0.7	301	2.52	0.022	5.6	0.27	0.73
		326	2.60	0.016	5.6	0.27	0.73
		353	2.63	0.012	5.5	0.26	0.74
Phe-Val [b]	0.9	302	2.96	0.008	6.2	0.33	0.67
		328	3.04	0.008	6.2	0.33	0.67
		338	3.06	0.008	6.4	0.35	0.65
Val-Phe [b]	12.6	302	1.83	0.100	5.8	0.29	0.71
		354	1.88		5.6	0.27	0.73
Phe-Val [b]	13.3	302	2.78	0.014	5.7	0.28	0.72
		328	2.85	0.018	5.6	0.27	0.73
		350	2.89	0.018	5.6	0.27	0.73
Tyr-Val [b]	12.7	302	2.78	0.018	5.8	0.29	0.71
		326	2.85	0.020	5.7	0.28	0.72
		354	2.90	0.022	5.8	0.29	0.71
Val-Tyr [b]	12.8	302	1.84	0.068	5.9	0.30	0.70
		317	1.88	0.070	5.8	0.29	0.71
		354	1.90	0.070	5.8	0.29	0.71

[a] Downfield from internal t-butyl alcohol. [b] Recorded at 100 MHz. [c] Recorded at 220 MHz. [d] Chemical shift difference between the geminal methy groups. [e] If population calculated is *b*, then final column lists $a + c$.

tronic thermometer; typical spectrometer conditions were: sweep width 100—2 500 Hz, sweep times 250 or 500 s, frequency response 0.4—2.0 Hz, and r.f. attenuator 10—20 db.

ABC/ABX Spectra were analysed as described previously.[11]

RESULTS AND DISCUSSION

Side-chain Conformations of Histidyl- and Tryptophyl-containing Peptides.—Table 1 shows the ^{1}H n.m.r. parameters and rotamer populations derived for the histidyl residues in four peptides. For the peptides in the cation form, the positively charged imidazole ring ensures that the side-chain ABX resonances are to low field of those in other peptides, such as those containing phenylalanine and tyrosine.[6] While all three rotamers appear appreciably populated, rotamer (I) with the imidazole group *trans* to the *C*-terminal peptide bond is most favoured. This implies an increase in the population of the *trans*-rotamer (I) relative to that in the free amino-acid at the same pH.[12] Although His-Gly has a deceptively simple spectrum at 220 MHz, comparison of $|J_{AX} + J_{BX}|$ for His-Gly with those for Gly-His and Gly-His-Gly suggests similar rotamer populations in all three peptides. For the peptides in acid solution, increase of temperature has very little effect on the populations. For the dipeptide His-Phe in basic solution, rotamer (III) is rather more populated than (II) at room temperature; the reverse holds when the temperature is increased above 350 K.

In most of the tryptophyl-containing peptides (Table 2), rotamer (I) predominates, although all rotamers have appreciable populations. Since the 100 MHz ABX spectrum of Trp-Tyr is deceptively simple in both acidic and basic solution, only the sum of the vicinal coupling constants can be extracted. This sum indicates that the populations are more disparate in acid than in base[13] at 358 K.

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Side-chain Conformations of Valyl-containing Peptides.—The rotamer populations of the valyl residues (Table 3) have been calculated with J_{trans} 13.6 and J_{gauche} 2.6 Hz.[7] For the –C–C– fragment, electronegativity values suggest that replacement of hydrogen by the more electronegative carbon is likely to reduce the average vicinal coupling constant by *ca.* 0.3 Hz. However, in view of the difficulty of assessing individual changes in J_{trans} and J_{gauche}, the Pachler values[9] have been adopted; any consequent small errors in the rotamer populations are unlikely to distort the trends.

In all the Val peptides examined, rotamer (I) has an occupancy of about one-third. Measurements of the vicinal coupling constant may be divided into two groups clustered around 5.7 and 6.3 Hz. The first group contains the tripeptide Val-Tyr-Val and the dipeptides Val-X in acid and base, with valine as the *N*-terminal residue, and two dipeptides (Phe-Val, Tyr-Val) in base with the valine residue *C*-terminal. The second group comprises the peptides Tyr-Val, Phe-Val, and Val-Tyr-Val in acid solution, with valine present as the *C*-terminal residue. Relative to those of the amino-acid[14] the vicinal coupling constants indicate a higher population of the rotamer with the vicinal hydrogens *trans*. Increase of temperature has little effect on the rotamer populations. For most peptides examined, the *gem*-methyl groups were non-equivalent; this small chemical-shift difference was reduced for most peptides by an increase of temperature.

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