# Proton Magnetic Resonance Spectra of Amino-acids and Peptides relevant to Wool Structure. Part III.[1] Relative Residence Times of Dipeptides of Asparagine, Aspartic acid, Phenylalanine, and Tyrosine

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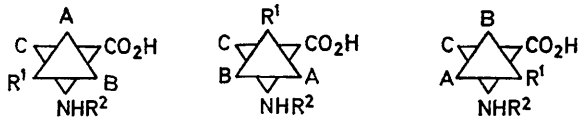
Iterative analyses have been carried out of the methine–methylene ABC spin systems in 60 MHz proton magnetic resonance ($^1$H n.m.r.) spectra, over the temperature range 283—353 K, of alkaline deuteriated aqueous solutions of aspartic acid and asparagine and of dipeptides of aspartic acid, asparagine, tyrosine, and phenylalanine with glycine and alanine. With increase of temperature, the fractional rotamer populations, deduced from the observed averaged vicinal coupling constants, exhibit three kinds of behaviour: invariance in aspartic acid, asparagine, glycyltyrosine and alanyltyrosine; a tendency towards equalization in glycylphenylalanine and alanylphenylalanine and, to some extent, in glycylaspartic acid and alanylaspartic acid; and a greater preference for one rotamer in tyrosine. In phenylalanine, increasing concentration of base causes linear downfield shifts of the methine and methylene resonances, while changes in coupling constants correspond to steady changes in relative rotamer populations.

By comparison with high resolution $^1$H n.m.r. spectra of proteins, the $^1$H n.m.r. spectra of solutions of amino-acids [1,2] and dipeptides are susceptible to relatively detailed analysis. The α-methine and β-methylene protons often form an ABC spin system in which the two methylene protons A and B are sufficiently distinct for spectral analysis, even at 60 MHz, to yield distinct couplings $J_{AC}$ and $J_{BC}$. For some sulphur-containing amino-acids in alkaline solution,[1] the Pachler [3,4] approach, whereby measured vicinal coupling constants may be associated with fractional populations (*a*, *b*, and *c*) of the three classical staggered rotamers (I), (II), and (III),




(I) (II) (III)

has revealed both invariance and temperature-dependence of these populations. While the gauche and *trans* coupling constants, $J_g$ and $J_t$, necessary for extracting relative rotamer residence times, may differ somewhat for different isomers,[4,5] *changes* in measured coupling constants $J_{AB}$, $J_{AC}$, and $J_{BC}$ with pH and temperature are likely to reflect genuine changes in relative rotamer lifetimes.[6]

## EXPERIMENTAL

*Materials.*—Samples of L-aspartic acid, glycyl-L-aspartic acid, L-alanyl-L-aspartic acid, L-asparagine, glycyl-L-asparagine, L-phenylalanine, glycyl-L-phenylalanine, L-alanyl-L-phenylalanine, L-leucyl-L-phenylalanine, phenylalanylglycine, L-phenylalanyl-L-leucine, L-tyrosine, glycyl-L-tyrosine, L-alanyl-L-tyrosine, and L-tyrosylglycine were purchased from Sigma London Chemical Co., Ltd. Solvents were deuterium oxide (99·7% isotopic purity) from Prochem Ltd., sodium deuterioxide (40% solution in 99% deuterium oxide) from Fluka AG, and 99% deuteriated trifluoroacetic acid from CIBA (ARL) Ltd. The internal reference was sodium 3-trimethylsilylpropane-1-sulphonate (Merck). Acidities, pD, were estimated [7] by adding 0·4 to the pH measurement made on an EIL GHM 23/B meter with a glass electrode and potassium bromide salt bridge.

*Spectra.*—$^1$H N.m.r. spectra were recorded for 0·5M-solutions of samples in $D_2O$, with additions of NaOD, borax, or $CF_3{\cdot}CO_2D$ as indicated, on a Varian A-60 spectrometer. The temperature of samples in the V-6057 variable-temperature probe ranged from 283 to 353 K and was estimated from the line separation in an ethylene glycol sample.

*Calculations.*—From ABX starting values for chemical shifts, ν, and coupling constants, ABC spectra were refined by the iterative program LAME (Mr. C. W. Haigh) modified for operation on the Bradford University I.C.T. 1909 computer to yield, for example, the envelope shown in the lower half of Figure 1.

For the ABX cases, alternative solutions are possible.[8,9] While computer refinement of conjugate solutions [10] of ABC/AMX systems converges to the solution nearer to the starting parameter, only one solution here is compatible with expected $J$ values. For 0·5M-phenylalanine in 4% NaOD, for example (Table 4), the ABX solutions (i) ($J_{AB}$ −13·4), $J_{AX}$ 8·1, $J_{BX}$ 4·7; and (ii) ($J_{AB}$ −13·4), $J_{AX}$ 12·4, $J_{BX}$ 0·5 Hz may be compared with the ABC set: $J_{AB}$ −13·5, $J_{AC}$ 7·6, $J_{BC}$ 5·4 Hz. Further, only one conjugate solution should agree with either solution at another frequency;[10] Cavanaugh [11] found close agreement between the following analyses of phenylalanine; at 60 MHz: $J_{AB}$ −13·45, $J_{AC}$ 7·75, and $J_{BC}$ 5·34 Hz; and at 100 MHz: $J_{AB}$ −13·48, $J_{AC}$ 7·77, and $J_{BC}$ 5·53 Hz.

## RESULTS AND DISCUSSION

*Asparagine and Aspartic Acid Dipeptides with Glycine and Alanine.*—Analysis of the aliphatic ABC spectra of

[1] Part II, K. D. Bartle, D. W. Jones, and R. L'Amie, preceding paper.
[2] K. D. Bartle, J. C. Fletcher, D. W. Jones, and R. L'Amie, *Biochim. Biophys. Acta*, 1969, **160**, 106.
[3] K. G. R. Pachler, *Spectrochim. Acta*, 1963, **19**, 2085; 1964, **20**, 581.
[4] R. J. Abraham, L. Cavalli, and K. G. R. Pachler, *Mol. Phys.*, 1966, **11**, 471.
[5] R. J. Abraham and G. Gatti, *J. Chem. Soc.* (*B*), 1970, 961.
[6] G. C. K. Roberts and O. Jardetzky, *Adv. Protein Chem.*, 1970, **24**, 448.
[7] P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, 1960, **64**, 188.
[8] K. D. Bartle and D. W. Jones, *J. Mol. Spectroscopy*, 1969, **32**, 353.
[9] P. L. Corio, ' An Introduction to the Analysis of Spin-Spin Splitting in High-resolution Nuclear Magnetic Resonance Spectroscopy,' Benjamin, New York, 1962, p. 71.
[10] R. J. Abraham and S. Castellano, *J. Chem. Soc.* (*B*), 1970, 49.
[11] J. R. Cavanaugh, *J. Amer. Chem. Soc.*, 1967, **89**, 1558.

TABLE 1

$^1$H N.m.r. spectral parameters and rotamer populations of aspartic acid, asparagine, and glycylasparagine in alkaline solution [a]

| | | Chemical shifts [b] (Hz) | | | | $J$/Hz | | | Fractional rotamer populations | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pD | $T$/K | $\nu_A$ | $\nu_B$ | $\nu_C$ | $\nu_B - \nu_A$ | $J_{AB}$ | $J_{AC}$ | $J_{BC}$ | $a$ | $b$ | $c$ |
| | | | | | (1) Aspartic acid | | | | | | |
| 10·4 | 283 | 145·9 | 155·2 | 229·5 | 9·3 | −16·8 | 8·7 | 3·7 | 0·55 | 0·10 | 0·35 |
| 10·4 | 303 | 145·6 | 154·9 | 229·2 | 9·3 | −16·8 | 8·7 | 3·7 | 0·55 | 0·10 | 0·35 |
| 10·4 | 333 | 145·0 | 154·3 | 228·6 | 9·3 | −16·8 | 8·7 | 3·7 | 0·55 | 0·10 | 0·35 |
| 10·4 | 353 | 145·6 | 154·9 | 229·2 | 9·3 | −16·8 | 8·7 | 3·7 | 0·55 | 0·10 | 0·35 |
| 13·4 | 283 | 138·8 | 157·9 | 214·1 | 19·1 | −15·4 | 9·5 | 3·9 | 0·63 | 0·12 | 0·25 |
| 13·4 | 303 | 138·8 | 157·9 | 214·1 | 19·1 | −15·4 | 9·5 | 3·9 | 0·63 | 0·12 | 0·25 |
| 13·4 | 333 | 137·8 | 156·9 | 213·0 | 19·1 | −15·4 | 9·5 | 3·9 | 0·63 | 0·12 | 0·25 |
| 13·4 | 353 | 138·0 | 157·1 | 213·0 | 19·1 | −15·4 | 9·5 | 3·9 | 0·63 | 0·12 | 0·25 |
| | | | | | (2) Asparagine | | | | | | |
| [NaOD]/M | | | | | | | | | | | |
| 1·0 | 303 | 145·2 | 159·0 | 213·7 | 13·8 | −14·7 | 8·8 | 4·8 | 0·56 | 0·20 | 0·24 |
| 1·5 | 303 | 145·9 | 159·3 | 214·2 | 13·4 | −14·6 | 8·7 | 4·7 | 0·55 | 0·19 | 0·25 |
| 2·0 | 303 | 147·1 | 160·3 | 215·3 | 13·2 | −14·7 | 8·8 | 4·9 | 0·56 | 0·21 | 0·23 |
| | | | | | (3) Glycylasparagine | | | | | | |
| pD | | | | | | | | | | | |
| 13·4 | 303 | 160·8 | 167·3 | 272·5 | 6·5 | −15·2 | 9·0 | 4·5 | 0·58 | 0·17 | 0·25 |

[a] Solutions 0·5M in $D_2O$, with 0·5—2·0M-NaOD. [b] Downfield from sodium 3-trimethylsilylpropane-1-sulphonate (internal reference).

TABLE 2

Variation with temperature and pD of solvent [a] of $^1$H n.m.r. spectral parameters of aspartic acid residues in dipeptides

| | | | Chemical shifts (Hz) | | | | $J$/Hz | | | Rotamer populations | | | Other shifts and couplings (Hz) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Peptide | pD of solvent | $T$/K | $\nu_A$ | $\nu_B$ | $\nu_C$ | $\nu_B - \nu_A$ | $J_{AB}$ | $J_{AC}$ | $J_{BC}$ | $a$ | $b$ | $c$ | $GlyCH_2$ | | |
| (4) Gly–Asp [c] | 4·4 | 303 | 153·5 | 165·7 | 268·6 | 12·2 | −15·8 | 9·9 | 3·9 | 0·66 | 0·12 | 0·22 | 232 | | |
| (4) Gly–Asp | 4·9 | 303 | 156·7 | 167·3 | 270·8 | 10·6 | −15·9 | 9·4 | 4·1 | 0·62 | 0·14 | 0·24 | 232 | | |
| (4) Gly–Asp | 7·2 | 303 | 150·9 | 163·8 | 270·0 | 12·9 | −15·7 | 9·6 | 3·9 | 0·61 | 0·13 | 0·26 | 228 | | |
| (4) Gly–Asp | 7·2 | 353 | 150·5 | 161·5 | 267·4 | 11·0 | −15·4 | 9·1 | 4·1 | 0·59 | 0·13 | 0·28 | 226 | | |
| (4) Gly–Asp | 13·2 | 303 | 152·0 | 160·7 | 269·0 | 8·7 | −15·6 | 9·2 | 4·1 | 0·60 | 0·13 | 0·27 | 200 | | |
| (4) Gly–Asp | 13·2 | 353 | 151·5 | 159·1 | 267·0 | 7·6 | −15·0 | 8·6 | 4·2 | 0·54 | 0·15 | 0·31 | 200 | | |
| (4) Gly–Asp | 13·5 | 303 | 152·1 | 160·0 | 264·5 | 8·1 | −15·3 | 9·0 | 4·2 | 0·57 | 0·15 | 0·28 | 200 | | |
| | | | | | | | | | | | | | $AlaCH_3$ | CH | $J_{CH_3CH}$ |
| (5) Ala–Asp | 13·0 | 303 | 151·2 | 158·9 | 263·5 | 7·7 | −15·4 | 9·1 | 4·3 | 0·59 | 0·15 | 0·26 | 76 | 210 | 6·7 |
| (5) Ala–Asp | 13·0 | 353 | 151·3 | 157·9 | 262·3 | 6·6 | −15·0 | 8·4 | 4·2 | 0·52 | 0·14 | 0·34 | 75 | 208 | 6·7 |
| (5) Ala–Asp | 13·3 | 303 | 152·4 | 158·9 | 262·0 | 6·5 | −15·4 | 9·1 | 4·1 | 0·59 | 0·13 | 0·28 | 72 | 207 | 6·7 |
| (5) Ala–Asp | 13·3 | 353 | 152·1 | 157·5 | 261·1 | 5·4 | −15·0 | 8·2 | 4·4 | 0·51 | 0·16 | 0·33 | 74 | 206 | 6·7 |

[a] Solvent $D_2O$ with 0·25—2·0M NaOD for all except first row. [b] Downfield from sodium 3-trimethylsilylpropane-1-sulphonate (internal reference). [c] 1% Borax in $D_2O$.

TABLE 3

Comparison of room temperature [a] spectral parameters of alkaline solutions [b] of phenylalanine and phenylalanyl residues in dipeptides

| | | Chemical shifts (Hz) | | | | $J$/Hz | | | Rotamer populations | | | Aromatic shift (Hz) | Other shifts and couplings (Hz) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Peptide | pD | $\nu_A$ | $\nu_B$ | $\nu_C$ | $\nu_B - \nu_A$ | $J_{AB}$ | $J_{AC}$ | $J_{BC}$ | $a$ | $b$ | $c$ | | | | |
| Phe | 13·2 | 170·9 | 182·5 | 211·4 | 11·6 | −13·5 | 7·6 | 5·4 | 0·45 | 0·25 | 0·29 | 441 | | | |
| | | | | | | | | | | | | | $GlyCH_2$ | | |
| Gly–Phe | 13·1 | 176·8 | 192·3 | 269·0 | 15·5 | −13·7 | 8·5 | 5·0 | 0·54 | 0·22 | 0·24 | 440 | 192 | | |
| | | | | | | | | | | | | | $AlaCH_3$ | CH | $J_{CH_3CH}$ |
| Ala–Phe | 13·2 | 175·4 | 191·3 | 263·0 | 15·9 | −13·7 | 8·6 | 5·0 | 0·55 | 0·22 | 0·23 | | 67 | 198 | 6·8 |
| | | | | | | | | | | | | | $Leu(CH_3)_2$ | $CH{\cdot}CH_2$ | CH |
| Leu–Phe | 13·1 | 177·3 | 194·7 | 268·0 | 17·4 | −13·8 | 9·2 | 4·8 | 0·60 | 0·20 | 0·20 | 440 | 48 | 75 | 195 |
| | | | | | | | | | | | | | | $GlyCH_2$ | |
| Phe–Gly | 13·0 | 174·0 | 181·0 | 225·0 | 7·0 | −13·4 | 8·1 | 5·1 | | | | 438 | | 229 | |
| | | | | | | | | | | | | | $AlaCH_3$ | CH | $J_{CH_3CH}$ |
| Phe–Ala | 13·0 | 172·0 | 180·7 | 220·0 | 8·7 | −13·4 | 8·0 | 4·8 | | | | | 80 | 248 | 7·0 |
| | | $\nu_{AB}$ | | | | | | | | | | | $LeuCH_3$ | $CH{\cdot}CH_2$ | CH |
| Phe–Leu | 13·0 | 177·0 | | 220 | | | 6·3 | | | | | 438 | 52 | 91 | 251 |

[a] 303 K. [b] 0·5M-Peptide in 1·0M-NaOD.

alkaline solutions of aspartic acid (Asp) (1) $CH_2{\cdot}(CO_2H){\cdot}CH{\cdot}(NH_2){\cdot}CO_2H$ shows no change of coupling constants, and hence of normalized rotamer populations, with temperature (Table 1); presumably solvation, enhanced by the presence of the second negative group, hinders rotation. The larger *a* and smaller *c* at pD 13·4 than at 10·4 may be a consequence of stabilization of electrostatic repulsion between the two $CO_2^-$ groups; at pD $\leqslant$ 10·4, those amino-groups still present as $NH_3^+$ will stabilize rotamer (III) by attraction to the $CO_2^-$ groups. This explanation is consistent with the smaller *a*, almost independent of pD (Table 1), when $CO{\cdot}NH_2$ replaces $CO_2H$, as in asparagine ($AspNH_2$) (2) $CH_2{\cdot}(CONH_2){\cdot}CH{\cdot}(NH_2){\cdot}CO_2H$.

In aspartyl (Asp) residues of the dipeptides glycylaspartic acid (Gly-Asp) (4) and alanylaspartic acid (Ala-Asp) (5) (Table 2), $J_{AC}$ decreases with pD, just as for aspartic acid.[12] In contrast to phenylalanyl (Phe) residues in the XPhe peptides, Asp residues in XAsp peptides exhibit a small convergence of the vicinal coupling constants by comparison with the parent amino-acids at pD 13·4 (Table 2). Replacement of the amino-group by the peptide bond and the glycyl residue would make rotamer (I) sterically less attractive. For asparagine, where $R^1$ is $CO{\cdot}NH_2$, and for Gly-Asp (Table 1), there is a small divergence in the vicinal coupling constants from asparagine to the Asp residue in Gly-$AspNH_2$. Such a difference in behaviour between $AspNH_2$ and Asp may be due to the high population of rotamer *a* in Asp (0·63; 0·56 in $AspNH_2$).

In acidic solution (pD <1·5), both Asp and Gly-Asp give deceptively simple (five-line) ABX spectra at 60 MHz (although at 220 MHz the spectra[13] have a well defined eleven-line ABX form); $(J_{AX} + J_{BX})/2$ is 5·3 in Asp and 5·8 Hz in glycyl and alanyl peptides.

*Phenylalanine and Tyrosine Dipeptides with Glycine and Alanine.*—From room-temperature spectra (Figure 1) of

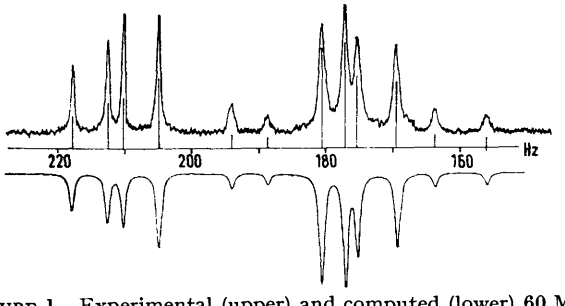



FIGURE 1 Experimental (upper) and computed (lower) 60 MHz $^1H$ n.m.r. spectra of 0·5M-phenylalanine in 0·5M-NaOD solution. Shifts are downfield from sodium 3-trimethylsilylpropane-1-sulphonate (internal reference)

0·5M-phenylalanine in NaOD concentrations of from 1·0 to 4·0M, chemical shifts and coupling constants have been extracted (*e.g.* see top line of Table 3 for the case of 1·0M-NaOD, *i.e.* pD = 13·2) and fractional rotamer populations (Table 4) deduced. With pK = 9·22,[14] the amino-acid should be fully ionized to the anionic form

[12] F. Taddei and L. Pratt, *J. Chem. Soc.*, 1964, 1553.
[13] B. J. Dale and D. W. Jones, unpublished measurements.

TABLE 4

Variation with [NaOD] of coupling constants and rotamer populations of phenylalanine in aqueous solution [a] at room temperature [b]

| | *J*/Hz | | | Rotamer populations | | |
|---|---|---|---|---|---|---|
| [NaOD]/M | $J_{AB}$ | $J_{AC}$ | $J_{BC}$ | *a* | *b* | *c* |
| 1·0 [c] | −13·5 | 7·6 | 5·4 | $0{\cdot}45_5$ | $0{\cdot}25_5$ | $0{\cdot}29_0$ |
| 1·5 | −13·5 | 7·75 | 5·2 | $0{\cdot}47_0$ | $0{\cdot}24_0$ | $0{\cdot}29_5$ |
| 2·0 | −13·5 | 8·0 | 5·2 | $0{\cdot}49_0$ | $0{\cdot}23_5$ | $0{\cdot}27_5$ |
| 2·5 | −13·5 | 8·15 | 5·2 | $0{\cdot}50_5$ | $0{\cdot}23_5$ | $0{\cdot}26_0$ |
| 3·0 | −13·5 | 8·25 | 4·95 | $0{\cdot}51_0$ | $0{\cdot}21_5$ | $0{\cdot}27_5$ |
| 4·0 | −13·5 | 8·8 | 4·7 | $0{\cdot}56_0$ | $0{\cdot}19_0$ | $0{\cdot}25_0$ |

[a] 0·5M in $D_2O$. [b] 303 K. [c] pD = 13·2 in 1·0M-NaOD.

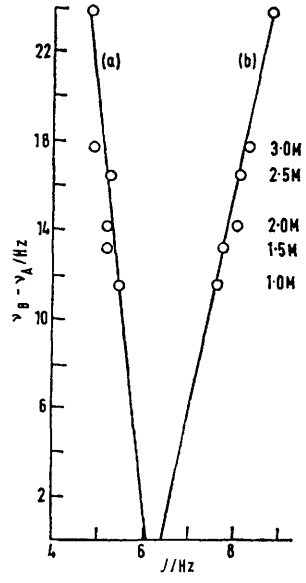



FIGURE 2 Chemical shift differences, $\nu_B - \nu_A$, *vs.* coupling constants, $J_{BC}$ (a) and $J_{AC}$ (b), for phenylalanine at a series of concentrations of NaOD

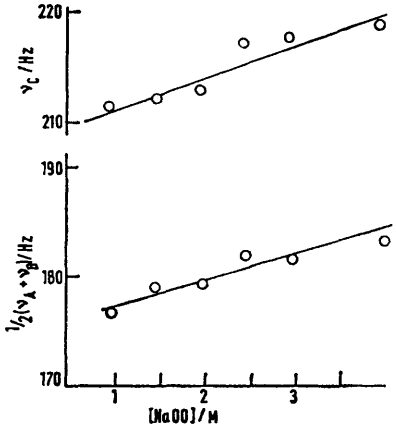



FIGURE 3 Chemical shifts $(\nu_A + \nu_B)/2$ and $\nu_C$ in phenylalanine *vs.* concentration of NaOD

even at pD 13·2, so that changes in $J_{AC}$ and $J_{BC}$ (Table 4 and Figure 2) and in chemical shifts (Figures 2 and 3)

[14] R. J. F. Nivard and G. I. Tesser, 'Comprehensive Biochemistry,' eds. M. Florkin and E. H. Stotz, Elsevier, Amsterdam, 1965.

can hardly be attributed to incomplete ionization; further ionization of the carboxy-group should affect the nearer α-methine proton more than the β-methylene protons, whereas their chemical shifts change to the same extent (Figure 3). The $J_{av}$ of 6·30 Hz extracted from the lines of $(\nu_B - \nu_A)$ *vs.* $J_{AO}$, $J_{BO}$ (Figure 2) is close to the 6·25 found by Pachler [3] in amino-acids.

In Phe, increase in concentration of NaOD (Table 4) results in a downfield drift (Figure 3) of $(\nu_A + \nu_B)/2$, together with an increase in population of rotamer (I); in this, both protons A and B can experience some deshielding from the carboxylate group, whereas in rotamers (II) and (III) only A or B can experience the full effect of $CO_2^-$. Such a mechanism cannot explain the downfield shift of $\nu_O$ (Figure 3). The chemical shift of the aromatic protons remains constant at 442 Hz, indicating the predominance of the ring-current contribution to their chemical shifts. The implied fall in relative potential energy * of rotamer (I) may arise from solvent–solute interactions, which are likely to be complex since Phe possesses both hydrophobic and hydrophilic groups. A second possibility is the formation of ion pairs between the phenylalanate and sodium ions; concomitant increased bulk of the carboxylate group could then stabilize structure (I).

The temperature behaviour of the spectra of Phe [16] emphasises the importance of concentration: for 0·3M, the vicinal coupling constants diverge between 283 and 383 K; for 2·0M, they remain nearly constant; and for 3·0M, they converge. Cavanaugh [10,13] attributed the consequent changes in rotamer population of Phe to changes in the solvent–solute interactions as the dielectric constant of water falls. For 0·5M-Phe, increase of concentration of NaOD changed the rotamer population in the same direction as increase in temperature of 0·3M-Phe. Further, addition of electrolytes to an aqueous solution reduced the dielectric constant,[17] presumably because the charged ions [18] prevent rotation of the water molecules; this could alter structuring of the solvent and hence its interaction with the solute. Decrease in dielectric constant will also enhance the effectiveness of local electric fields, which will influence solvent–solute interactions; increase in field strength would make ion-pair formation more probable.

In Table 3, spectral parameters of phenylalanine are compared with those of Phe residues in the peptides XPhe and PheX(X = glycyl, alanyl, and leucyl residues) in alkaline solution. Similarity of the Phe rotamer populations of Gly-Phe and Ala-Phe peptides means that, by comparison with Phe, rotamer (I) becomes more populated at the expense of rotamers (II) and (III). Steric considerations would support reduction in *c*, with bulky groups in close proximity, and would favour a smaller *a* and a larger *b*. Such behaviour is even more evident in leucylphenylalanine (Leu-Phe). Direct comparison between the parameters of phenylalanine and phenylalanyl residues in the PheX peptides is not possible because the carboxy-group has been lost during the formation of the peptide bond. For reasons which are not clear, the spectrum of the phenylalanyl in Phe-Leu has a five-line rather than a full ABC, appearance.

Spectral parameters of the peptides Gly-Phe, Phe-Gly, and Phe-Leu, recorded in acidic solution (pD <1·4), are summarised in Table 5. As before, the vicinal coupling constants are more divergent in the phenylalanyl residue in Gly-Phe than in Phe itself. Whereas there is little difference between the respective values of $(\nu_B - \nu_A)$ in alkaline solution, $(\nu_B - \nu_A)$ nearly doubles from amino-acids to peptide residue in acidic media.

Coupling constants of glycylphenylalanine and alanylphenylalanine (Table 6) in alkaline solution converge with increasing temperature over the range 283—353 K; the rotamer population *c* increases at the expense of *a*. For Gly-Phe in alkaline solution, neither change in peptide nor change in NaOD concentration appears to have any marked effect on the phenylalanyl coupling constants (Table 6). Two changes in the chemical shift are noticeable. First, as NaOD concentration increases from 1 to 3M, there is a similar small downfield shift of the Phe resonances to that in phenylalanine under the same conditions. Second, reduction of the concentration of NaOD to 0·25M shifts the glycyl methylene resonances 5 Hz downfield; this is in the direction of the methylene resonances in the dipolar ion.[19] Since the glycyl peak has collapsed to a broad hump, it is concluded that exchange is taking place between the dipolar and anionic forms of the peptide, an explanation consistent with the pD of the solution (10·9).

In tyrosine (Tyr), coupling constants (which are in satisfactory agreement with other recent measurements, where they overlap [20]) diverge over the temperature range 278—333 K (Table 7), with rotamer (I) gaining at the expense of (III). Neither reduction of concentration of Tyr to 0·1M nor increase of concentration of NaOD to 2·0M has any significant effect on the spectral parameters. In all the Tyr spectra at 303 K, lines were of such a breadth ($W_{\frac{1}{2}}$ 1·0 Hz) that small changes (0·1 Hz) in $J$ would remain undetected. This greater breadth of Tyr than Phe lines is puzzling in view of the similarity of the amino-acid structures. It is likely to be a consequence of slow interconversion and hence incomplete averaging of individual ABC spin systems from the several rotational isomers, perhaps as a result of self-association or solvent–solute interaction. Unlike Phe residues (Table 5), Tyr residues in Gly and Ala peptides give aliphatic spectral parameters which vary very little

* Throughout, we assume that rotamer (I) has lower energy than (II). In a publication following completion of our work, Newmark and Miller [15] are inclined to regard (II) [labelled (I) in their Figure 1] as more stable than (I) [their (II)].

[15] R. A. Newmark and M. A. Miller, *J. Phys. Chem.*, 1971, **75**, 505.
[16] J. R. Cavanaugh, *J. Amer. Chem. Soc.*, 1968, **90**, 4533.
[17] J. B. Hasted, D. M. Ritson, and C. H. Callie, *J. Chem. Phys.*, 1948, **16**, 1.
[18] G. H. Haggis, J. B. Hasted, and T. J. Buchanan, *J. Chem. Phys.*, 1952, **20**, 335.
[19] A. Nakamura and O. Jardetzky, *Proc. Nat. Acad. Sci., U.S.A.*, 1967, **58**, 2212.
[20] J. R. Cavanaugh, *J. Amer. Chem. Soc.*, 1970, **92**, 1488.

TABLE 5

Room-temperature [a] spectral parameters of phenylalanine, aspartic acid, and their simple peptides in acid solution [b]

| Amino-acid or peptide | Chemical shifts (Hz) | | | | J/Hz | | | Other chemical shifts (Hz) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\nu_A$ | $\nu_B$ | $\nu_C$ | $\nu_B - \nu_A$ | $J_{AB}$ | $J_{AC}$ | $J_{BC}$ | | | | | | |
| Phe | 195·3 | 202·1 | 263·1 | 6·8 | −14·9 | 7·7 | 5·4 | | | | | | |
| Gly–Phe | 184·7 | 196·9 | 291·2 | 12·2 | −14·8 | 9·0 | 5·9 | $GlyCH_2$ | 233 | | | | |
| Phe–Gly | | 195·0 | 262·0 | | | 7·6 | | $GlyCH_2$ | 241 | | | | |
| Phe–Leu | | 198·0 | 265·0 | | | 7·0 | | $LeuCH_3$ | 53 | $CH{\cdot}CH_2$ | 97 | CH | 265 |
| Asp | | 189·5 | 263·9 | | | 5·3 | | | | | | | |
| Gly–Asp | | 180·1 | 289·6 | | | 5·8 | | $GlyCH_2$ | 234 | | | | |
| Ala–Asp | | 182·8 | 292·0 | | | 5·7 | | $AlaCH_3$ | 94 | CH | 249·5 | $J_{CH_3CH}$ | 7·0 |

[a] 303 K. [b] 0·5M in $D_2O$ containing 10% w/w $CF_3{\cdot}CO_2D$. [c] Downfield from sodium 3-trimethylsilylpropane-1-sulphonate (internal reference).

TABLE 6

Effect of temperature and concentration on $^1H$ n.m.r. parameters of phenylalanine residues in alkaline solution

| [Peptide] | [NaOD]/M | T/K | Chemical shifts [a] (Hz) | | | | J/Hz | | | Rotamer populations | | | Other chemical shifts and couplings (Hz) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $\nu_A$ | $\nu_B$ | $\nu_C$ | $\nu_B - \nu_A$ | $J_{AB}$ | $J_{AC}$ | $J_{BC}$ | *a* | *b* | *c* | Ph | $GlyCH_2$ | |
| 0·5M-Gly–Phe (pD 13·1) | 1·0 | 283 | 176·6 | 192·5 | 268·5 | 15·9 | −13·7 | 8·8 | 5·0 | 0·56 | 0·22 | 0·22 | 440 | 192 | |
| | | 303 | 176·8 | 192·3 | 269·0 | 15·5 | −13·75 | 8·5 | 5·0 | 0·54 | 0·22 | 0·24 | 440 | 193 | |
| | | 323 | 177·4 | 192·4 | 269·0 | 15·0 | −13·8 | 8·4 | 5·0 | 0·53 | 0·22 | 0·25 | 440 | 193 | |
| | | 343 | 179·2 | 193·5 | 270·0 | 14·2 | −13·8 | 8·1 | 5·0 | 0·50 | 0·22 | 0·28 | 439 | 193 | |
| | | 353 | 179·8 | 193·8 | 270·0 | 14·0 | −13·75 | 8·1 | 4·9 | 0·50 | 0·21 | 0·29 | 439 | 194 | |
| | | | | | | | | | | | | | $AlaCH_3$ | CH | $J_{CH_3CH}$ |
| 0·5M-Ala–Phe | 1·0 | 303 | 175·4 | 191·3 | 263·0 | 15·9 | −13·75 | 8·6 | 5·0 | 0·55 | 0·22 | 0·23 | 67 | 198 | 6·8 |
| | | 353 | 176·3 | 191·6 | 265·0 | 15·3 | −13·7 | 8·05 | 5·1 | 0·49 | 0·23 | 0·28 | 67 | 200 | 6·8 |
| | | | | | | | | | | | | | Ph | $GlyCH_2$ | |
| 0·25M-Gly–Phe (pD 13·3) | 1·0 | 303 | 176·8 | 191·7 | 268·3 | 14·9 | −13·75 | 8·6 | 4·8 | 0·54 | 0·20 | 0·26 | 440 | 193 | |
| 0·1M-Gly–Phe (pD 13·3) | 1·0 | 303 | 177·0 | 192·0 | 268·7 | 15·0 | −13·7 | 8·5 | 5·0 | 0·53 | 0·22 | 0·25 | 440 | 193 | |
| 0·5M-Gly–Phe (pD 10·8) | 0·25 | 303 | 175·0 | 190·6 | 268·9 | 15·6 | −14·0 | 8·7 | 4·9 | 0·56 | 0·21 | 0·23 | 440 | 198 | |
| 0·5M-Gly–Phe (pD 13·6) | 3·0 | 303 | 183·2 | 198·3 | 274·5 | 15·1 | −14·1 | 8·6 | 5·2 | 0·55 | 0·24 | 0·21 | 440 | 195 | |

[a] Downfield from sodium 3-trimethylsilylpropane-1-sulphonate (internal reference).

TABLE 7

$^1H$ N.m.r. spectral parameters and rotamer populations of tyrosine and tyrosyl residues in dipeptides in alkaline solution

| [Peptide/amino-acid] | [NaOD]/M | pD | T/K | Chemical shifts [a] (Hz) | | | | J/Hz | | | Rotamer populations | | | Other shifts and couplings (Hz) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $\nu_A$ | $\nu_B$ | $\nu_C$ | $\nu_C - \nu_A$ | $J_{AB}$ | $J_{AC}$ | $J_{BC}$ | *a* | *b* | *c* | | | |
| 0·5M-Tyr | 2·0 | >13 | 303 | 161·8 | 172·9 | 204·9 | 11·1 | −13·4 | 7·4 | 5·2 | 0·44 | 0·23 | 0·34 | | | |
| 0·5M-Tyr | 1·0 | 12·7 | 278 | 163·2 | 169·5 | 205·2 | 6·3 | −13·4 | 6·8 | 5·2 | 0·39 | 0·23 | 0·38 | | | |
| | 1·0 | 12·7 | 308 | 160·5 | 171·5 | 204·6 | 11·0 | −13·4 | 7·4 | 5·2 | 0·44 | 0·23 | 0·34 | | | |
| | 1·0 | 12·7 | 338 | 158·8 | 171·8 | 204·2 | 13·0 | −13·6 | 7·8 | 5·1 | 0·47 | 0·21 | 0·31 | | | |
| 0·1M-Tyr | 1·0 | 12·7 | 303 | 158·4 | 169·8 | 205·6 | 11·4 | −13·4 | 7·4 | 5·1 | 0·44 | 0·23 | 0·34 | | | |
| | | | | | | | | | | | | | | $GlyCH_2$ | | |
| 0·5M-Gly–Tyr | 1·0 | 13·0 | 283 | 167·5 | 179·3 | 263·0 | 11·8 | −13·9 | 7·7 | 5·3 | 0·46 | 0·24 | 0·30 | 194 | | |
| | 1·0 | 13·0 | 303 | 167·5 | 178·9 | 262·0 | 11·9 | −13·9 | 7·7 | 5·2 | 0·46 | 0·23 | 0·31 | 194 | | |
| | 1·0 | 13·0 | 333 | 167·4 | 179·2 | 262·8 | 11·8 | −13·8 | 7·7 | 5·3 | 0·46 | 0·24 | 0·30 | 193 | | |
| | 1·0 | 13·0 | 353 | 167·2 | 179·1 | 262·5 | 11·9 | −13·9 | 7·6 | 5·3 | 0·45 | 0·24 | 0·31 | 193 | | |
| | | | | | | | | | | | | | | $AlaCH_3$ | CH | $J_{CH_3CH}$ |
| 0·5M-Ala–Tyr | 1·0 | 13·1 | 283 | 166·2 | 177·9 | 268·5 | 11·7 | −13·9 | 7·6 | 5·3 | 0·45 | 0·24 | 0·31 | 68 | 201 | 6·8 |
| | 1·0 | 13·1 | 303 | 166·0 | 177·8 | 269·0 | 11·8 | −13·8 | 7·6 | 5·2 | 0·45 | 0·23 | 0·32 | 69 | 202 | 6·7 |
| | 1·0 | 13·1 | 333 | 166·3 | 178·0 | 269·5 | 11·7 | −13·9 | 7·7 | 5·2 | 0·46 | 0·23 | 0·31 | 68 | 202 | 6·8 |
| | 1·0 | 13·1 | 353 | 166·5 | 178·4 | 269·9 | 11·9 | −13·9 | 7·7 | 5·2 | 0·46 | 0·23 | 0·31 | 68 | 202 | 6·8 |

[a] Downfield from sodium 3-trimethylsilylpropane-1-sulphonate (internal reference).

with temperature. Further, vicinal constants of Tyr residues in peptides differ less from those in tyrosine (Table 7) than do Phe couplings in peptides from those in phenylalanine (Tables 5 and 6).

Overall, the differences in $^1H$ n.m.r. spectral behaviour between parent α-amino-acids in isolation and the corresponding residues in dipeptides emphasise the care needed in predicting the behaviour of residues in larger peptides. Even in dipeptides, conformational isomerism about the NH–CH bond is superimposed on that about the $C_\alpha$–$C_\beta$ bonds of the amino-acid; the peptide bond is taken to be planar and *trans*. $C_\alpha$–$C_\beta$ Rotamer populations are calcu-

lated from measured coupling constants, together with theoretical coupling constants $J_g$ and $J_t$ which depend mainly on the atoms directly bonded; such calculations will not be influenced much by the nature of any N–C rotations.

For alanyl dipeptides, Bystrov *et al.*[21] found from $^1$H n.m.r. and i.r. studies that the rotamers with the amido-hydrogen atom eclipsed by some other group (NH and CH *cis*-O, gauche-120, and gauche-240°) were preferred to those with NH gauche to other groups (*trans*-180, gauche-300, and gauche-60°) [Figure 8 of ref. 21]. Models of Gly-Asp and Gly-Phe, constructed with $C_\alpha$–$C_\beta$ in its preferred conformation (I), show that, of the six classical NH–CH rotamers, *cis*-O and *trans*-180° would be most hindered sterically and gauche-240 and gauche-60° least hindered; gauche-300° would be somewhat hindered and gauche-120° might just be possible. Thus the preferred $C_\alpha$–$C_\beta$ conformation (I) would be compatible with both eclipsed and gauche conformations about NH–CH.

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[21] V. F. Bystrov, S. L. Portnova, V. I. Tsetlin, V. T. Ivanov, and Yu. A. Ovchinnikov, *Tetrahedron*, 1969, **25**, 493.