Conformational Analysis of Tryptophan in Solution using Nuclear Magnetic Resonance Methods

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The conformation of the amino-acid tryptophan in solution has been examined by n.m.r. methods. Coupling constants, nuclear Overhauser effects, and lanthanide perturbations were all analysed in terms of six conformers, each having one of three values for the C^{α}–C^{β} dihedral angle (χ_1) and one of two values for the C^{β}–C^{γ} dihedral angle (χ_2). A set of populations was obtained which was consistent, within reasonable limits, with all the n.m.r. data. It was found that one conformer, that having the side-chain *trans* to the carboxylic acid group and the ring perpendicular to the C^{α}–C^{β} bond, was the dominant species in solution. There are similarities between the population distribution in solution and the distribution of dihedral angles found for tryptophan in protein crystal structures.

In protein structures, the distribution of side-chain dihedral angles is far from random, and the number of configurations actually observed in proteins for a given type of amino-acid residue is small.[1-5] It appears that in the folded protein structure, little strain is placed on the side-chains each of which generally adopts one of a few permitted conformations.[3,5] In this paper we examine the conformation of tryptophan in solution, and compare the results with the distribution of conformations found for residues of this amino-acid in protein structures.

Three different types of method were used to define the molecular conformation in solution. All the methods involve n.m.r., but are very different in their approach. The first involves direct interpretation of coupling constants in terms of dihedral angles.[6,7] The second involves measurement of relative interproton distances using nuclear Overhauser effects.[8,9] In the third method, a paramagnetic lanthanide ion is bound to the carboxylate group, and the resulting shift and relaxation perturbations to the spectrum are analysed in term of vectors between the different protons and the bound metal ion.[10-12]

Studies of tryptophan and tryptophan-containing peptides by analysis of coupling constants have shown [13-16] that a distribution of conformers with different C^{α}–C^{β} dihedral angles exists. On the other hand, a study of tryptophan using the lanthanide ion method showed that the data were consistent with a single preferred conformation for the amino-acid complexed to a lanthanide ion.[17] The nature of the metal complex was, however, suggested to differ for different lanthanides.[17] In the present study, the different methods are considered together, so as to arrive at a population distribution for tryptophan (free and complexed to lanthanides) which is in best agreement with all the data. Such a procedure has proved successful in the analysis of nucleotide conformations.[18-20]

EXPERIMENTAL

DL-Tryptophan was obtained from the Sigma Chemical Company and freeze dried from 99.8% ^2H_2O. Lanthanide chloride solutions were prepared by dissolution of the anhydrous chlorides (Research Organic/Inorganic Chemicals) in ^2H_2O. Solutions were, unless otherwise stated, 30mM in tryptophan and at pH 5.5 ± 0.5 (meter reading in ^2H_2O).

^1H N.m.r. spectra were recorded at 270 MHz using a Bruker spectrometer, at 25 °C unless otherwise indicated. Chemical shifts were measured relative to acetone and dioxan as internal standards. T_1 Values were measured using a 180°–τ–90° pulse sequence and coupling constants by spectral simulation. Nuclear Overhauser effects were measured at steady state using a pre-saturation pulse and difference spectroscopy.[21,22] Assignments for the tryptophan resonances have been given previously.[15,23]

Co-ordinates for tryptophan were taken from the crystal structure data,[24] except that the hydrogen atoms were generated in accepted positions from the heavy-atom co-ordinates. Computer programs for calculating lanthanide shift and relaxation effects were based on those described previously.[25,26]

RESULTS

Relaxation rates ($1/T_1$ values) were measured for each of the protons of 30mM-tryptophan in ^2H_2O solution at pH 5.5 ± 0.5. Relaxation rates were then measured under identical conditions for solutions of tryptophan containing various concentrations of lanthanide ions. In Table 1 are listed values of relaxation rate enhancements corrected for diamagnetic and non-specific effects for different protons of tryptophan, relative to that of H^{α}.

For all paramagnetic lanthanides other than Gd^{3+}, dipolar shifts were observed in the tryptophan proton resonances, as described previously.[17] In this work the shifts with Pr^{3+} were measured in detail, at a range of concentrations between 0.001 and 0.15M. Values of shifts relative to that of H^{α} were found to be independent of concentration of added lanthanide and to be, corrected for diamagnetic effects, within experimental error of those reported previously [17] (see Table 5).

The coupling constants between H^{α} and $H^{\beta 1}$ and $H^{\beta 2}$ were 8.2 ± 0.2 and 4.8 ± 0.2 Hz, respectively, both for tryptophan itself and for tryptophan in the presence of 0.5M-La^{3+}, where it is more than 80% bound.

Nuclear Overhauser effects were observed at steady state following saturation of the fully resolved H^{α} resonance. Relative nuclear Overhauser effects on the different protons were measured by observing peak areas in difference spectra.

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The effect on $H^{\beta 1}$ was 0.48 ± 0.10 of the effect on $H^{\beta 2}$. The effect on $H^{\delta 1}$ was 0.43 ± 0.10 that on $H^{\epsilon 3}$.

DISCUSSION

Homology of Lanthanide Complexes.—The paramagnetic lanthanides, except for Gd^{3+}, give rise to dipolar shifts in the n.m.r. spectrum of a ligand molecule. The relative shifts of resonances of different nuclei in the molecule depend upon the molecular conformation, and also on the symmetry of the ligand field exerted by the molecule on the lanthanide ion.[10-12] Gd^{3+}, and to a good approximation the other lanthanide ions, give rise to isotropic dipolar relaxation and the relative enhancement of relaxation rates of different nuclei depends simply on the inverse sixth power of the relative distances of different nuclei from the lanthanide ion.[10-12,27]

In previous work it was found that the induced transverse relaxation rates for tryptophan protons differed significantly with different lanthanides.[17] In particular, it was found that the induced relaxation rate ratio for $H^{\beta 1}$ relative to that of H^{α} varied from 0.70 with Gd^{3+} to 0.10 with Tm^{3+}, suggesting a conformational difference between the two complexes. No such variation is observed with the longitudinal relaxation rates (Tables 1 and 2) which, within experimental error, are the same for given protons with all lanthanides. These data indicate that the complexes of tryptophan with different lanthanides are homologous. It is possible that the transverse, unlike the longitudinal, relaxation is not purely dipolar but contains contributions from scaler or exchange mechanisms.

The conformation of a tryptophan molecule is defined fully by the torsional angles about the C^{α}–C^{β} and C^{β}–C^{γ} bonds. The coupling constants between H^{α} and the β protons, $J_{\alpha-\beta 1}$ and $J_{\alpha-\beta 2}$, are strongly dependent on the C^{α}–C^{β} dihedral angle. These values, within experimental error, are the same in the presence as in the absence of La^{3+} showing that the conformational equilibrium about the C^{α}–C^{β} bond of tryptophan is not affected significantly by complexation with a lanthanide. The chemical shifts of the $H^{\beta 1}$ and $H^{\beta 2}$ resonances have been shown to be sensitive to the C^{β}–C^{γ} torsional angle.[13] The values are the same within experimental error, for tryptophan in the presence of La^{3+} as in its absence. We conclude that the conformational state of the molecule complexed with La^{3+}, and by inference with other lanthanides, is very close to that of the free molecule.

TABLE 1

Relative rate enhancements with different lanthanides [a]

	H^{α}	$H^{\beta 1}$	$H^{\beta 2}$	$H^{\delta 1}$	$H^{\epsilon 3}$	$H^{\zeta 3}$	$H^{\eta 2}$	$H^{\zeta 2}$
Pr^{3+}–Eu^{3+} [b]	1.00	0.87	0.75	0.29	0.45	0.13	0.10	
Gd^{3+}	1.00	0.78	0.73	0.22	0.44	0.10	0.09	0.04
Tb^{3+}–Er^{3+}, Yb^{3+} [c]	1.00	0.65	0.66	0.25	0.44	0.12	0.08	0.05
Tm^{3+}	1.00	0.68	0.59	0.25	0.46	0.13	0.11	0.05
Mean [d]	1.00	0.70	0.67	0.25	0.43	0.11	0.09	0.05
		±0.12	±0.08	±0.03	±0.07	±0.03	±0.02	±0.01
Mean (70 °C) [e]	1.00	0.75	0.79	0.28	0.41	0.12	0.08	0.05
		±0.09	±0.15	±0.03	±0.05	±0.01	±0.02	±0.01

[a] Ratios to those for the H^{α} resonance, at 25 °C unless otherwise stated. Corrected for diamagnetic effects by measurement of effects of La^{3+} on tryptophan relaxation rates and chemical shifts; corrected for non-specific effects by measurement of effects of lanthanides on relaxation rates and shifts of protons of indole. All corrections were small. Error is less than *ca.* ±0.05 for all measurements, except for relaxation data on β protons where it is *ca.* ±0.10. For groups of lanthanides, within experimental error all gave the same ratios. [b] Mean of data for Pr^{3+}, Nd^{3+}, and Eu^{3+}. [c] Mean of data for Tb^{2+}, Dy^{3+}, Ho^{3+}, Er^{3+}, and Yb^{3+}. [d] Mean of all above. [e] Mean of data for Pr^{3+}, Gd^{3+}, and Tm^{3+}, measured at 70 °C.

TABLE 2

Populations of the C^{α}-C^{β} rotamers [a]

	g+	g−	t
J [b]	0.32 ± 0.10	0.53 ± 0.10	0.15 ± 0.10
NOE [c]	0.15 ± 0.15	0.75 ± 0.10	0.10 ± 0.10
Ln^{3+} [d]	⩽0.15	⩾0.65	⩽0.20

[a] g+ has χ_1 (the N–C^{α}–C^{β}–C^{γ} angle) defined to be 62.7°, as found in the crystal structure; g− and t are then defined by χ_1 values of −57.3 and −177.3°, respectively. The nomenclature corresponds to that used in refs. 15 and 16; in ref. 3, the labels g+ and g− are reversed. In ref. 6 g+, g−, and t are denoted III, II, and I, and in ref. 13 by A, C, and B. [b] Using K_1 9.4, K_2 1.4, K_3 1.6. Error limits are those estimated for this approach by Feeney.[6] Use of the alternative parameters K_1—K_3 summarised by Bystrov [7] gives a range of 0.27—0.46 for g+, 0.43—0.56 for g−, and 0.11—0.20 for t. [c] Error bars assume a 20% error in the n.O.e. ratio. [d] These populations predict the experimental ratios within 0.10 in each case.

Conformational Equilibrium about the C^{α}–C^{β} *Bond.*—In order to study the C^{α}–C^{β} bond, the usual simplifying assumption was made that only those rotamers having torsional angles N–C^{α}–C^{β}–C^{γ} of *ca.* 60, 180, and −60° (Figures 1 and 2) need be considered. The populations of these three rotamers were estimated independently from the coupling constant, the nuclear Overhauser effect, and the lanthanide perturbation data.

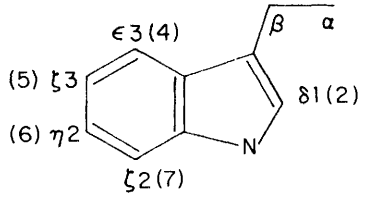

FIGURE 1 Numbering scheme for carbon atoms of tryptophan. The other commonly used notation is given in parentheses

The observed coupling constants, $J_{\alpha-\beta i}$, between H^{α} and $H^{\beta i}$ ($i = 1$ or 2) can be defined by equation (1)

$$J_{\alpha-\beta i} = \sum_{j=1}^{3} P_j J_{\alpha-\beta i}^{j} \qquad (1)$$

where P_j is the fractional population of rotamer j. $H^{\beta 1}$ and $H^{\beta 2}$ are defined as in Figure 2, and are assigned experimentally on the basis of previous work;[6] the resonance of $H^{\beta 1}$ is to high field of that of $H^{\beta 2}$. $J^j_{\alpha-\beta i}$ is the coupling constant for a specific rotamer j, which is not determined experimentally in a direct way but may be defined[7] by an equation of the form (2) where ϕ is the

$$J_{\alpha-\beta i} = K_1 \cos^2 \phi - K_2 \cos \phi + K_3 \qquad (2)$$

N–C^α–C^β–$H^{\beta i}$ torsional angle. K_1—K_3 are constants that depend on the steric and electronic properties of the molecule, and are taken to be 9.4, 1.4, and 1.6, respectively, from experimental work on related molecules.[28] Using these relationships in the usual way,[6,7] the populations given in Table 2 were determined. These values are close to those determined previously for tryptophan itself and in small peptides,[13-16] and for phenylalanine and tyrosine.[6,13] The error limits[6] cover the spread of populations obtained using different estimates for K_1—K_3 as well as the experimental errors in measurement. It is, however, clear that the calculated populations are highly dependent on the co-ordinates of the protons, and on the dihedral angles chosen for the three rotamers.

The nuclear Overhauser effects, $\eta_{\alpha-\beta i}$, observed on the $H^{\beta i}$ resonances as a consequence of saturation of the H^α resonance are, as the T_1 values are the same within experimental error for the two β protons, to a good approximation[8,9] given by equation (3) where K is a

$$\eta_{\alpha-\beta i} = K \sum_{j=1}^{3} P_j / r^6_{\alpha-\beta i}(j) \qquad (3)$$

constant and $r_{\alpha-\beta i}$ (j) is the distance between the H^α and $H^{\beta i}$ in rotamer j. From the relative effects on the two β protons, the populations of the three rotamers were calculated (Table 2). The limits are estimated from experimental errors and neglect of possible cross-relaxation effects.

Yet a further method of estimating the populations comes from the lanthanide shift and relaxation data. On the reasonable assumption that the lanthanide ion is co-ordinated identically to each rotamer, it is possible to write equations (4) and (5) where R^i and S^i are

$$R^i = \sum_{j=1}^{3} P_j R_j^i \qquad (4)$$

$$S^i = \sum_{j=1}^{3} P_j S_j^i \qquad (5)$$

respectively the observed ratios of induced relaxation and shift for the $H^{\beta i}$ resonances relative to that of the H^α resonance, and R_j^i and S_j^i are the ratios that correspond to a specific rotamer j. In order to determine the latter, reference was made to previous studies of molecules containing carboxylate groups.[12,17,29,30] In the light of these studies, the Pr^{3+} ion was positioned along the C^α–COOH bond direction at a distance of 3.0 Å from the carboxy-carbon. The shifts for Pr^{3+} were taken to be proportional to $(3 \cos^2 \theta - 1)/r^3$ where r is the distance between the metal ion and the proton, and θ the angle between the vector joining the metal to the proton and the C^α–COOH bond. The relaxation rate enhancements were assumed to be proportional to $1/r^6$, as expected for a dipolar mechanism. Using this information, R_j^i and S_j^i were calculated for the three

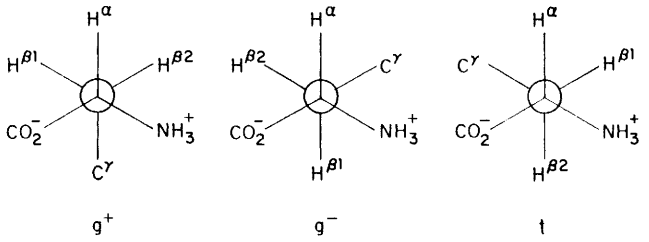

FIGURE 2 Representation of the g^+, g^-, and t configurations of tryptophan

tryptophan rotamers and from these the limits on the populations given in Table 2 were defined. In accord with earlier work,[17] it was found that the lanthanide data were fully compatible with 100% of rotamer g^- being present in solution, but it was found here that within experimental error, up to 35% of other rotamers were allowed. Taken together, however, the three very different methods of estimating the populations are by no means incompatible. It is clear from each method that g^- has substantially the highest population, whilst the coupling constants and nuclear Overhauser effects require that g^+ and t exist as minor species.

Conformational Equilibrium about the C^β–C^γ Bond.— The first attempt to define the ring orientation was to examine, by an extension of the procedures described above, whether any single χ_2 dihedral angle (C^α–C^β–C^γ–$C^{\delta 1}$) was consistent with the lanthanide shift and relaxation data. The lanthanide shift data were easy to fit and indeed, as shown earlier,[17] even without considering any rotamer other than g^- good fits to single χ_2 dihedral angles can be obtained. These fits are, however, incompatible with the longitudinal relaxation data (see below and Table 5), and no satisfactory fit to both shift and relaxation data could be obtained for any distribution of C^α–C^β rotamers whilst requiring a unique value of the C^β–C^γ dihedral angle.

In crystal structures of small peptides and proteins, the tryptophan ring is almost invariably found in perpendicular (χ_2 *ca.* 90°) or antiperpendicular (χ_2 *ca.* −90°) orientations[1-3] (Figure 3). For each of the three rotamers, therefore, it was assumed that these two orientations of the ring were allowed. For the six conformations of tryptophan thus generated, shift and relaxation ratios were calculated as described above. Then, the populations of the six conformations were varied in steps of 0.05, and for each of the mixtures of populations generated in this way, the shift and broadening ratios were calculated. The mixtures with calculated ratios corresponding to the observed ratios, within experimental error, were selected and the results

of these calculations are given in Table 3. First, it is clear that the conformation of g$^-$ is again found to be the dominant one, but it is no longer acceptable that no other rotamers are populated. This result for the lanthanide data is, therefore, now fully consistent with

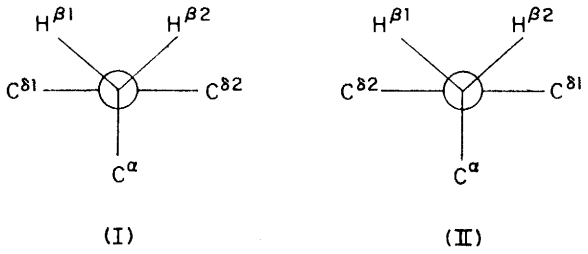

FIGURE 3 Representation of the perpendicular (I) and antiperpendicular (II) configurations of tryptophan

the coupling constant and nuclear Overhauser data. Second, it is apparent that despite the large amount of experimental data, it is not possible to distinguish between a perpendicular and an antiperpendicular ring orientation, and no information at all is available as to their relative populations.

TABLE 3

Populations of conformations determined from lanthanide shift and relaxation data [a]

	Perpendicular [b]	Antiperpendicular	Total
g$^+$	0.00 ⩽ 0.10	0.00 ⩽ 0.20	0.05—0.20
g$^-$	0.00 ⩽ 0.80	0.00 ⩽ 0.80	0.65—0.80
t	0.00 ⩽ 0.10	0.00 ⩽ 0.20	0.00—0.20
Total	0.00—1.00	0.00—1.00	

[a] Fractional populations were incremented from 0 to 1 in steps of 0.05, maintaining $\sum_{j=1}^{6} P_j = 1$. The ranges given in this Table correspond to calculated broadening and shift ratios within 0.10 of the experimental values (Table 5) for each proton. [b] Perpendicular has χ_2 (the Cα–Cβ–Cγ–Cδ1 angle) defined to be 102.9° as found in the crystal structure; antiperpendicular is defined as having χ_2 of −77.1.

Saturation of the H$^{\alpha}$ resonance gives significant nuclear Overhauser effects on only the H$^{\delta 1}$ and H$^{\epsilon 3}$ resonances of the aromatic ring. Because the T_1 values of these protons are different (4.75 and 2.44 s respectively under the conditions used for the nuclear Overhauser experiment) expression (6), similar to equation (3), where K' is a constant and T_1 (δ1) is the

$$\eta_{\alpha-\delta 1} = K' T_1\,(\delta 1) \sum_{j=1}^{6} P_j / r^6_{\alpha-\delta 1}\,(j) \qquad (6)$$

T_1 value of H$^{\delta 1}$, and an analogous expression for $\eta_{\alpha-\epsilon 3}$ were used to relate the effects to rotamer populations. Using these relationships, for all the mixtures of populations generated in the lanthanide analysis ratios of nuclear Overhauser effects on H$^{\delta 1}$ and H$^{\epsilon 3}$ were calculated. This calculation showed that for the antiperpendicular ring conformation of g$^-$ the ratio of $\eta_{\alpha-\delta 1}/\eta_{\alpha-\epsilon 3}$ was 13.9. As the experimental value is 0.43 ± 0.10 only mixtures of conformations with low populations of the g$^-$ antiperpendicular conformation are possible. Table 4 summarises populations obtained from the lanthanide and nuclear Overhauser effect data.

Comparison of Different Methods.—The populations calculated and listed in Table 4 are based on the results of the different n.m.r. methods. In Tables 5 and 6 are listed the calculated parameters for the conformational mixture of Table 4, along with the experimental data. Data are also given for the dominant conformation in the mixture.

TABLE 4

Populations of conformations determined from lanthanide shift and relaxation data in conjunction with nuclear Overhauser data [a]

	Perpendicular	Antiperpendicular	Total
g+	0.05 ± 0.05	0.15 ± 0.05	0.20 ± 0.10
g−	0.70 ± 0.05	0.00 ± 0.05	0.70 ± 0.10
t	0.05 ± 0.05	0.05 ± 0.05	0.10 ± 0.10
Total	0.80 ± 0.15	0.20 ± 0.15	1.00

[a] Determined as in Table 3, but requiring that $\eta_{\alpha-\delta 1}/\eta_{\alpha-\epsilon 3} = 0.43 \pm 0.43$. The actual range found to be compatible with the shift data was 0.50—0.78.

These data show that the lanthanide shift ratios are rather insensitive to the conformational state of tryptophan, at the level of accuracy at which the experiments and calculations are done. The relaxation data, however, are somewhat more sensitive. For example, the relatively large relaxation effects for the protons of the tryptophan ring are compatible with the calculations for the mixture but not for any single conformation. The coupling constant and nuclear Overhauser data (Table 6) taken together are at least as compatible with the

TABLE 5

Lanthanide shift and relaxation ratios

	Shift			Relaxation		
Proton	Observed [a]	Calculated for mixture [b]	Calculated for g$^-$, perpendicular [b]	Observed [c]	Calculated for mixture [b]	Calculated for g$^-$, perpendicular
Hα	1.00	1.00	1.00	1.00	1.00	1.00
Hβ1	0.66	0.65	0.68	0.70	0.64	0.70
Hβ2	0.69	0.63	0.66	0.67	0.58	0.66
Hδ1	0.18	0.23	0.24	0.25	0.30	0.07
Hε3	0.27	0.23	0.23	0.43	0.43	0.11
Hζ3	0.00	0.01	0.09	0.11	0.13	0.02
Hη2	−0.02	0.01	0.07	0.09	0.04	0.01
Hζ2	0.02	0.04	0.09	0.05	0.03	0.01

[a] With Pr^{3+}. Experimental errors are *ca.* ±0.03. [b] Populations from Table 4. [c] Mean with different lanthanides, see Table 1.

mixture as with any single conformation. Of all the measurements, only $J_{\alpha-\beta 1}$ and $\eta_{\alpha-\delta 1}/\eta_{\alpha-\epsilon 3}$ are not predicted within experimental error for the conformational mixture. Closer agreement, however, requires only minor changes in populations or dihedral angles (Table 2). A fundamental reason for these discrepancies probably lies in the accuracy of bond lengths and angles, and in the

TABLE 6

Calculated [a] coupling constants and nuclear Overhauser effects

	Observed	Calculated for mixture [b]	Calculated for g−, perpendicular
$J_{\alpha-\beta 1}$	8.2 Hz	9.7 Hz	12.3 Hz
$J_{\alpha-\beta 2}$	4.8 Hz	4.6 Hz	4.0 Hz
$\eta_{\alpha-\beta_1}/\eta_{\alpha-\beta_2}$	0.48	0.51	0.25
$\eta_{\alpha-\delta_1}/\eta_{\alpha-\epsilon_3}$	0.43	0.71 [c]	0.50 [c]

[a] Calculations were carried out as described in Table 2 and in text. [b] Populations from Table 4. [c] Ratios of r^{1-6} are 0.36 for the mixture and 0.26 for g⁻, perpendicular.

definition of the conformers to be included in the conformational mix. Given these limitations, the different methods agree remarkably well in their overall conclusions.

Conclusions.—The predominant conformation of tryptophan in solution is that which has the C^{α}–C^{β} bond in the g⁻ configuration and has the ring in an orientation perpendicular to the C^{α}–C^{β} bond. The experimental data require, however, that other conformations contribute to at least 20% and possibly 40% of the population. A rather simple picture of six contributing conformers, whilst being a considerable approximation, is sufficient to obtain reasonably consistent agreement with coupling constant, nuclear Overhauser effect, and lanthanide shift and relaxation data.

It is interesting to compare these conclusions with *X*-ray structural data. For tryptophan hydrochloride in crystals,[24] the ring is in the perpendicular orientation found for the predominant conformer in solution, but

TABLE 7

Distribution of conformations for tryptophan in proteins [a]

	Perpendicular	Antiperpendicular	Total
g⁺	0.11 (9)	0.04 (3)	0.15 (12)
g⁻	0.37 (30)	0.09 (7)	0.46 (37)
t	0.19 (16)	0.19 (16)	0.40 (32)
Total	0.68 (55)	0.32 (26)	1.00 (81)

[a] From ref. 2. Perpendicular conformations are defined as those with χ_2 closest to 90°, and antiperpendicular those with χ_2 closest to −90°. In fact, *ca.* 10% of the conformations should be described as parallel or antiparallel. Numbers in parentheses refer to the number of times the conformation appears, from which the fractional 'populations' of the different conformations were calculated. Similar data are given in ref. 3.

the conformation about the C^{α}–C^{β} bond corresponds to the g⁺, not to the g⁻ configuration. In proteins, tryptophan is found in a variety of conformations, and the distribution amongst these is summarised in Table 7 using data from a number of globular proteins.[2] In proteins, the most common configuration is that found to be dominant in solution. Further, although insufficient data are available for close comparison, there are similarities between the distribution of tryptophan conformations in proteins (Table 7) and the distribution of tryptophan amongst its different conformations in solution (Table 4). This result is an example of that expected generally for amino-acids on the basis of calculations involving the bovine pancreatic trypsin inhibitor protein.[4] In that study the minimum energy positions of residues in the protein were found to be close to those of free amino-acids despite the large restrictions in conformational space resulting from non-bonded interactions. This was suggested to be not simply a consequence of the energies involved but of the requirements of the folding process.[4] The effects of peptide bond formation, removal from solvent and inter-residue contacts appear to be limited to occasional stabilisation of these conformations which are unusual in solution, to give a slightly more even conformational distribution for tryptophan in proteins compared to tryptophan in solution.

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