2 is even larger, since the amide groups are rigidly attached to the azobenzene residue. The isomerization also changes from 62 to 0° the angle between the bonds connecting the $-CONHC_6H_4N{=}NC_6H_4NHCO-$ (or, the $-NHCOC_6H_4N{=}NC_6H_4CONH-$) groups to the rest of the chain molecule. We must then consider the question of how this restraint can be without a significant effect on the rate of the process.

The result may be rationalized in the following manner. The necessity to rearrange the conformation of a considerable portion of the polymer chain as the transition state in the *cis–trans* isomerization is being approached will, indeed, slow down the approach to the transition state, but it will reduce equally the rate at which the geometry of the strained azobenzene group returns to that characterizing its ground state. Thus, the equilibrium concentration of any intermediate state will not be altered as long as the energy of the azobenzene moiety, in any given geometric form, is unaffected by the incorporation of this group into the polymer chain. We should, therefore, not expect the isomerization rate to be reduced unless the time required for the change of the chain conformation to a form consistent with the transition state of the azo group is comparable with the relaxation time of the *cis–trans* isomerization in the absence of such added restraints. The requirement for extensive changes in the chain conformation is apparently not rate limiting for the *cis–trans* isomerization process even at polymer concentrations corresponding to extensive chain entanglements. We hope to explore in the future the isomerization behavior at still higher polymer concentrations and particularly the characteristics of such processes in bulk polymers, both above and below the glass transition point.

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# The Conformation about the Nitrogen—α-Carbon Bond in Random-Coil Polypeptides

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ABSTRACT: The conformation about the N—C$^\alpha$ bond in random-coil polypeptides is studied by computing the average vicinal coupling $J_{N\alpha}$ of the peptide NH and C$^\alpha$ proton. Previously calculated conformational energies of random-coil polypeptides make possible the computation of the vicinal proton coupling by taking an average over the calculated conformations and assuming a reasonable Karplus-like dependence of $J_{N\alpha}$ on the dihedral angle $\varphi'$. The calculated coupling agrees within the probable experimental error with the values observed in solution for three different random-coil polypeptides.

Measurements such as light scattering,[1,2] viscosity,[1,2] and dielectric increments[3] yield information concerning the overall extension of a random-coil polypeptide chain in solution, as expressed by the mean square end-to-end distance $\langle r^2\rangle$. By contrast, the measurement of the nuclear magnetic resonance spectra of random-coil polypeptides in solution gives information concerning the local conformation about the N—C$^\alpha$ bond through the magnitude of the vicinal coupling $J_{N\alpha}$ of the peptide NH and C$^\alpha$ proton (see Figure 1).

The assumption of a reasonable Karplus-like dependence[4] of $J_{N\alpha}$ on the dihedral angle $\varphi'$ leads to the following relation

$$J_{N\alpha}(\text{Hz}) = \begin{cases} 8.5\cos^2\varphi' & (0° \le \varphi' \le 90°) \\ 9.5\cos^2\varphi' & (90° \le \varphi' \le 180°) \end{cases} \tag{1}$$

where $\varphi'$ is the customarily defined dihedral angle between N–H and C$^\alpha$–H$^\alpha$ and is directly related to the rotation angle[5a] $\varphi$ about the N—C$^\alpha$ bond. A similar relation is given by Bystrov, *et al.*[6]

$$J_{N\alpha}(\text{Hz}) = 8.9\cos^2\varphi' - 0.9\cos\varphi' + 0.9\sin^2\varphi' \tag{2}$$

From the conformational energy calculations of Brant and Flory[7] and Brant, Miller, and Flory[8] ap-

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propriate to random-coil poly-L-peptides whose side chains R are of the type $CH_2R'$, the average vicinal coupling constant $J_{N\alpha}$ of this class of polypeptides may be evaluated. This calculated vicinal coupling constant may be compared to the values observed in solution for poly-L-alanine[9] ($R{\equiv}CH_3$), poly-L-methionine[10,11] ($R{\equiv}CH_2CH_2SCH_3$), and poly($\beta$-benzyl L-aspartate[12]) ($R{\equiv}CH_2CO_2CH_2C_6H_5$) in their random-coil conformations.

## Method of Calculation

Because the amide bonds in open-chain polypeptides are almost exclusively in the planar *trans* conformation,[13-17] the interactions of atoms and groups in neighboring peptide units which depend jointly on adjacent pairs of the rotation angles ($\varphi,\psi$) may be neglected.[7,8] Thus, if the randomly coiled polypeptide chain is assumed to be unperturbed by intra- or intermolecular excluded volume effects,[18] then the conformation of each peptide unit depends only on those interactions whose magnitudes are solely functions of one or both of the pair of rotation angles ($\varphi,\psi$) of the peptide unit in question.

Brant and Flory[7] and Brant, Miller, and Flory[8] have calculated the conformational energies, $E(\varphi,\psi)$, of a peptide unit in the class of randomly coiling poly-L-peptides whose side chains are of the form $R{\equiv}CH_2R'$ by assuming $R{\equiv}CH_3$. Their calculations took appropriate account of the intrinsic torsional potentials about the N—C$^\alpha$ and C$^\alpha$—C bonds, the nonbonded van der Waals interactions, and the electrostatic interactions between adjacent peptide group dipoles. From these conformational energies, $E(\varphi,\psi)$, the average vicinal coupling $J_{N\alpha}$ may be calculated by evaluating the conformational averages of $\cos^2 \varphi'$ (see eq 1) or ($8.9 \cos^2 \varphi' - 0.9 \cos \varphi' + 0.9 \sin^2 \varphi'$) (see eq 2).

$$\langle \cos^2 \varphi' \rangle = \frac{\sum\limits_{\varphi,\psi} (\cos^2 \varphi') \exp[-E(\varphi,\psi)/RT]}{\sum\limits_{\varphi,\psi} \exp[-E(\varphi,\psi)/RT]} \tag{3}$$

$$\langle 8.9 \cos^2 \varphi' - 0.9 \cos \varphi' + 0.9 \sin^2 \varphi' \rangle = \frac{\sum\limits_{\varphi,\psi} (8.9 \cos^2 \varphi' - 0.9 \cos \varphi' + 0.9 \sin^2 \varphi') \exp[-E(\varphi,\psi)/RT]}{\sum\limits_{\varphi,\psi} \exp[-E(\varphi,\psi)/RT]} \tag{4}$$

Both $\cos^2 \varphi'$ and ($8.9 \cos^2 \varphi' - 0.9 \cos \varphi' + 0.9 \sin^2 \varphi'$) were averaged at 25° over all $\varphi$ and $\psi$ in 10° increments corresponding to energies $E(\varphi,\psi)$ less than 5.0 kcal/mol above the minimum energy. The averaging of $\cos^2 \varphi'$ was carried out separately over ($\varphi,\psi$) corresponding to $0° \leq \varphi' \leq 90°$ and $90° \leq \varphi' \leq 180°$ for use in eq 1.

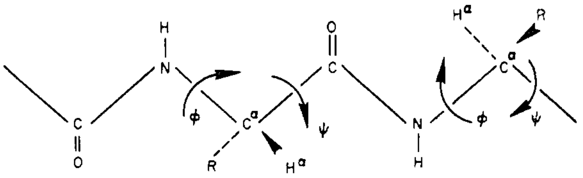

Figure 1. A schematic representation of a portion of a poly-L-peptide in the planar *trans* conformation.

TABLE I
A COMPARISON OF THE CALCULATED AND EXPERIMENTAL COUPLING CONSTANTS $J_{N\alpha}$ OF RANDOM-COIL POLY-L-PEPTIDES

| Polypeptide | Solvent | $J_{N\alpha(\text{exptl})}$, Hz | $J_{N\alpha(\text{calcd})}$, Hz |
|---|---|---|---|
| Poly-L-alanine | 80:20 TFA–$CDCl_3$ | 6.5[b] | 6.1 (6.7)[a] |
| Poly-L-methionine | TFA | 6.3[c]<br>(5.3)[d] | 6.1 (6.7)[a] |
| Poly($\beta$-benzyl L-aspartate) | 30:70 TFA–$CDCl_3$ | 7.0[e] | 6.1 (6.7)[a] |

[a] Calculated according to eq 2. [b] See ref 9. [c] See ref 10. [d] See ref 11. [e] See ref 12.

## Results

A comparison of the calculated vicinal coupling $J_{N\alpha}$ with those observed in solutions of random-coil poly-L-alanine, poly-L-methionine, and poly($\beta$-benzyl L-aspartate) is made in Table I. The values of $J_{N\alpha}$ are obtained from the spacing of the peptide NH doublet. The agreement is within the probable experimental error of the measured couplings and leads to several conclusions. Even though the experimental couplings were obtained under conditions where the random coil poly-L-peptides are extended from their unperturbed conformations by intramolecular excluded volume effects,[18] the agreement with the coupling constant calculated for an unperturbed random-coil poly-L-peptide[7,8] is good. Thus, it appears that the conformation about the N—C$^\alpha$ bond, as manifested by $J_{N\alpha}$, is little affected by the dissolution of the poly-L-peptide in a good solvent[18] which promotes randomly coiled conformations.

The conformational characteristics of this class of random-coil poly-L-peptides are apparently adequately described by the energy calculations of Brant and Flory[7] and Brant, Miller, and Flory.[8] Interactions between the side chains and between side chains and solvent which might influence backbone conformations appear to be negligible,[7] as witnessed by the similarity of vicinal couplings $J_{N\alpha}$ of three random-coil poly-L-peptides with widely differing side chains (see Table I). In addition, both the Karplus[4] (eq 1) and Bystrov, *et al.*[6] (eq 2), relations satisfactorily represent the dependence of the vicinal proton coupling $J_{N\alpha}$ on the dihedral angle $\varphi'$ for this class of random-coil poly-L-peptides.

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