On the existence of *cis/trans* peptide mixtures in poly(*N*-methylglycine)

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(Received 4 May 1976; revised 30 August 1976)

Unperturbed dimensions have been computed for poly(*trans*-*N*-methylglycine), poly(*cis*-*N*-methylglycine) and poly(*cis/trans*-*N*-methylglycine) by a Monte Carlo simulation technique using potential energy calculations. Computed characteristic ratios for the above polypeptides vary within a narrow range supporting the view that both *cis* and *trans* units could be present in the polymer in different proportions under different solvent conditions.

INTRODUCTION

Since Bovey *et al.*[1] made the suggestion from n.m.r. studies that both *cis* and *trans* imide bonds could be present in poly(*N*-methylglycine) (PNMG) in organic solvents, many research workers have tried to interpret this experimental finding in terms of calculations of conformational properties of this polymer molecule. Tanaka and Nakajima[2] made calculations on the unperturbed dimensions of this polymer with all imide bonds in the *trans* configuration and also indicated the possibility of theoretically incorporating *cis* imide bonds in the chain. Warvari *et al.*[3] made Monte Carlo studies assuming the hard-sphere model and *trans* imide bonds. Recently Burgess *et al.*[4] considered this polymer with all *cis* imide bonds by the matrix method and Sisido[5] *et al.* have reported their hard-sphere Monte Carlo studies incorporating *cis* and *trans* imide bonds in the polymer chain. The assumptions made in all these theoretical studies, however, do not correspond to the situation where both *cis* and *trans* imide bonds occur at random in the chain which is suggested by the experimental results.

In the present study we have tried to investigate the conformational properties of random poly(*N*-methylglycine) by a Monte Carlo simulation technique using potential energy calculations. Unperturbed dimensions have been calculated for poly(*cis*-*N*-methylglycine) (PCNMG), poly(*trans*-*N*-methylglycine) (PTNMG) and poly(*cis/trans*-*N*-methylglycine) chains with *cis* and *trans* peptide units distributed at random. The computed results show that the characteristic ratios for PCNMG, PTNMG and poly(*cis/trans*-*N*-methylglycine) chains vary within a narrow range supporting the view that both *cis* and *trans* units could be present in different proportions in the polymer under different solvent conditions.

THEORETICAL TREATMENT

Geometry and energy parameters

A segment of PNMG chain in its fully extended conformation is shown in *Figure 1*. Bond lengths and bond angles used to fix various atoms are indicated in this Figure. The methyl hydrogens were positioned so that one of the C–H bonds eclipses the C′–N bond. The total conformational energy $V(\phi, \psi)$ was taken to be the sum of non-bonded, electrostatic and torsional contributions and was computed using expressions and constants given by Ooi *et al.*[6] and Srinivasan and Rao[7]. The dihedral angles ϕ and ψ were varied in 10° intervals and were at 0° or 180° when computing the conformational energy maps. The convention followed is that of IUPAC–IUB Commission[8]. $V(\phi, \psi)$ maps have been constructed for *cis–cis*, *cis–trans*, *trans–cis* and *trans–trans* dipeptide segments neglecting interactions beyond the dipeptide range as a first approximation.

Monte Carlo method of unit selection

The relative probability of occurrence of a conformation of energy $V(\phi, \psi)$ with reference to the whole (ϕ, ψ) space may be given by:

$$P(\phi, \psi) = \exp[-\beta V(\phi, \psi)] / \sum_{\phi,\psi} \exp[-\beta V(\phi, \psi)] \qquad (1)$$

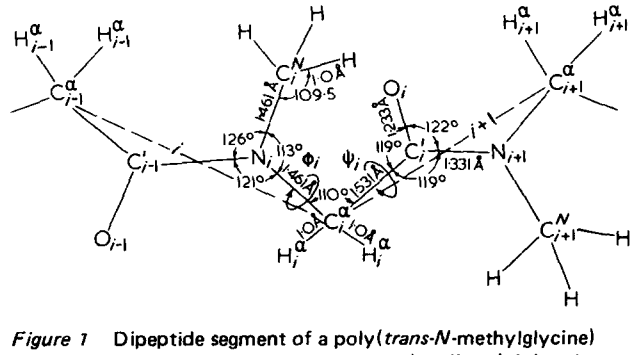

Figure 1 Dipeptide segment of a poly(*trans*-*N*-methylglycine) chain. Virtual bonds are shown by the broken lines joining the successive α-carbon atoms. Bond lengths and bond angles are given. Poly(*cis*-*N*-methylglycine) has the same dimensions but with $C^{\alpha}NC'$ and $C^{N}NC'$ angular values interchanged

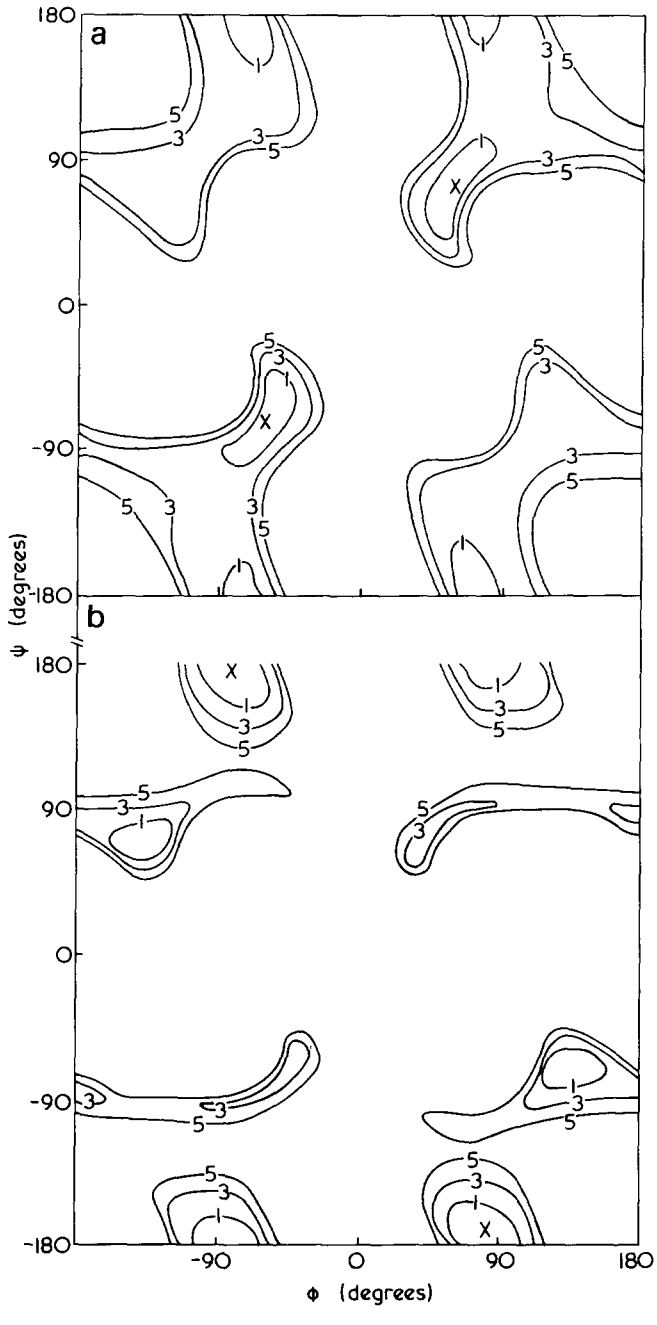

Figure 2 Conformational energy maps for the dipeptide segments of (a) PCNMG and (b) PTNMG. Contours are drawn at intervals of 1 kcal/mol above the minimum, marked X

where $\beta = 1/RT$, R being the universal gas constant and T the absolute temperature. Using the conformational energy $V(\phi, \psi)$ for all the conformations ($\phi = 0°$ to $350°$ and $\psi = 0°$ to $350°$) the corresponding probabilities of occurrence $P(\phi, \psi)$ were computed with the use of equation (1). Then a collection of conformations was obtained for the dipeptide by assigning each conformation a frequency of occurrence of $1000 \times P(\phi, \psi)$ [as many of the $P(\phi, \psi)$ values were found to be very small the values were multiplied by 1000 to obtain whole numbers]. This collection of conformations were then used to generate the random coil polypeptide chains. A chain of N residues was obtained by selecting N conformations at random within the total collection of conformations. The characteristic ratio $C = \langle r^2 \rangle_0 / n\overline{l}^2$ may be computed by generating a large number of such chains; $\langle r^2 \rangle_0$ is the unperturbed mean square end-to-end distance, n the total number of chemical bonds in the chain backbone and $\overline{l}^2$ is the mean square bond length. (N, the total number of virtual bonds in the chain is equal to $n/3$ and $\overline{l}^2$ has the same value for the *cis* and the *trans* units as $\overline{l}^2 = (a^2 + b^2 + c^2)/3$, where a, b, c are the lengths of the bonds C^α–C′, C′–N and N–C^α, respectively. The experimental values quoted in the original articles have been recalculated accordingly for use in this paper.)

RESULTS AND DISCUSSION

The energy maps corresponding to the *cis–cis* and *trans–trans* units are given in *Figure 2*. The *cis–trans* and *trans–cis* maps were found to be very similar to those of *cis–cis* and *trans–trans* maps, respectively, differing only in minor details and hence are not presented here.

Characteristic ratios were computed for PCNMG and PTNMG chains by using the energy maps shown in *Figures 2a* and *2b* and generating the respective polymer chains with 256 residues, by the Monte Carlo method described above (this chain length is justified because our earlier studies[9] have shown that the C_∞ value of PTNMG, reaches its asymptotic value even at low molecular weight).

The 36 x 36 matrix for the conformational energy $V(\phi, \psi)$ was converted to the corresponding 36 x 36 matrix for the probabilities of occurrence $P(\phi, \psi)$ using equation (1), for each of the dipeptide segments. Thus, collections of 986 and 988 conformations were obtained for the *trans–trans* and *cis–cis* units by assigning each conformation a frequency of occurrence of $1000 \times P(\phi, \psi)$. Using the appropriate transformation matrices and the Monte Carlo method of residue selection discussed earlier, 200 chains were generated for the 256-mer of PTNMG and PCNMG. The distributions of the computed C values and the progress of the cumulative average after the generation of every chain for these polymers are shown in *Figures 3a* and *3b*. It is seen from these Figures that the C values for the PCNMG and PTNMG polymers are distributed over a range, varying between 0 and 12 and 0 and 9, respectively. However, the cumulative average fluctuates to start with, and reaches an asymptotic value (3.0 ± 0.5 for PCNMG and 4.5 ± 0.7 for PTNMG), as the number of chains generated increases. It is interesting to see from the curves of *Figure 3* that the cumulative average of C values reaches the steady state even before the generation of about 1000 chains.

To calculate the characteristic ratios of poly(*cis/trans-N*-methylglycine) all the four energy maps constructed for *cis–cis*, *cis–trans*, *trans–cis* and *trans–trans* dipeptide segments of PNMG were considered. The frequency of occurrence for the different conformations in these four segments were then computed. A total collection of 3938 conformations were obtained including 978 conformations for the *cis–trans* segment and 986 for the *trans–cis* case. In generating the poly(*cis/trans-N*-methylglycine) chains, the following procedure has been followed. Two types of chains, one (PCTNMG) with a *cis* imide bond in the first unit and another (PTCNMG) with a *trans* imide bond in the first unit were considered. To start with, the first unit was selected, say a *trans* unit, corresponding to the PTCNMG chain. Then a random number was generated between 1 and 3938. If it corresponded to the *trans–trans* case in the total collection of conformations then a *trans* unit was built as the next unit with the conformation corresponding to the generated random number. A *cis* unit was constructed

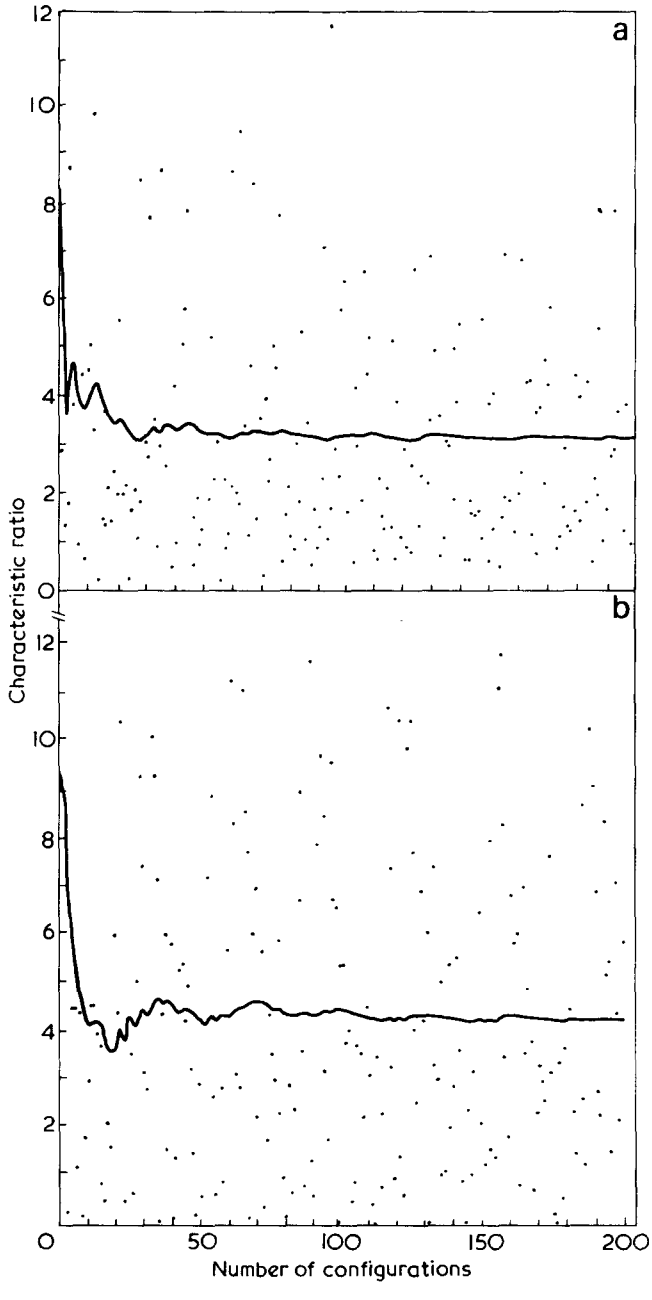

Figure 3 Dependence of characteristic ratio values of a 256-mer random poly(*N*-methylglycine) on the number of configurations used to compute the average. ●, Represents the individual value $\langle r^2\rangle_0/n\bar{l}^2$ for the particular random coil configuration; ——, represents the cumulative average of $\langle r^2\rangle_0/n\bar{l}^2$ as each configuration is generated: (a) PCNMG, (b) PTNMG

in a similar way if the random number corresponded to the *trans–cis* combination. If the random number corresponded to the *cis–cis* or *cis–trans* case, it was rejected and the next random number was generated. In this way a *trans* unit was followed by a *trans* or a *cis* unit and *vice versa*, at random. After the addition of the 256th unit, the chain was terminated and the next one generated. PCTNMG chains starting with a *cis* unit as the first were also generated in a similar way.

The distributions of the characteristic ratios and the progress of the cumulative average at each stage for the PCTNMG and PTCNMG chains are shown in *Figures 4a* and *4b*. It is seen from these curves that chains with the *trans* imide bond in the first unit reach the asymptotic C value of 2.2 ± 0.3 and those with the *cis* imide bond in the first unit, a value of 2.5 ± 0.4. The slight discrepancy between the C values of chains with *trans* and *cis* imide bonds, respectively, in the first unit is within the expected error limit of the present approximate calculations neglecting interaction beyond the dipeptide range. These values are comparatively lower than the values of 3.0 ± 0.5 and 4.5 ± 0.7 obtained respectively for PCNMG and PTNMG cases.

One can approximate the random poly(*cis/trans N*-methylglycine) as a mixture of PCTNMG and PTCNMG chains and the average of the cumulative asymptotic C values of the later two types of chains as the representative average asymptotic C value for the random poly(*cis/trans-N*-methylglycine) polymer. The C value 2.35 thus obtained for the random poly(*cis/trans-N*-methylglycine) is lower compared with that obtained for a completely *cis* polymer and also with that of a completely *trans* polymer and is indicative of the chain adopting a comparatively compact

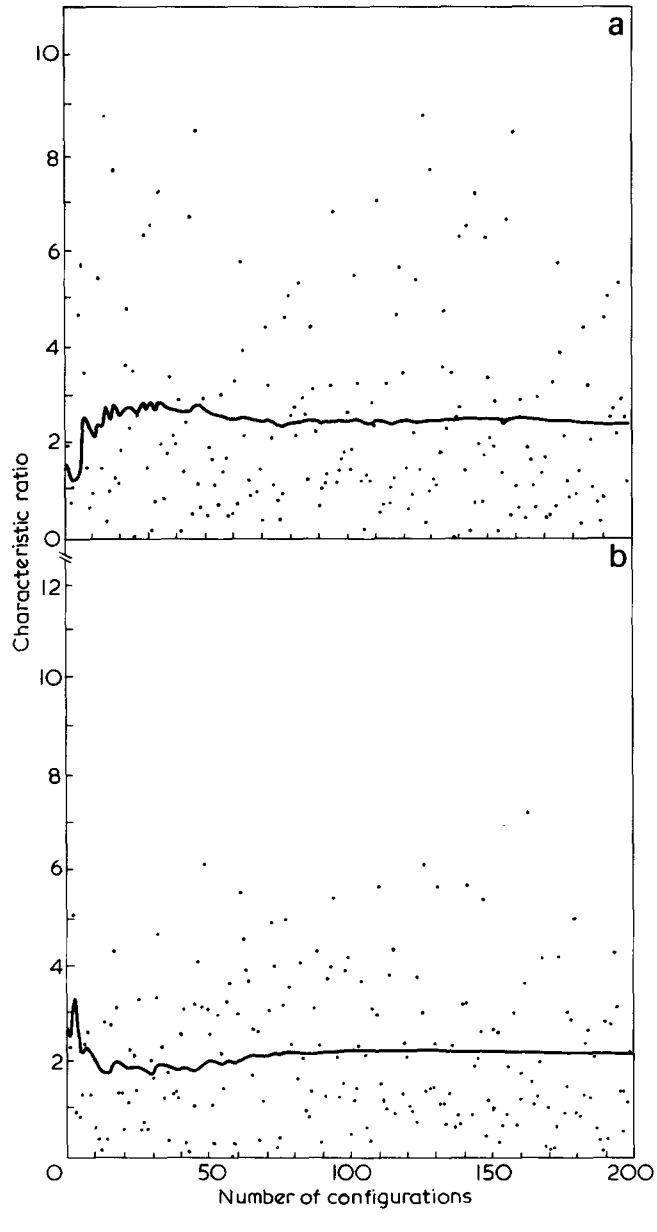

Figure 4 Dependence of characteristic ratio values of a 256-mer random poly(*cis/trans-N*-methylglycine) with *cis* and *trans* peptide units distributed at random. ● and ——, have same meaning as in *Figure 3*. (a) Poly(*cis/trans-N*-methylglycine) chain with a *cis* imide bond in the first unit; (b) poly(*cis/trans-N*-methylglycine) chain with a *trans* imide bond in the first unit

coil structure when it consists of *cis* and *trans* units at random and the compactness is related to the *cis/trans* ratio which could be influenced by external conditions such as different organic solvents as in the case of experiments performed by Burgess *et al.*[4].

Experimental measurements of characteristic ratios for PNMG are available in three different solvents; from the studies of Burgess *et al.*[4] we have values of 2.1 in chloroform, 3.8–5.2 in water and 6.6 in glacial acetic acid; from Tanaka and Nakajima[10] a value of 4.2 in water is obtained (both these groups used the experimental data in water obtained by Fessler and Ogston[11]; however, the former group calculated the value by the method of Brant and Flory[12] while the latter calculated the value by the method of Stockmayer and Fixman[13]). It is to be noted that the characteristic ratio in chloroform is comparatively lower than that in water and that in glacial acetic acid is comparatively higher than that in water. As it has been pointed out earlier[14] and found in the present calculations, the presence of a fraction of *cis* imide bonds markedly decreases the chain dimensions and hence it is likely that the polymer adopts a more compact structure in chloroform giving a higher proportion of *cis* imide bonds, whereas it adopts a comparatively less compact structure in glacial acetic acid, compared to the structure adopted in water. However, all the values of characteristic ratios determined from experimental data have been computed using the virtual bond length of only the *trans* imide units and hence are not directly comparable with the presently calculated results including both *trans* as well as *cis* imide bonds. However, the general trend is in accordance with the results obtained in the present study. It is of interest to experimentally determine the *cis/trans* contents in the above mentioned solvents.

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