Polymer Nuclear Magnetic Resonance Spectroscopy. XV. The Conformation of Polysarcosine

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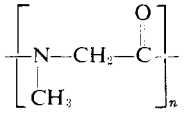
ABSTRACT: The 220-MHz nmr spectrum of polysarcosine (poly-N-methylglycine) in DMSO-d_6 shows eight peaks in the N-methyl region. This is interpreted as indicating that, in contrast to poly-L-proline and poly-N-methylalanine in most solvents, polysarcosine in DMSO-d_6 has nearly equal numbers of *cis*- and *trans*-peptide units. The N-methyl group shielding is sensitive not only to the conformation of the peptide unit to which it is attached, but also to the conformations of the nearest neighboring units. One can thus detect all eight of the possible peptide triad conformational sequences. It can also be shown by nmr spectroscopy that the preferred peptide conformation varies widely with the solvent employed, although a model compound, N-acetylsarcosine methyl ester, is always strongly *trans*.

Among the vast number of studies of poly(α-amino acids) by all possible physical techniques, including nmr,[1-10] comparatively little attention has been given to polysarcosine (poly-N-methylglycine). Probably one



of the principal reasons for this neglect is that polysarcosine (which is isomeric with polyalanine) has no asymmetric carbon atom and hence is not subject to optical rotatory dispersion or circular dichroism studies. The sarcosine unit is present in certain proteins and natural polypeptides but its occurrence is rather rare.

Fessler and Ogston[11] measured the viscosity, sedimentation, and diffusion of polysarcosine in aqueous solution and concluded that it was probably a random coil. Glazer and Rosenheck[12] reached a similar conclusion from a study of its ultraviolet spectrum, and believed that the absence of hydrogen bonding was responsible. This, however, is not an adequate criterion, for it now appears to be established from optical studies and energy calculations that poly-L-proline,[13-17] poly-L-acetoxyproline,[18] and poly-N-methylalanine[19-22] assume helical conformations in solution despite a lack of stabilizing hydrogen bonds. It was therefore of some interest to investigate further the conformation of polysarcosine, which is the simplest polypeptide of this type, and in which steric restrictions could be expected to be the least demanding.

In this paper, it will be demonstrated by high-resolution nmr that in some organic solvents polysarcosine is nearly random with respect to the conformation of the ω bonds,[23] *i.e.*, the peptide units are *cis* or *trans* with nearly equal probability, although they are appreciably influenced by their neighbors' ω-bond conformations. It follows that no regular helical structure can be present.

Experimental Section

Materials. Poly-L-sarcosines of mol wt 6700 (DP 94) and 3500 (DP 49) were obtained from Mann Research Laboratories Inc., New York, N. Y. N-Acetylsarcosine methyl ester was obtained from Cyclo Chemical Corp., Los Angeles, Calif. DMSO-d_6 was supplied by Merck Sharp and Dohme of Canada, Montreal. Other solvents were of reagent grade or showed no evident impurities by nmr spectroscopy.

Methods. Spectra were obtained on a Varian 220-MHz spectrometer, using Wilmad "Imperial" grade sample tubes. Samples were spun at approximately 100 cps, the field homogeneity being adjusted so that side-band intensities

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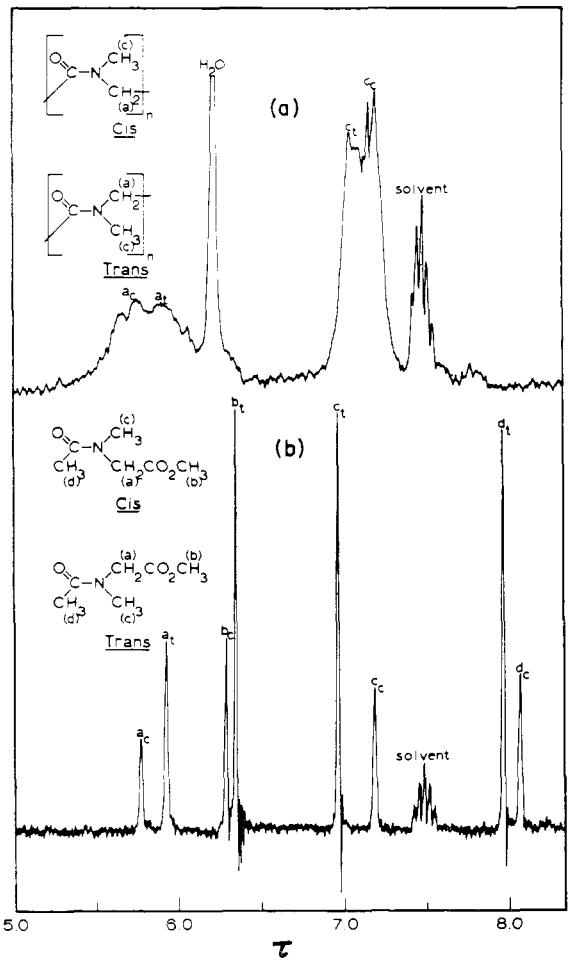


Figure 1. Spectra (60 MHz) of (a) polysarcosine (DP 94) and (b) N-acetylsarcosine methyl ester in DMSO-d_6 (*ca.* 10% w/v) at 35°. The peak assignments are indicated by letters, the subscripts denoting the *cis* and *trans* conformers.

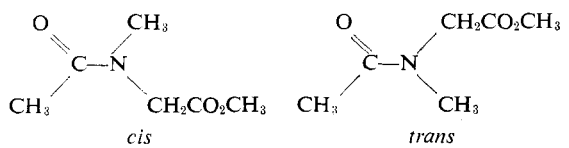
were less than 3% of those of the main peaks. Spectra were also taken at 60 MHz, using a Varian A-60 spectrometer. Spectral peaks were analyzed with the aid of a Du Pont Model 310 curve resolver.

Results and Discussion

In Figure 1 are shown the 60-MHz spectra of polysarcosine of DP 94 (a) and that of the model compound N-acetylsarcosine methyl ester (b), employed to assist

$$CH_3CONCH_2CO_2CH_3$$
$$CH_3$$

in making assignments in the polymer spectrum; both spectra were observed in DMSO-d_6 at *ca.* 35°, using 2% tetramethylsilane as internal reference. (The multiplet at τ 7.47 is due to residual DMSO-d_5.) In the model compound spectrum, it is observed that all the peaks are doubled, giving a total of eight, although there would at first sight appear to be only four different kinds of protons. The reason for this of course is that the compound can exist in both *cis* and *trans* conformations.



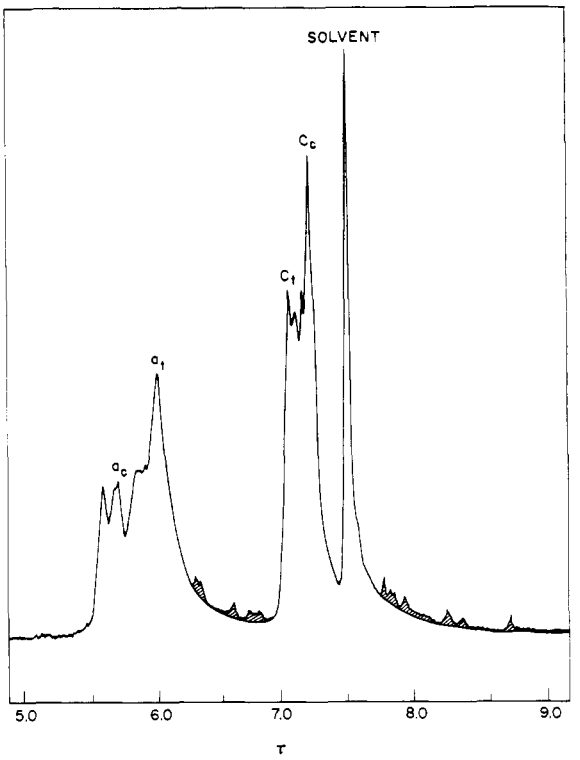


Figure 2. Spectrum (220 MHz) of polysarcosine (DP 94) in DMSO-d_6 at 35°. (The small cross-hatched peaks are spinning side bands; H_2O was more effectively excluded here than in Figure 1.)

(These are defined in the sense appropriate to the polymer chain.) The exchange between them is slow on the nmr time scale at the observing temperature, and so each conformer gives sharp peaks. The assignments are as given in Figure 1, and are based on the results of Anet and Bourn,[24] who showed by means of the nuclear Overhauser effect that in N,N-dimethylformamide, the most shielded methyl group is that *cis* to the carbonyl group. It can be seen from the relative intensities of all peaks in b that the *trans* conformer is preferred by about 2.5:1 in DMSO-d_6. (Relative *cis* and *trans* peak heights vary slightly for the a, b, c, and d protons because of differential broadening by weak, unresolved coupling.) The *trans*/*cis* ratio varies somewhat in other solvents (methanol-d_4, $CDCl_3$, trifluoroacetic acid, trifluoroethanol, and pyridine), but the *trans* is always strongly preferred.

Turning to the polymer spectrum a, we find the N-methyl and methylene peaks seemingly much broadened. (There are of course no peaks corresponding to the b and d protons of spectrum b.) The N-methyl region shows clear evidence of more than two peaks; the methylene region also shows this higher multiplicity, but is more difficult to interpret. It becomes better resolved in the 220-MHz spectrum (Figure 2).

In Figure 3, the N-methyl multiplet of Figure 2 is expanded fivefold. One can now distinguish seven peaks by inspection; analysis of the multiplet with the Du Pont analyzer (see Experimental Section) makes it clear that there must be at least one additional peak to

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Figure 3. Expanded 220-MHz N-methyl spectrum of polysarcosine under the same conditions as for Figure 2. The "stick" spectrum is the result of an analysis of the experimental band intensities (see text).

match the observed spectrum. A set of peak positions and relative intensities (*i.e.*, areas) which matches the observed spectrum within the probable experimental error is indicated as "sticks" beneath the spectrum.

It is believed that these peaks reflect the fact that N–CH_3 shielding is not only a function of the ω-bond conformation of the peptide unit to which it is attached but also of the conformations of the nearest neighbors as well. We can then recognize eight species of peptide triad sequences: *trans–trans–trans*, *trans–trans–cis*, *cis–trans–trans*, *cis–trans–cis*, *cis–cis–cis*, *cis–cis–trans*, *trans–cis–cis*, and *trans–cis–trans*. If the chain were truly random with respect to this bond the spectrum would consist of eight equal peaks. This clearly is not the case. Yet if we assume that the four upfield peaks correspond to sequences with *cis* central units and the four downfield peaks to sequences with *trans* central units, it is found that the probabilities of *cis* and *trans* units are nearly equal, the *trans*/*cis* ratio being *ca.* 0.9. This contrasts to the strong *trans* preference of the model compound.

It is difficult to make specific triad peak assignments. It appears that certain triad sequences tend to be favored and others excluded, the energy differences being as great as *ca.* 1 kcal in some cases. Thus, not surprisingly the ω-bond conformation of a peptide unit influences that of its neighbors to some degree. Molecular models do not suggest severe steric limitations for any of these sequences, and so it will be of interest to see what potential energy calculations predict. These have not yet been carried out. One might conjecture that the *cis–cis–cis* and *trans–trans–trans* conformations would have the lowest potential energy, since the latter appear to be exclusively preferred for poly-N-methyl-L-alanine[20–22] and poly-L-proline can exist in both conformations, depending upon the solvent.[25–29]

The ω-bond conformational preference of polysarcosine varies markedly with solvent. All measurements were made at 35°. In methanol-d_4 the spectrum is similar to that in DMSO-d_6. In $CDCl_3$, the *cis* conformation is strongly preferred. In pyridine, the *trans* conformation is of somewhat lower energy. In trifluoroacetic acid, trifluoroethanol, and water the *trans* conformation is quite strongly preferred. However, in all these solvents, fine structure due to neighboring peptide conformations is either more difficult to discern than in DMSO-d_6, or is entirely absent.

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