N-Substituted Poly(α-amino acids). 2. Conformational Properties of Poly(γ-ethyl N-methyl-L-glutamate) in Various Solvent Mixtures

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ABSTRACT: The conformational properties of poly(γ-ethyl N-methyl-L-glutamate) have been investigated under various experimental conditions, using ^{1}H NMR and CD spectroscopy. It was found that in a number of solvents including water, trifluoroethanol, and trifluoroacetic acid, poly(γ-ethyl N-methyl-L-glutamate) assumes an extremely stable helical conformation, whose chiroptical properties are very close to those of poly(N-methyl-L-alanine). Computer-simulated model studies have been carried out assuming the ϕ and ψ dihedral angles of the poly(N-methyl-L-alanine) helix to be 30 and 240°, respectively, according to 1966 convention. The structure reveals a hydrophobic groove which in aqueous solution could be partly responsible for the intrinsic conformational stability. Side chains are well separated from one another in the structure, and interactions among them appear unlikely.

In a previous paper,[1] we have reported the synthesis and some preliminary results on the characterization of N-methyl derivatives of poly(γ-methyl L-glutamate) and poly(γ-ethyl L-glutamate). On the basis of the optical rotatory properties, it was concluded that in TFE solution both polymers do assume an ordered helical conformation. Comparison with the chiroptical properties of poly(N-methyl-L-alanine) led to the tentative hypothesis that the structure in the solution of the N-methyl derivatives of poly(L-glutamic acid) methyl and ethyl esters is that of a right-handed helix whose ϕ and ψ dihedral angles are 30 and 250°, respectively.[2] The configuration at the peptide bonds has been assumed to always be trans. In the case of N-substituted poly(α-amino acids), it is well known that solvent-induced conformational transitions occur through isomerization of peptide bonds.[3,4] Besides the classical example of the helix I–helix II transition of $[\text{L-Pro}]_n$, there are cases in which a random distribution of cis and trans configurations of peptide bonds along the chain induces a disordered conformation of the whole polypeptide molecule.[5] Goodman and co-workers[6] have shown that a conformational transition of poly(N-methyl-L-alanine) from a right-handed helix with all-trans peptide configurations to a random chain occurs in the presence of TFA. In 1:1 TFA–TFE mixtures, there is approximately a 1:1 ratio of cis and trans configurations along the chain. However, complete isomerization to an all-cis configuration has never been achieved with poly(N-methyl-L-alanine).

In the present paper, we have extended conformational studies to poly(γ-ethyl N-methyl-L-glutamate) in various solvent mixtures in the attempt to obtain more information on the conformational stability of the helical structure of this new polymer. The present investigation has been carried out mainly by using CD and NMR techniques.

Experimental Section

Materials and Solvents. High molecular weight poly(γ-ethyl N-methyl-L-glutamate) was synthesized according to our previous papers.[1] The percent of N-methylation was evaluated to be 94%, and the percent of trans esterification to the methyl ester was 13% by the methods previously reported.[1] The last figure has also been confirmed by ^{1}H NMR measurements of the peak areas relative to the OCH_3 and OCH_2CH_3 resonances.

Reagent grade concentrated sulfuric acid, 2,2,2-trifluoroethanol (TFE), deuteriochloroform, trifluoroacetic acid (TFA), butanesulfonic acid, triethylamine (Et_3N), LiCl, $CaCl_2$, and KBr were used throughout our experiments.

Measurements. Proton NMR measurements were performed on a Varian HR-220 spectrometer. All chemical shifts are given in ppm from the internal standard (Me_4Si). CD measurements were carried out with a Cary 61 recording spectropolarimeter. Fused quartz optical cells with Suprasil windows were used. IR measurements were performed on a Perkin-Elmer 580 spectrometer in the solid state, using KBr disks.

Results

Measurements in Trifluoroacetic Acid and Chloroform. CD and NMR measurements on poly(γ-ethyl N-methyl-L-glutamate) have been carried out in pure TFA in order to find out if the acid provokes isomerization of peptide bonds and therefore a conformational change in the polymer. The CD spectrum of the polymer is shown in Figure 1; the CD pattern recorded in TFE is also reported for comparison. The two spectra overlap completely, thus indicating that even in a strongly denaturing solvent such as TFA the helical structure remains stable. This result is rather surprising and leads to the conclusion that the helical form of poly(γ-ethyl N-methyl-L-glutamate) is remarkably more stable than that of poly(N-methyl-L-alanine). This conclusion has been further supported by NMR experiments carried out in $CDCl_3$ and in $CDCl_3$–TFA mixtures. In $CDCl_3$, the CD pattern is identical with that shown in Figure 1, implying that the polymer exhibits the same conformation in this solvent as in TFE or TFA. The proton NMR spectrum in $CDCl_3$ exhibits a single N–CH_3 peak at 2.97 ppm, confirming that only one peptide configuration is present (Figure 2). The chemical shift of N-methyl protons corresponds to that observed for the trans configuration of poly(N-methyl-L-alanine) and of the model compound N-acetyl-N-methyl-L-alanine methyl ester in the same solvent.[6] In pure TFA, a single N–CH_3 peak is once again observed, slightly shifted downfield with respect to that found in $CDCl_3$. A shift in the same direction of the N–CH_3 trans peak has also been observed for poly(N-methyl-L-alanine)[6] and is due to a solvent effect.

Measurements in Sulfuric Acid–TFE Mixtures. In the attempt to provoke trans-cis isomerization of the peptide bonds in the polymer, we used sulfuric acid as the solvent.[7] We could not obtain meaningful CD spectra in H_2SO_4, since a rapid and extensive chain cleavage takes place in this solvent. Evidence of acid-induced chain cleavage has been obtained by the fact that slightly water-soluble poly(γ-ethyl N-methyl-L-glutamate) is

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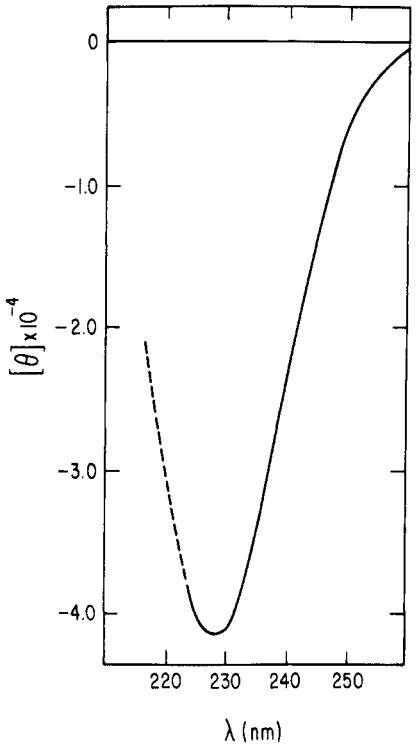


Figure 1. CD spectrum of poly(γ-ethyl *N*-methyl-L-glutamate) in TFA. The dashed line represents the corresponding CD spectrum in TFE. The remaining cruve in TFE is identical with the curve found in TFA.

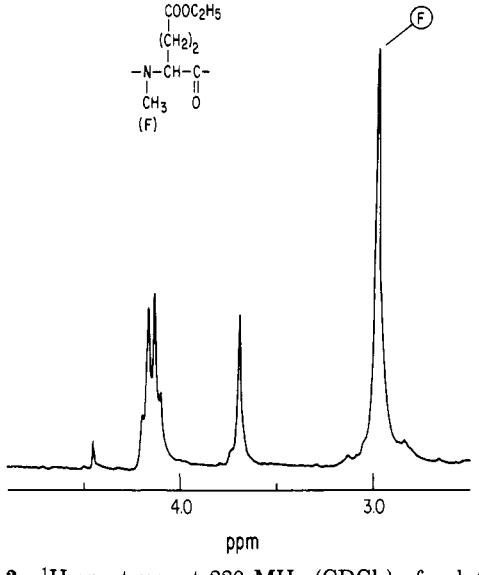


Figure 2. ^1H spectrum at 220 MHz ($CDCl_3$) of poly(γ-ethyl *N*-methyl-L-glutamate). The peak at 2.97 ppm is due to the N-CH_3 group.

rapidly converted into water-soluble fragments by sulfuric acid. The fragments dialyze completely through a membrane with a 3500 molecular weight cutoff.

Measurements in Aqueous Solutions. As we reported in our previous paper, the polymer is slightly soluble in water; the CD pattern is identical with that observed in TFE except for a small shift of the negative band. This is again consistent with the assignment of this band, at least in part, to an $n \rightarrow \pi^*$ transition.[8]

We also did not observe dramatic spectral changes in concentrated salt solutions. Addition of $CaCl_2$ up to 5 M causes only a small (≃20%) increase in the intensity of the 226-nm negative band but no frequency shift. In line with our findings in organic solvents (TFA, TFE, and $CDCl_3$), we can conclude that the ordered conformation of poly(γ-ethyl *N*-methyl-L-glutamate) is also rather stable in aqueous solutions. Further support to the above statement arises from CD measurements at high temperatures in TFE and in water. No significant spectral change is observed on raising the temperature to 75 °C in both cases.

Discussion and Conclusions

The results presented in this paper have shown that the all-trans conformation of poly(γ-ethyl *N*-methyl-L-glutamate) is stable in a number of solvents, including TFA. Addition of a strong acid such as H_2SO_4 to polymer solutions in TFE causes rapid chain cleavage of the *N*-methyl peptide chain. In no case have we been able to observe isomerization of peptide bonds with consequent conformational changes.

As we also pointed out in the previous paper,[1] there are small differences between the CD spectra of poly(*N*-methylalanine) and poly(γ-ethyl *N*-methyl-L-glutamate). We note, in fact, that the band intensities are lower, and the whole spectrum is shifted by 5 nm in the case of the *N*-methylalanine polymer. We have already suggested that one of the sources of these differences could be the different molecular weights of the polymer samples used in the two cases.[1] Actually, it could also be that the two structures are not exactly identical. From theoretical calculations carried out by Mattice,[9] it turns out that the conformational free-energy minima are very sensitive to the orientation of *N*-methyl and α-carbon substituents. Different side chains might have different orientations, which in turn could lead to conformational free-energy minima with a slightly different set of ϕ and ψ dihedral angles. Again from calculations, we learn that a change in the dihedral angles of the order of 10° accounts for the observed spectral variation. The remarkable CD spectral variation of $[L\text{-Pro}]_n$ II in water and in TFE solution has been interpreted as being due to changes of the ψ angles of the order of 10°.[10]

From these considerations, we might conclude that the polymers of γ-ethyl *N*-methyl-L-glutamate and *N*-methylalanine have by and large the same conformation in TFE, perhaps with small differences in the ϕ and ψ dihedral angles.

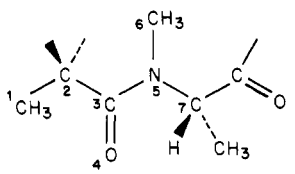
In the light of the results presented in this work, we can summarize briefly what is known at the present time on the conformational properties of N-substituted polypeptides (Table I). The simplest polymer of the series is $[Sar]_n$ which is not able to form any ordered structure in solution. The trans-peptide configuration, as shown by NMR experiments, appears to be quite strongly preferred in TFE.[5] The next polymer of the series, namely poly(*N*-methyl-L-alanine), forms the all-trans, right-handed helix described by Goodman and co-workers.[2] The next two terms of the series, namely poly(γ-methyl *N*-methyl-L-glutamate) and poly(γ-ethyl *N*-methyl-L-glutamate), formed an ordered helical structure in solution, which, on the basis of the CD properties, can be assumed to be very similar to, even if not exactly identical with, that of the *N*-methylalanine polymer. In general, in TFE we observed an increasing conformational stability toward denaturation by TFE on going from the first to the last polymer of the series. Stability also increases in water toward the action of ions such as Li^+, Ca^{2+}, and H^+. When discussing the helix stability of different polymers, we must face the problem of comparing samples of nearly equal degree of polymerization. This is not the case of the polymers listed in Table I; however, the molecular weights appear high enough to make the comparison of the relative

Table I
Conformation of N-Substituted Polypeptides in TFE

polymer	structural unit	peptide config	conformation
$(Sar)_n$	$-N(CH_3)CH_2CO-$	cis and trans	random
$(N$-methyl-L-alanine$)_n$	$-N(CH_3)CH(CH_3)CO-$	trans	right-handed helix; $\phi = 30°$; $\psi = 240°$
(γ-methyl L-glutamate)$_n$	$-N(CH_3)CH[(CH_2)_2COOCH_3]CO-$	trans	right-handed helix; $\phi = 30°$; $\psi = 240°$
(γ-ethyl L-glutamate)$_n$	$-N(CH_3)CH[(CH_2)COOC_2H_5]CO-$	trans	right-handed helix; $\phi = 30°$; $\psi = 240°$

helix stability still significant. A different approach to the problem will be the determination of the critical chain length required to form the helix in each case. Work is in progress in this direction.

In the attempt to suggest an explanation for the increasing stability in the polymer series, let us consider in more detail the model of the *N*-methyl-L-alanine polymer poposed by Goodman and co-workers.[2] When the ψ angle is 240° (or 60°, according to the most recent IUPAC convention), the carbonyl oxygen atom and the C_α methyl group are exactly in the cis position. Since the peptide configuration is always trans, it turns out that the C_α-methyl substituent of a given residue and the *N*-methyl substituent of the subsequent residue along the chain are as far as possible from one another. This is clearly shown below.



Actually, the ψ values arising from theoretical energy calculations are 250° (Goodman) and 245° (Mattice). Both values are very close to the value of 240° which defines the steric situation shown in the structure. This also implies that the 7 atoms labeled in the structure are thereby forced to stay in a plane. A computer-drawn stereo view of the structure is shown in Figure 3. In the central portion, the 7-atom plane perpendicular to the plane of the paper is evident.

The relative positions of the *N*-methyl and C_α substituents of the same residue, with the suggested value of 30° (150°) for the ϕ dihedral angle, are eclipsed with respect to each other, with the hydrogen atom of the C_α shown slightly out of the plane previously defined.

The interesting aspect of this structure is the presence of a pronounced nonpolar helical groove, along which the C_α-methyl and the *N*-methyl substituents interact rather strongly. The space-filling model and the computer-simulated model studies suggest that methyl–methyl interactions could be one of the forces responsible for conformational stability. The nonpolar region appears more crowded if the C_α-methyl substituent is replaced by the CH_2CH_2COOEt group. There are strong interactions between the C_α and C_β methylene groups and the *N*-methyl group, and this could explain why the conformational stability increases from sarcosine to *N*-methylalanine and to γ-ethyl *N*-methyl-L-glutamate polymers. Obviously, hydrophobic interactions could account for conformational stability only in aqueous media or in polar-organized solvents.

From computer-simulated model studies, we also learn that the side chain groups are well away from one another. It is therefore unlikely that side chain–side chain interactions can contribute to the stability of the helical structure. In other words, there is an intrinsic stability of the polymer backbone due to NCH_3 and C_β methylene interactions. On this basis, it is also possible to predict that poly(*N*-methylglutamic acid) will assume in aqueous solution the same conformation as the parent ethyl ester

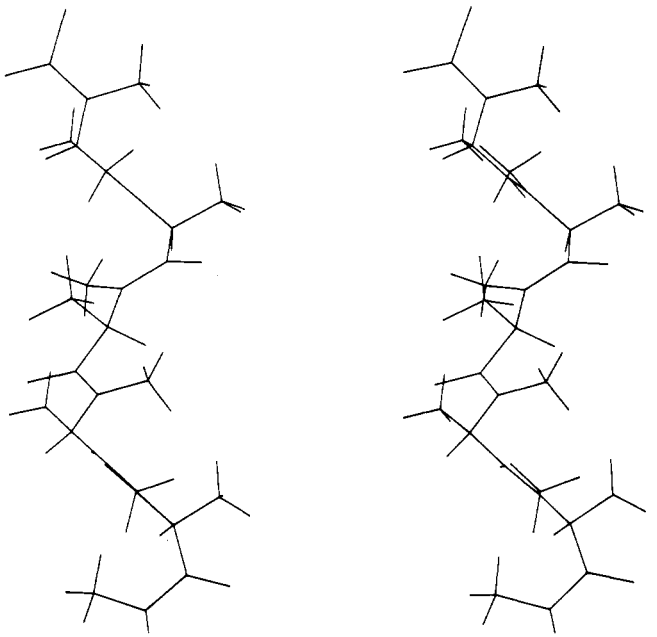


Figure 3. Computer-drawn stereo view of the structure of poly(*N*-methyl-L-alanine) on assuming ϕ and ψ dihedral angles of 30 and 240°, respectively.

and that the structure should be rather insensitive to the ionization of side chain carboxylate groups.

Interestingly, ordered conformations containing all-cis peptide bonds have not been detected in any of the systems investigated thus far. In N-substituted poly(glycines), the cis configuration becomes increasingly preferred on increasing the bulkiness of N substituents from ethyl to propyl and butyl groups.[3] Apparently, when N substituents and α-carbon substituents such as methyl groups or larger are simultaneously present, the formation of ordered structures with the cis peptide configuration seems to be prevented by steric interference between the substituents.

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