SENSITIVE CRITERIA FOR THE CRITICAL SIZE FOR HELIX FORMATION IN OLIGOPEPTIDES

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Abstract.—We studied the conformation of a series of γ-ethyl-L-glutamate oligopeptides by circular dichroism and 220 MHz nuclear magnetic resonance spectroscopy. By use of the first technique we noted enhancement of the $n \rightarrow \pi^*$ and splitting of the $\pi \rightarrow \pi^*$ transitions commencing with the heptamer in trimethylphosphate and trifluoroethanol. With the second method we found changes in chemical shifts for the amide protons consistent with the onset of helicity at the heptamer in the solvents noted above. When DMSO-d_6 is used as a solvent, no such chemical shift changes occur because the oligopeptides do not assume helical conformations.

Far-ultraviolet circular dichroism[1–3] and 220 MHz nuclear magnetic resonance[4–7] have been applied to the conformational analysis of polypeptides and proteins. We wish to report a definitive application of these techniques to the problem of the critical size for helix formation in oligopeptides. These measurements provide a more direct assessment of conformation than is possible by studying far ultraviolet absorption and optical rotatory dispersion spectra.[8–15] The circular dichroism spectra for a series of carbobenzoxy-L-glutamate oligomers in trimethylphosphate are shown in Figure 1.[16] Our results demonstrate the existence of a critical size for helix formation below which the circular dichroism spectrum is composed of broad weak bands at about 235 mμ, weak positive bands at about 218 mμ and a relatively strong negative band below 200 mμ. The low wave-length band is due to the $\pi \rightarrow \pi^*$ transition of the amide chromophore.

We attribute the very weak band above 230 mμ (for the dimer through hexamer) to the carbobenzoxy group in agreement with the findings of Weinryb and Steiner who studied carbobenzoxy dipeptides.[17] The weak positive band at about 218 mμ most probably arises from an $n \rightarrow \pi^*$ transition of the amide group in non-helical conformations.

Above the critical size for the onset of helicity the circular dichroism bands change dramatically (Fig. 1) and are substantially like spectra of α-helices. The first negative band at 222 mμ is therefore assigned to the $n \rightarrow \pi^*$ transition while the negative band near 205 mμ and the large positive band below 200 mμ result from the exciton splitting of the $\pi \rightarrow \pi^*$ transition of the peptide groups in a helical array.

A sharp increase in molar ellipticity occurs with the onset of helicity for the $n \rightarrow \pi^*$ transition in addition to the above noted spectral shifts (Fig. 1). A plot of molar ellipticity at 222 mμ versus number of residues in the peptide chain

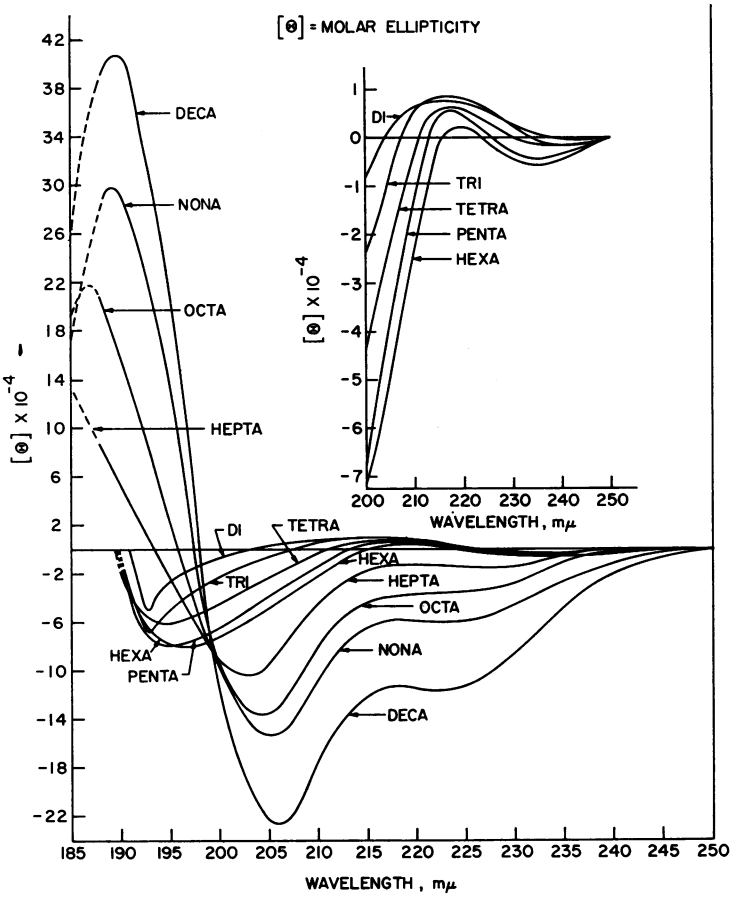


FIG. 1.—Circular dichroism spectra of *N*-carbobenzoxy-γ-ethyl-L-glutamate oligomers in trimethylphosphate as a solvent. Values for the molar ellipticity of each oligomer are recorded.

is shown in Figure 2. The increase in slope provides obvious support for the onset of helicity.

With a DuPont 310 Curve Resolver[18] we were able to resolve the circular dichroism curves for the helical oligomers into three gaussian bands centered at 222 mμ (negative), 206 mμ (negative), and 190 mμ (positive). Table 1 contains our results on the percentage contribution for these bands. It is clear that the contribution of the $n \rightarrow \pi^*$ band increases substantially but not abruptly as one passes through the critical region for helix formation. The appearance of splitting for the $\pi \rightarrow \pi^*$ transition seen in Figure 1 and the criteria of helicity based on the $n \rightarrow \pi^*$ transition are totally consistent with each other.

We have also examined these oligomers with the aid of 220 MHz nuclear magnetic resonance. The N-H protons are extremely valuable in deducing conformational changes since they are intramolecularly hydrogen-bonded in helical structures. Shielding of these protons depends on the peptide conformation and

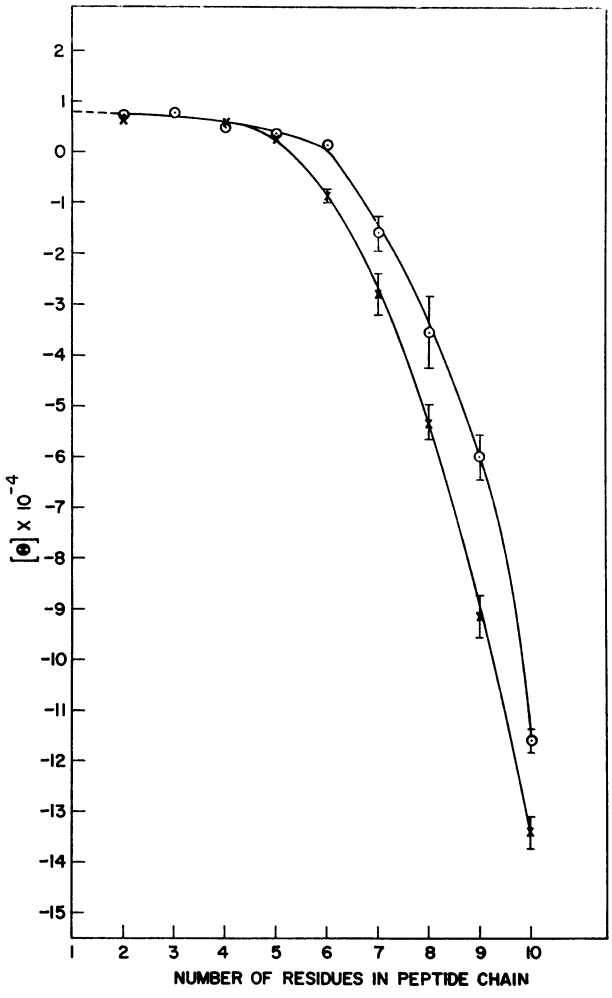


FIG. 2.—Plot of molar ellipticity at 222 mμ versus number of residues in peptide chain for *N*-carbobenzoxy-γ-ethyl-L-glutamate oligomers in trimethylphosphate (⊙) and in trifluoroethanol (×).

TABLE 1. *Relative contributions of the peptide electronic transitions to the circular dichroism spectrum of L-glutamate oligomers in trifluoroethanol[a] and trimethylphosphate[b].*

Wavelength of resolved Gaussian curves (mμ)	Area (%) of the resolved bands								
	Hexamer	Heptamer		Octamer		Nonamer		Decamer	
	a	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
222	9.8	10.1	10.1	21.5	18.4	22.3	19.1	26.3	20.2
206	55.2	50.8	50.5	33.0	35.9	33.3	32.4	31.6	32.8
190	34.8	39.1	39.4	45.5	45.8	44.5	48.5	42.0	47.0

other environmental factors.[19–22] Figure 3 contains the N-H and phenyl proton regions of the nuclear magnetic resonance spectrum for the carbobenzoxy glutamate hexamer in trifluoroethanol.[23] The aromatic protons from the amine blocking group provide a convenient internal standard to assign the chemical shifts for the magnificently resolved amide protons of the hexamer and the other oligomers seen in Figure 4.

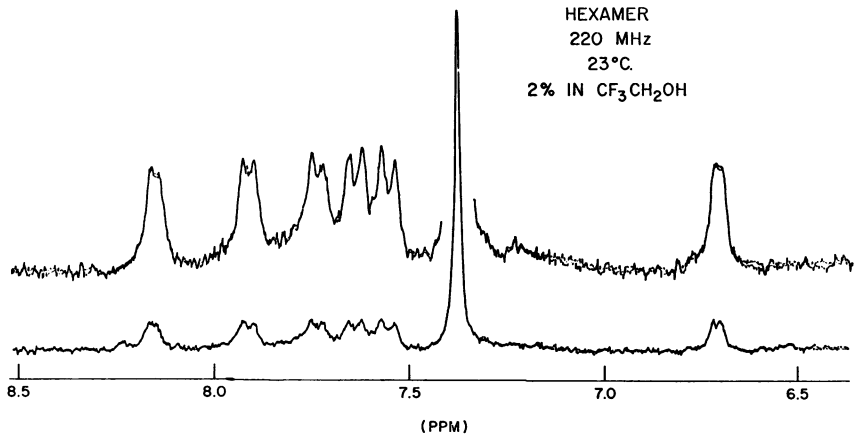


FIG. 3.—Partial 220 MHz nmr spectrum for the *N*-carbobenzoxy-γ-ethyl-L-glutamate hexamer as a 2% solution in trifluoroethanol at 23°. This spectrum shows the amide and phenyl protons. The upper spectrum shows the resonances of the lower spectrum at higher gain.

It is interesting to note that resonances of the amide protons are nearly all resolved for the N-H groups of the dimer through the hexamer. For the heptamer of higher peptides coincidences are common for internal N-H resonances.

From model compound studies, we assigned the lowest and highest field peaks to the NH groups of N and C terminal residues, respectively. The resonances for these terminal residues show a downfield shift with chain length as can be seen from Figures 3 and 4. Figure 5 presents this dependence as a plot of the relative chemical shifts as a function of number of residues in the oligomer. The most substantial change in frequency occurs between the dimer and the hexamer. We interpret these results as arising from the interconversion of folded and extended forms for the oligopeptides. For the dimer, conformational alterations do not materially affect the geometric relationship of the two amide groups. From the trimer to the hexamer alterations of the θ and ψ internal rotation angles most dramatically alter the proximity of the N and C terminal amide groups. As a result, a substantial change in environment is felt by these terminal groups. The equilibrium among all these conformations must be rapidly established on a nuclear magnetic resonance time scale (less than about 10^{-2} sec.) since we observe only a single, averaged one resonance for each amide proton. Commencing with the heptamer, stable helical structures exist which cause specific ϕ and ψ angles to be cooperatively preferred. The terminal groups are conformationally frozen with respect to each other.

These results suggest that nuclear magnetic resonance offers us a tool to study conformational preferences of peptides and proteins irrespective of the over-all helicity of the structure. If our explanation for the nuclear magnetic resonance results is correct, no change in chemical shift should be observed for the oligomers if the peptides are solvated to the extent that they do not form folded and stable helical forms. We were able to confirm this by 100 MHz spec-

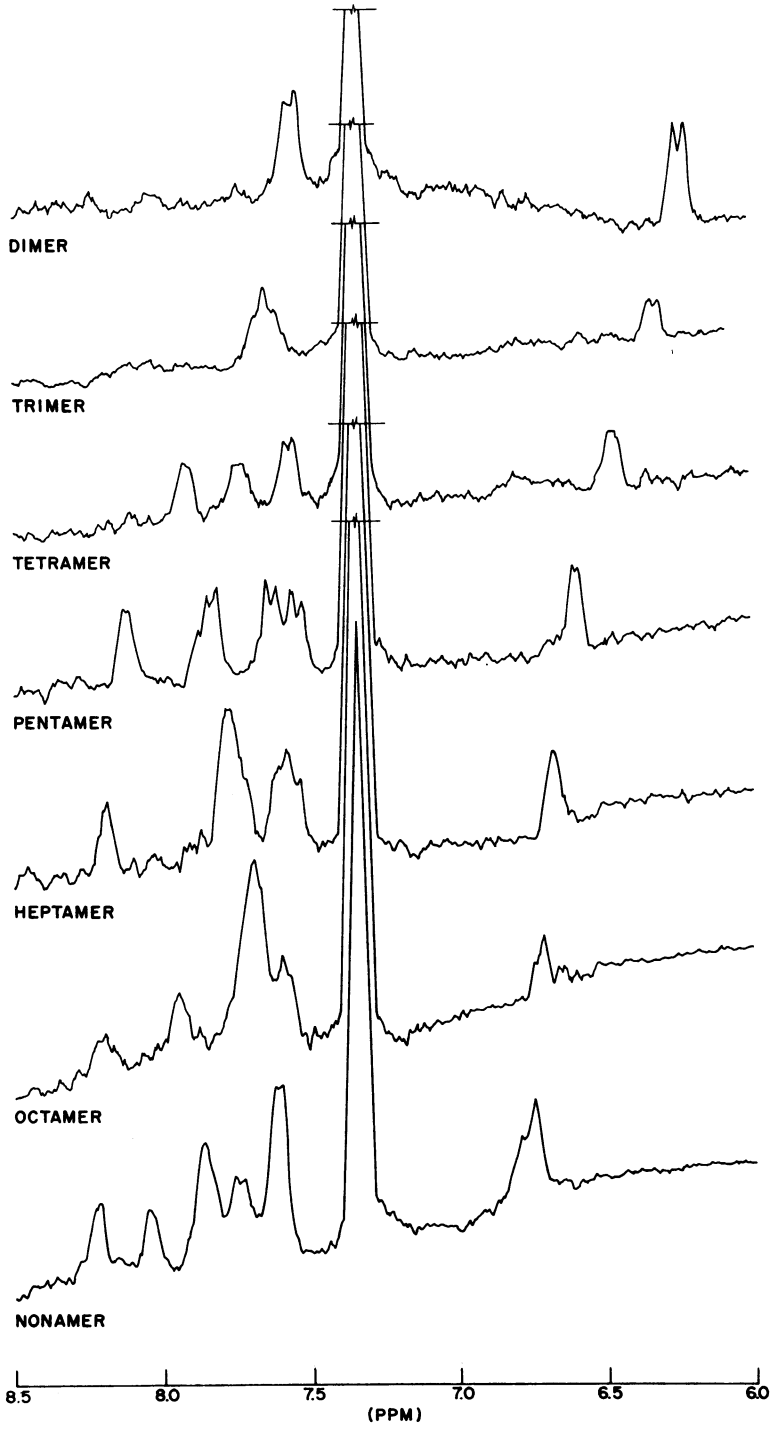


FIG. 4.—Partial 220 MHz nmr spectra of the *N*-carbobenzoxy-γ-ethyl-L-glutamate oligomers as 2% solutions in trifluoroethanol at 23°. These spectra cover the regions of the amide and aromatic proton resonances.

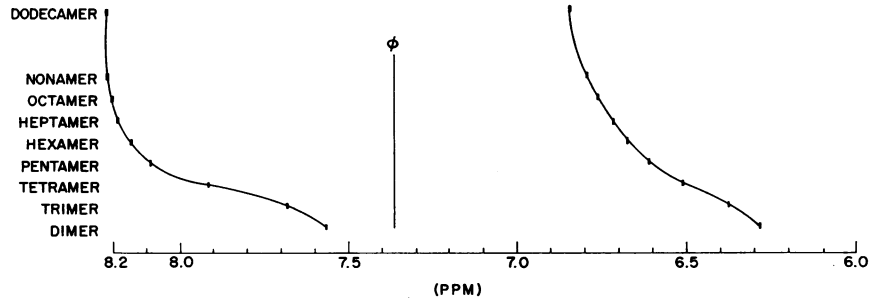
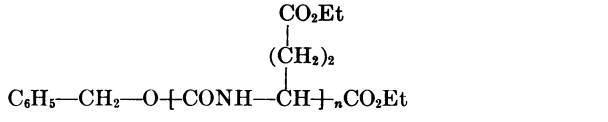


FIG. 5.—Chemical shift dependence of the highest and lowest field N-H resonances as a function of the number of residues in the peptide chain (220 MHz in CF_3CH_2OH at 23°C).

TABLE 2. *Amide proton chemical shifts for L-glutamate oligopeptides DMSO-d_6 at 23°C and 100 MHz (in ppm).*



		Residues*	
n	N-terminal	Internal	C-terminal
2	8.30(1)	—	7.42(1)
3	8.27(1)	8.03(1)	7.45(1)
5	8.27(1)	ca. 8.04(3)	7.45(1)
7	8.27(1)	ca. 8.00(5)	7.48(1)
8	8.27(1)	ca. 8.00(6)	7.48(1)
9	8.25(1)	ca. 8.00(7)	7.42(1)

* Relative intensities are shown in parentheses.

tra for the glutamate series in DMSO-d_6 as a solvent. Table 2 contains our results which clearly show that no significant change in chemical shift occurs for N-H's of given residues of the oligomer series. To substantiate our claim that DMSO does not support helical structure for these oligomers, we measured the values for the derived constants b_0 (Moffitt-Yang equation) which remain near zero for the entire oligomer series. We used the Moffitt-Yang constant because DMSO is opaque in the peptide chromophore absorption region.

In conclusion, we obtained a picture of critical size for helicity from circular dichroism and nmr spectra for the L-glutamate oligopeptides. Both techniques clearly indicate the onset of helicity at about the heptamer for these peptides in solvents such as trifluoroethanol and trimethylphosphate.

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