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Conformations of Alanine Oligopeptides in Solution[1]

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The conformations of four series of alanine oligomers containing solubility-enhancing blocking groups were investigated using circular dichroism (CD) in trifluoroethanol (TFE), hexafluoroisopropanol (HFIP), and TFE–sulfuric acid, TFE–water mixtures. These studies show that alanine oligomers may exist in α-helical, β-associated, or disordered conformations, depending upon the solvent.

The β-conformation, which appears in the higher oligomers, could be disrupted by dilution or by the addition of small amounts of sulfuric acid (1 %). A mixture of 99 % TFE–1 % H_2SO_4 was found to be helix supporting and was used to determine a critical size for helix formation. Studies in TFE–water suggest that the higher oligomers should exist in a β-conformation in water.

INTRODUCTION

Studies from our laboratories on alanine oligopeptides reported earlier were limited by the insolubility of the peptides in helix-supporting solvents (*1*). In order to increase the solubility of these materials, we synthesized four series of L-alanine oligomers with different blocking groups.

Z–$(Ala)_n$–OEt	$n = 2$–7
MEEA–$(Ala)_n$–OEt	$n = 3$–7
MEEA–$(Ala)_n$–MO	$n = 3, 5$–9
Z–$(Ala)_n$–MO	$n = 2$–9

where MEEA = 2-Methoxy-[2-ethoxy-(2-ethoxy)]acetyl; Mo = morpholine amide; Z = benzyloxycarbonyl; OEt = ethyl ester.

The synthesis of these oligomers is described in the paper immediately preceding this report (*2*). We found that significant increases in solubility were realized through the use of these solubilizing groups. Earlier studies in other laboratories used peptide-derived blocking groups to enhance the solubility of alanine oligomers and polymers (*3*, *4*). These studies were complicated by the possible influence of the blocking groups upon the oligomer conformation being studied. We feel that the use of low molecular-weight nonpeptide blocking groups should minimize this problem.

This paper reports CD studies with the aforementioned oligomers in organic media, trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP), and in mixed solvents (TFE–H_2SO_4 and TFE–water).

EXPERIMENTAL

Circular dichroism studies were carried out using a Cary 60 spectropolarimeter modified with a Model 6001 circular dichroism attachment. The experimental solutions

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were prepared by weighing the desired sample into a volumetric flask and adding the solvent. The studies performed in TFE–H_2SO_4 were conducted by first dissolving the oligomer in TFE. The sulfuric acid (on a volume basis) was then added and the resulting solution was stirred for 20–30 min to insure equilibrium. The spectra were obtained using a 0.1-mm pathlength cell. Dry prepurified nitrogen was employed to purge the instrument before and during the experiments. All spectra were recorded at ambient temperature.

The trifluoroethanol was purchased from the Halocarbon Corporation and the hexafluoroisopropanol from Columbia Organic Chemicals. Both solvents were used without further purification. Concentrated sulfuric acid was used in the preparation of all TFE–H_2SO_4 samples. Care was taken to exclude water from these solutions.

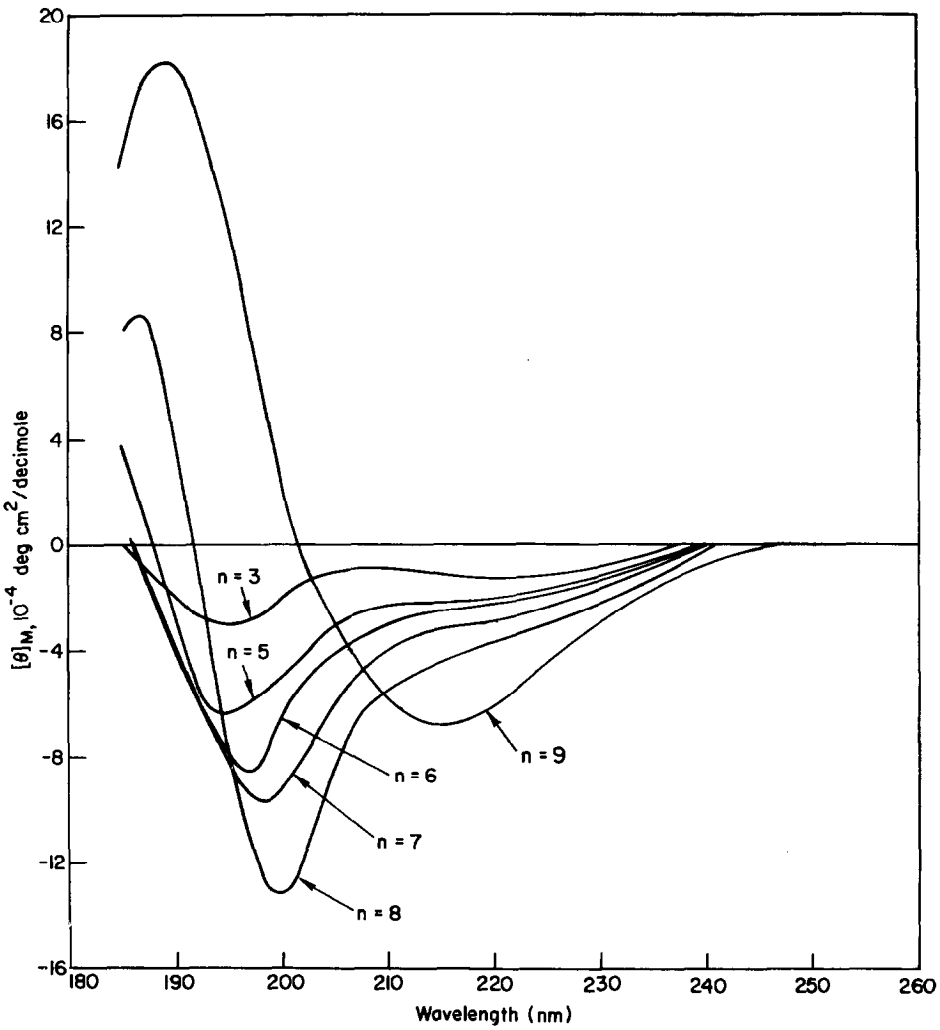

FIG. 1. Circular dichroism of MEEA–$(Ala)_n$–Mo in TFE.

RESULTS AND DISCUSSION

After demonstrating that our oligopeptides were optically pure (*2*), we examined the dependence of their secondary structure on chain length. Circular dichroism in the far ultraviolet region has been shown to be a sensitive tool for determining the secondary structure of oligopeptides (*5*). We undertook a detailed examination of the alanine oligomers using this technique.

A. Circular Dichroism of Alanine Oligomers in TFE, HFIP, and TFE–HFIP Mixtures

Figure 1 presents the CD spectra of the MEEA–$(Ala)_n$–Mo series in TFE. We observe two negative Cotton effects for trimeric to octameric oligomers. We attribute the long wavelength band to $n \rightarrow \pi^*$ transition and the short wavelength absorption to a $\pi \rightarrow \pi^*$ transition. These assignments are based on a comparison with previous CD examinations of polypeptides and oligopeptides (*6*). In the octamer [MEEA–$(Ala)_8$–Mo], we also observe a positive band centered at 187 nm. We believe that this band arises from exciton splitting of the $\pi \rightarrow \pi^*$ transition of the amide chromophore. The appearance of this splitting indicates the presence of some helical character in this oligomer. The change in CD pattern in going from the trimer to the octamer consists of two effects:

TABLE 1

SUMMARY OF CD DATA FOR THE ALANINE OLIGOMERS STUDIED

Oligomer	$[\theta]_M \times 10^{-3}$ at 215 nm	Position of $\pi \rightarrow \pi^*$	$[\theta]_M \times 10^{-3}$ of $\pi \rightarrow \pi^*$	Crossover	Assigned structure
MEEA–$(Ala)_3$–Mo	−11.4	195 nm	−30.3	185 nm	Disordered
MEEA–$(Ala)_5$–Mo	−21.7	194 nm	−63.8	186 nm	Disordered
MEEA–$(Ala)_6$–Mo	−24.9	197 nm	−86.6	186 nm	—
MEEA–$(Ala)_7$–Mo	−35.7	198.5 nm	−98.7	188 nm	Some helical character
MEEA–$(Ala)_8$–Mo	−45.3	200 nm	−132.0	192 nm	Some helical character
MEEA–$(Ala)_9$–Mo	−68.7	190 nm	+181.9	201.5 nm	β-Conformation
Z–$(Ala)_3$–Mo	−3.9	195 nm	−13.0	190 nm	Disordered
Z–$(Ala)_4$–Mo	−9.9	195 nm	−36.0	—	Disordered
Z–$(Ala)_5$–Mo	−10.4	196.5 nm	−41.7	189 nm	Disordered
Z–$(Ala)_6$–Mo	−19.4	197 nm	−71.4	189 nm	—
Z–$(Ala)_7$–Mo	−34.1	190 nm	+ 74.2	197 nm	Some β-character
Z–$(Ala)_8$–Mo	−83.6	194 nm	+421.8	207 nm	β-Conformation
Z–$(Ala)_9$–Mo	−103.2	194 nm	+416.0	206.6 nm	β-Conformation
MEEA–$(Ala)_3$–OEt	−5.6	195 nm	−37.5	—	Disordered
MEEA–$(Ala)_4$–OEt	−9.8	194 nm	−55.7	—	Disordered
MEEA–$(Ala)_5$–OEt	−10.6	194 nm	−74.0	—	Disordered
MEEA–$(Ala)_6$–OEt	−15.2	197 nm	−79.7	185 nm	—
MEEA–$(Ala)_7$–OEt	−21.6	198.5 nm	−90.8	188 nm	Some helical character
Z–$(Ala)_3$–OEt	−3.6	190 nm	−33.3	—	Disordered
Z–$(Ala)_4$–OEt	−5.8	194 nm	−58.0	—	Disordered
Z–$(Ala)_5$–OEt	−6.3	195 nm	−65.1	187.5 nm	Disordered
Z–$(Ala)_6$–OEt	−18.8	190 nm	+46.0	198 nm	Some β-character
Z–$(Ala)_7$–OEt	−72.4	196 nm	+971.0	211 nm	β-Conformation

(1) a gradual red shift of the $\pi \rightarrow \pi^*$ band from 195 nm in the trimer to 200 nm in the octamer; (2) an increase in intensity of both the $n \rightarrow \pi^*$ and the $\pi \rightarrow \pi^*$ bands as the oligomer chain length increases. On a molar basis, the variation in intensity in proceeding from the trimer to the octamer is small and does not appear to be conformationally relevant.

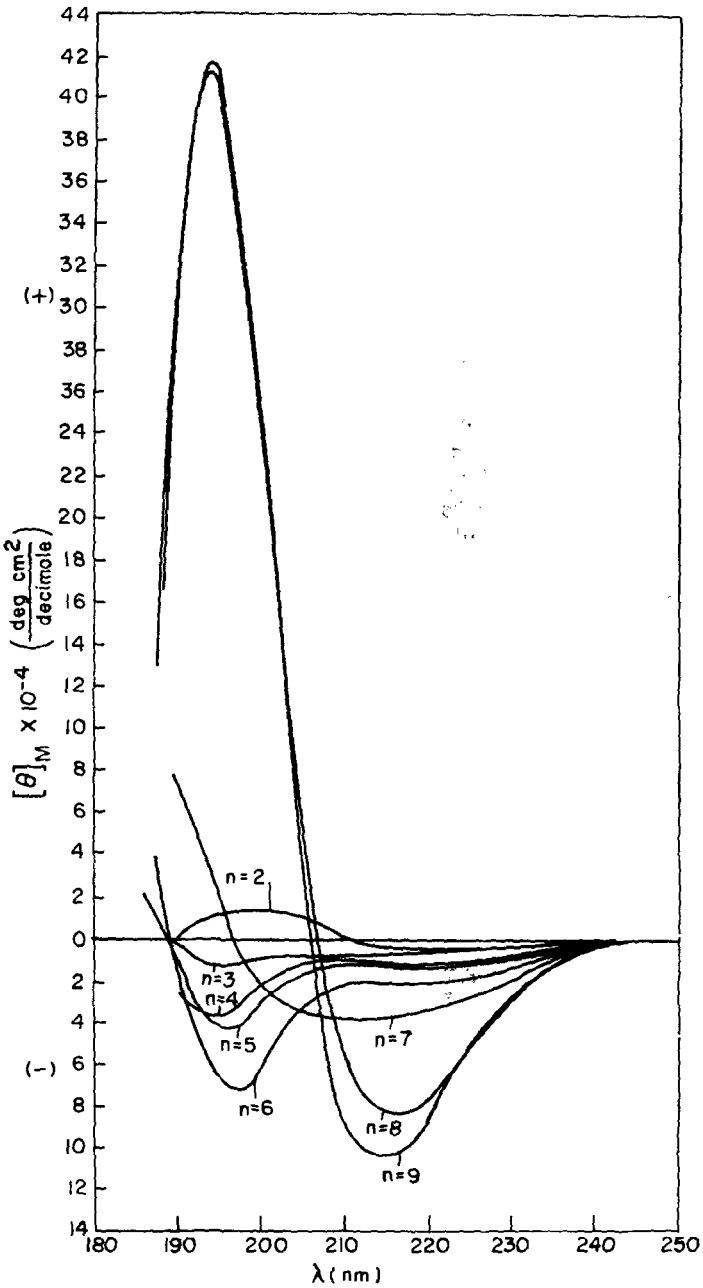

FIG. 2. Circular dichroism of Z-(Ala)$_n$-Mo in TFE.

The above effects result in a gradual evolution of the CD pattern toward that expected for a helical structure (*6*). This trend changes abruptly at the nonamer. The CD spectrum of this compound consists of a broad negative band centered at ~ 215 nm and a positive band at 190 nm. The shape of this spectral pattern is typical of that reported for β-conformations in solution (*7*).

Figure 2 and Table 1 present the results of CD studies on each of the oligomer series. We observe that both the Z-$(Ala)_n$-Mo and Z-$(Ala)_n$-OEt oligomers exhibit the appearance of β-conformations at the heptamer. In the MEEA-$(Ala)_n$-OEt oligomers, however, no β-conformations were observed up to the heptamer. Unfortunately, we were not able to study the higher members of this series because of solubility problems. We would expect oligomers of this type to behave analogously to those of the MEEA-$(Ala)_n$-Mo series and would eventually form β-structures. In all of the series examined, we observed a gradual increase in intensity of the ellipticity from both the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions and a red shift of the Cotton effect attributed to the $\pi \rightarrow \pi^*$ transition as the oligomer chain increases. Once again the variation in intensity as we proceed from the trimer to the octamer is small and does not appear to be conformationally relevant.

Because of the appearance of β-conformations in two of the oligomer series and the unavailability of the higher members of the MEEA-$(Ala)_n$-OEt series, it is difficult to assign the onset of helicity for these L-alanine oligomers from this data. It is our belief, however, that the red shift of the $\pi \rightarrow \pi^*$ transition which occurs between the pentamer and heptamer in the MEEA-$(Ala)_n$-OEt and between the pentamer and octamer in the MEEA-$(Ala)_n$-Mo oligomer series is indicative of some conformational change. In Section B we will present the results of CD studies on the alanine oligomers in TFE-1% sulfuric acid mixtures. Studies in this media shed further light on the conformational changes which occur with increasing chain length.

The stabilities of each of the β-conformations observed were investigated by examining their CD at different concentrations (Figs. 3 and 4 and Table 2). The molecules of

TABLE 2

THE EFFECT OF CONCENTRATION UPON OLIGOMER CONFORMATION

Oligomer	β-Conformation	Partial disruption of β-conformation	Complete disruption of β-conformation
MEEA-$(Ala)_9$-Mo	1.1 mg/ml	0.43 mg/ml	0.01 mg/ml
Z-$(Ala)_7$-Mo	1.27 mg/ml	—	0.49 mg/ml
Z-$(Ala)_8$-Mo	0.5 mg/ml	0.1 mg/ml	0.01 mg/ml
Z-$(Ala)_9$-Mo	0.1 mg/ml	0.02 mg/ml	—
Z-$(Ala)_7$-OEt	0.1 mg/ml	0.01 mg/ml	—

an oligomer in the β-associated form may be considered to be in an equilibrium between free and associated states. We can see that if the oligomer concentration is decreased the equilibrium will be driven toward the unassociated form. For each oligomer examined, dilution ultimately caused a disruption of the associated structures. This disruption resulted in spectral patterns similar to those observed for α-helical polypeptides although both the position of the $\pi \rightarrow \pi^*$ Cotton effect and the intensity of the $n \rightarrow \pi^*$ Cotton effect are not fully developed. In the case of Z-$(Ala)_7$-OEt and Z-$(Ala)_9$-Mo, we were unable to measure the CD at concentrations low enough to disrupt completely their

β-structures. The spectral patterns observed for these oligomers at 0.01 mg/ml and 0.02 mg/ml, respectively, may be due to the presence of more than one conformation.

A comparison of the conformations of the oligomers in the four series indicates that the competition between β and helical forms depends on the blocking group employed. It is obvious that the solubility of an oligomer influences its tendency to form associated structures. We must also consider the electronic and steric differences between the MEEA and "Z" blocking groups. The Z group is joined to the alanine oligomers

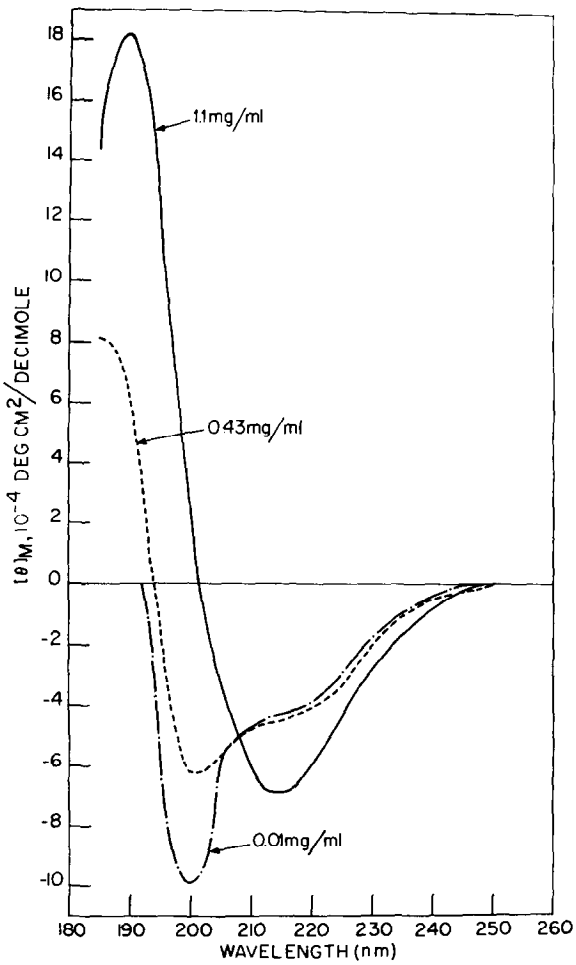

FIG. 3. Circular dichroism of MEEA–$(Ala)_9$–Mo in TFE.

through a urethane linkage. Such a linkage is chemically and electronically different from the peptide linkage between internal alanine residues. The MEEA blocking group, on the other hand, is coupled to the oligomer chain through an amide linkage. Although this linkage is not identical to that formed between adjacent alanine residues, it is expected to be chemically, electronically, and sterically far more similar to peptide bonds than the urethane linkage of the Z group. It is possible that these differences between the blocking groups are just sufficient to prevent the Z terminal residue from entering into intramolecular hydrogen bonds while allowing the MEEA terminal

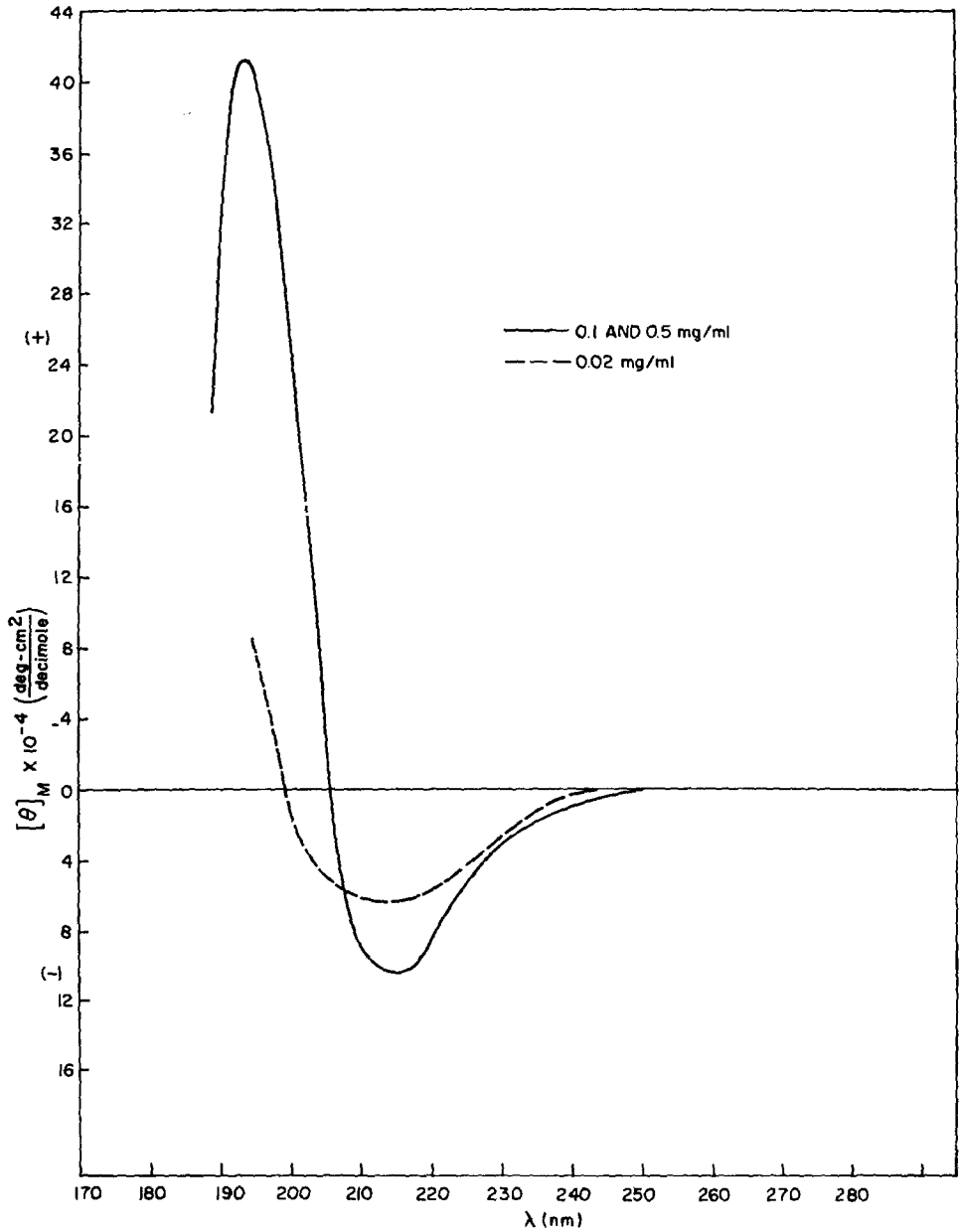

FIG. 4. Circular dichroism of Z–$(Ala)_9$–Mo in TFE.

residue to take part in such interactions. Thus, in the MEEA series additional residues will be available for intramolecular hydrogen bonds and helical forms should be stabilized.

In order to gain further insight into the solution conformation of the alanine oligomers, we attempted to find an organic solvent in which the higher oligopeptides

would be helical. Of all the known helix supporting solvents that are transparent in the far ultraviolet region, only hexafluoroisopropanol (HFIP) dissolves the entire oligomer series. Figure 5 shows the CD spectra for the MEEA–$(Ala)_n$–Mo oligopeptides. The CD patterns observed show no dramatic changes with increasing chain length. Similar results were also found for the Z–$(Ala)_n$–Mo series. In both series, as the oligomer chain length increases we notice a gradual increase in intensity of the two negative CD bands. The band positions, however, remain constant throughout each oligomer series.

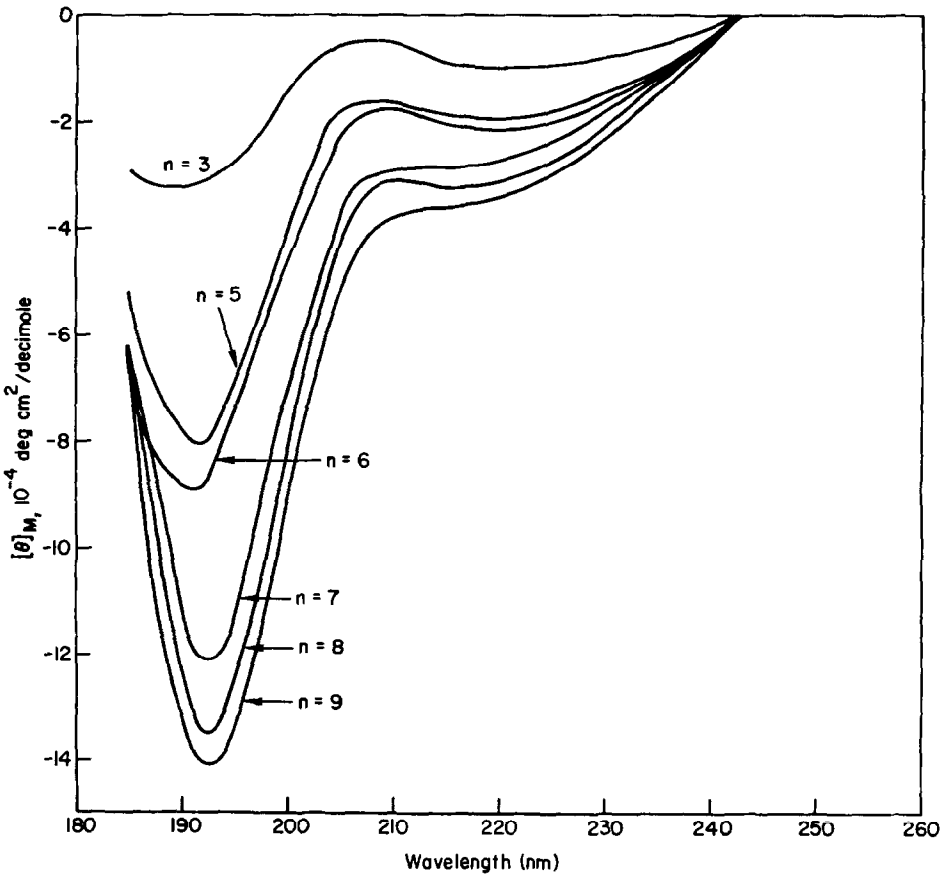

FIG. 5. Circular dichroism of MEEA-$(Ala)_n$–Mo in HFIP.

Although the CD patterns observed for these oligomers are not of the typical "random coil" type, we feel that these compounds exist in a disordered state in HFIP. This conclusion is based upon the similarity of the CD patterns throughout these series of oligomers. The absence of any significant change in the CD patterns, as the oligomer chain length increases, indicates that no conformational changes occur. HFIP appears to destabilize both the α-helix and β-conformations for alanine oligomers. This result is not surprising since HFIP is considerably more acidic than TFE and thus requires a longer chain length for the onset of secondary structures.

Since the higher Z–$(Ala)_n$–Mo oligomers are β in TFE and disordered in HFIP, we felt some mixture of these solvents might support helical structures. Figure 6 presents

the CD patterns of Z–$(Ala)_9$–Mo in several HFIP–TFE mixtures. From these spectra it seems that as the TFE concentration increases, the nonamer first appears to be going helical (50% HFIP), then gives way to a mixture of helical and associated forms (25%

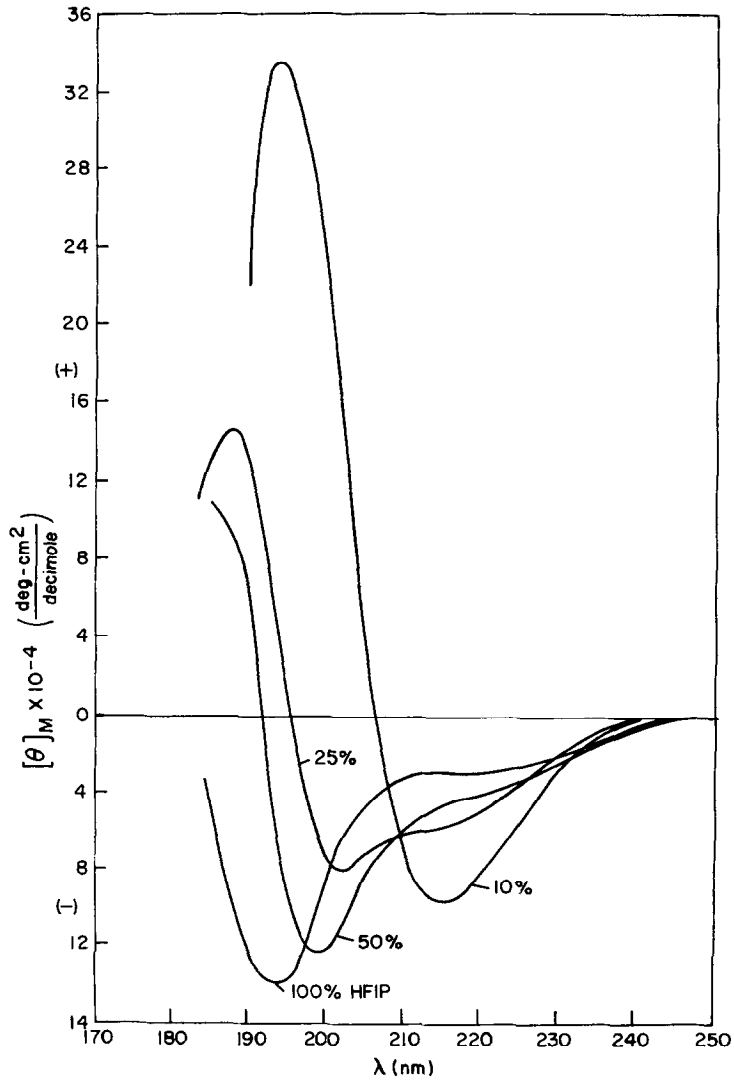

FIG. 6. Circular dichroism of Z–$(Ala)_9$–Mo in HFIP–TFE mixtures.

HFIP), and finally becomes completely β (10% HFIP). It is difficult to ascertain definitely which HFIP–TFE medium is "helix supporting". As a result we decided not to investigate the alanine oligopeptide series in these mixtures.

B. Circular Dichroism of Alanine Oligomers in Trifluoroethanol–Sulfuric Acid Mixtures

We studied the CD of alanine oligomers in different TFE–H_2SO_4 mixtures to determine the effects of the H_2SO_4 on association and on intramolecular helical structures.

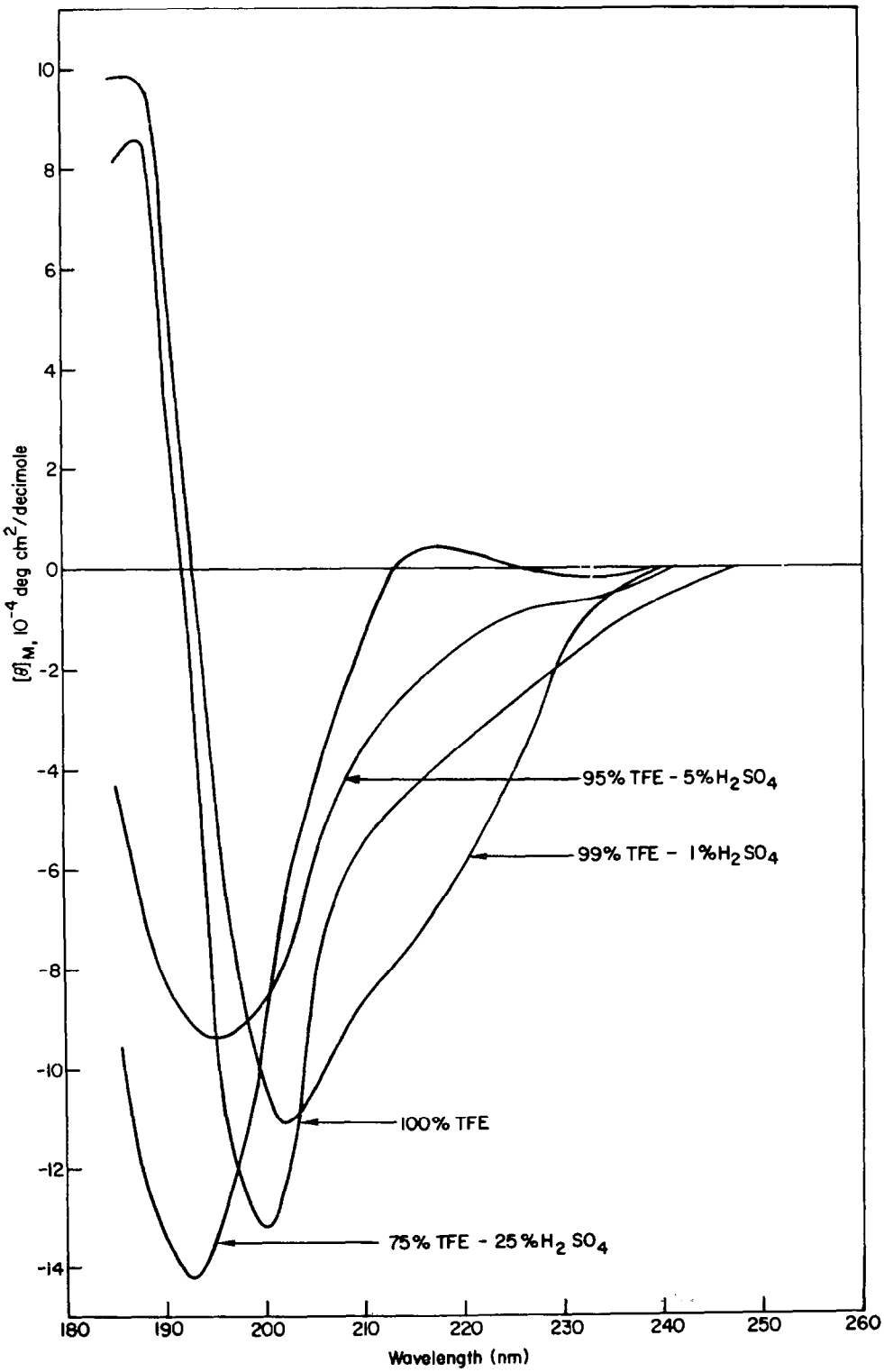

FIG. 7. Circular dichroism of MEEA–$(Ala)_8$–Mo in TFE–H_2SO_4 mixtures.

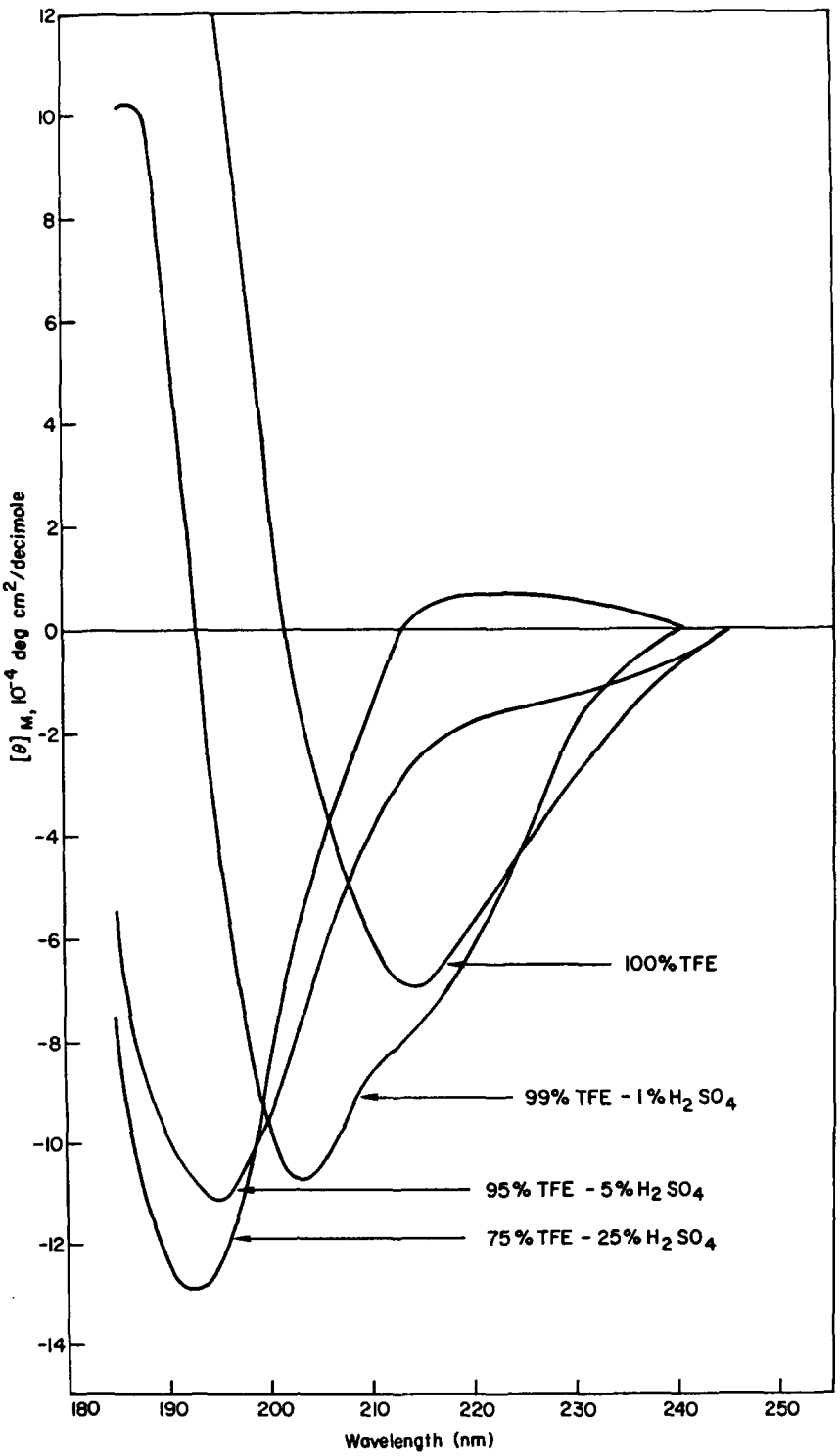

FIG. 8. Circular dichroism of MEEA-$(Ala)_9$-Mo in TFE-H_2SO_4 mixtures.

The CD of different oligomers was measured at different acid concentrations. We also measured the CD of the entire series MEEA–$(Ala)_n$–Mo and Z–$(Ala)_n$–Mo in 99% TFE–1% H_2SO_4.

The effect of different H_2SO_4 concentrations upon the CD patterns of alanine oligomers is illustrated in Figs. 7–9. Figure 8 shows the effect of adding acid to the β-type oligomer MEEA–$(Ala)_9$–Mo. We see that the addition of 1% H_2SO_4 to a TFE solution completely destroys the β-type CD pattern. The CD spectrum which results

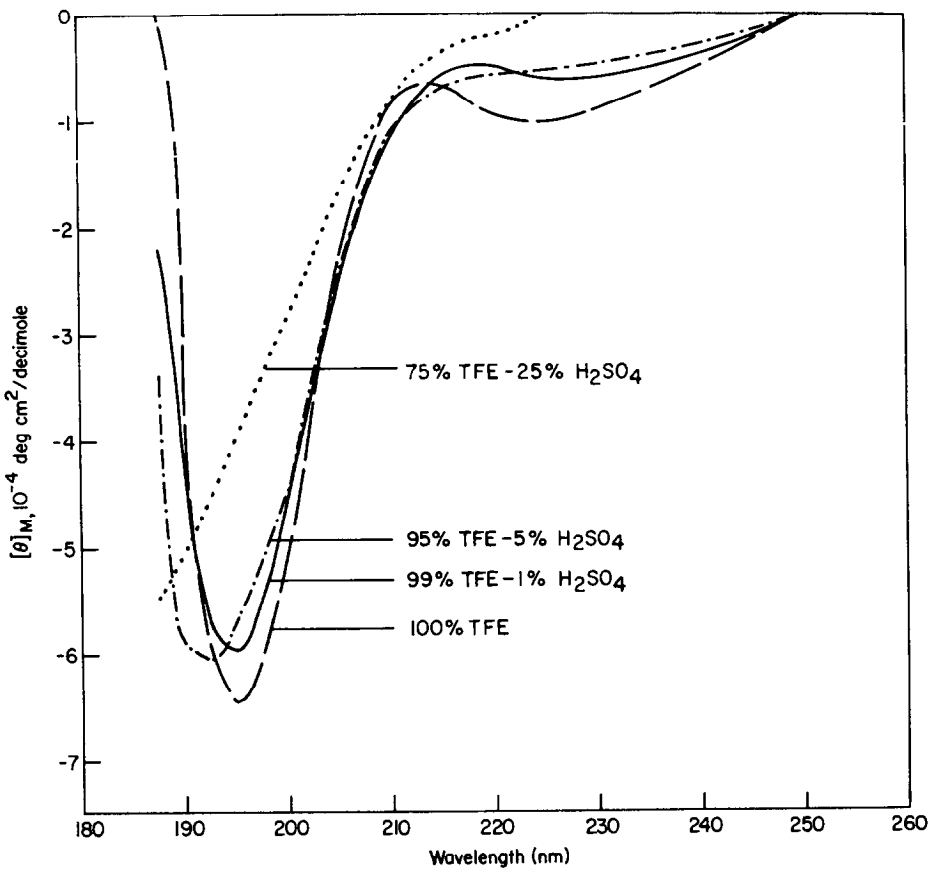

FIG. 9. Circular dichroism of Z–$(Ala)_5$–OEt in TFE–H_2SO_4 mixtures.

appears to be of the helical type. The CD patterns observed for this nonamer indicate that helical structures persist even in the presence of small amounts of a strong acid. The addition of larger amounts of acid breaks up the intramolecular hydrogen bonding and results in a CD pattern typical of a "random coil" solute. This effect is shown by a general blue shift of the negative $\pi \rightarrow \pi^*$ band, and a decrease in intensity of the $n \rightarrow \pi^*$ absorption as the acid concentration is increased. Similar results were found for Z–$(Ala)_7$–OEt, Z–$(Ala)_7$–Mo, Z–$(Ala)_8$–Mo, and Z–$(Ala)_9$–Mo.

The addition of small amounts of sulfuric acid to oligomers which show helical-type CD patterns in the absence of acid appears to enhance the helical nature of these compounds. This effect is shown by MEEA–$(Ala)_8$–Mo (Fig. 7). The addition of 1%

H_2SO_4 to a TFE solution of this oligomer results in a red shift of the negative $\pi \rightarrow \pi^*$ band and a marked increase in intensity of the $n \rightarrow \pi^*$ band. These effects are probably due to the influence of the sulfuric acid upon the general solvent structure and not to a specific interaction of the acid with the oligomer.

Recent work in our laboratories on a conformationally restricted camphorolactam shows that solvation of the amide chromophore by sulfuric acid results in complete loss of the $n \rightarrow \pi^*$ Cotton effect. In the higher alanine oligomers, however, we observe an enhancement of the spectral bands in the $n \rightarrow \pi^*$ region, as compared to pure TFE. These results suggest that stabilization of the helical oligomers, rather than solvation of the amide chromophore by sulfuric acid at these low concentrations, is responsible for the spectral changes.

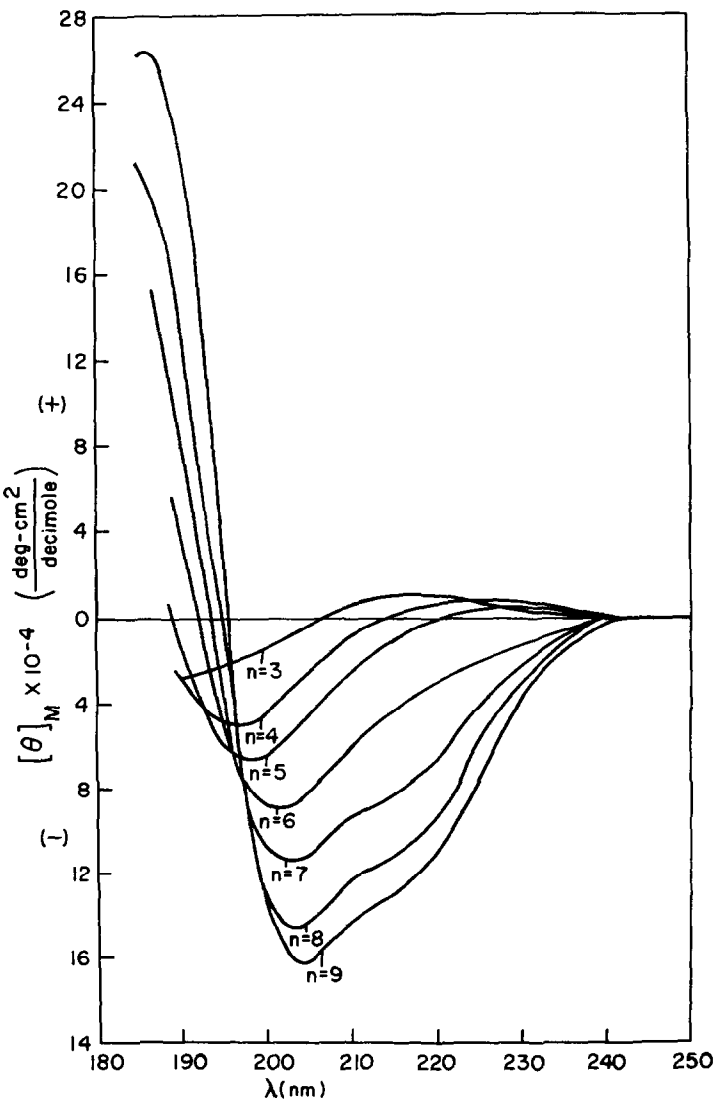

FIG. 10. Circular dichroism of Z-$(Ala)_n$-Mo in TFE–1% H_2SO_4.

A different effect is observed in the case of Z–$(Ala)_5$–OEt (Fig. 9), which shows a CD pattern in TFE typical of nonhelical solutes. In this case the addition of 1% H_2SO_4 has little effect upon the CD pattern. There was no change in the position of the $\pi \rightarrow \pi^*$ band, and a slight decrease in intensity of the $n \rightarrow \pi^*$ band. In all the oligomers studied, the addition of 5% or more H_2SO_4 resulted in a CD pattern indicative of a "random coil" conformation of the solute.

The results presented above may be explained as resulting from the effect of the acid on the type of hydrogen bonding present in the oligomer. The addition of small amounts (1%) of sulfuric acid appears to break up the intermolecular hydrogen bonds which stabilize the β-conformation. We feel that this effect was not due to specific interactions of the acid with the oligomer, since this small amount of acid was found to enhance the helical nature of the oligomers. The addition of larger amounts of sulfuric acid breaks up all intra- and intersolute hydrogen bonds by specific interactions of the acid with the amide chromophores.

After determining that helical species could exist in 1% sulfuric acid–TFE, we examined the series MEEA–$(Ala)_n$–Mo and Z–$(Ala)_n$–Mo in this solvent system. Since β-structures were not observed in this medium, we were able to determine the critical size necessary for helix formation in the alanine oligomers.

The results of our study are presented in Fig. 10. Although it is impossible to eliminate the effect of sulfuric acid solvation on the spectral patterns, the results suggest that stable helical forms do exist in this medium. As the chain length is increased, a definite splitting of the low wavelength Cotton effect and a marked increase in the molar ellipticity ($[\theta]_M$) at 215 nm is observed. Both of these phenomena are expected as a peptide changes from a disordered to a helical conformation.

We believe that perhaps the most positive indication of the onset of secondary structure is the difference in the shape of the CD patterns of the lower oligomers ($n = 3, 4, 5$) from that of the higher oligomers ($n = 7, 8, 9$). We suggest that these differences may only be explained if one assumes a nonregular structure for the lower oligomers and a folded structure for the higher members of the series. In the latter case the spectral differences may be attributed to the inability of the sulfuric acid to solvate the amide chromophores in a helical conformation. Evidence supporting our contention is presented in Figs. 8 and 9. Here the CD patterns of MEEA–$(Ala)_9$–Mo at higher acid concentrations are very similar in shape to those of Z–$(Ala)_{n=3,4,5}$–Mo in 1% sulfuric

TABLE 3

MOLAR ELLIPTICITIES IN 75% TFE–25% H_2SO_4

Compound	$[\theta]_M^{215} \times 10^{-3}$
Z–$(Ala)_5$–OEt	−2.5
Z–$(Ala)_6$–OEt	−9.6
Z–$(Ala)_7$–OEt	−8.5
MEEA–$(Ala)_7$–Mo	+1.3
MEEA–$(Ala)_8$–Mo	+3.7
MEEA–$(Ala)_9$–Mo	+2.8
Z–$(Ala)_5$–Mo	+8.8
Z–$(Ala)_6$–Mo	+7.4
Z–$(Ala)_7$–Mo	+3.3
Z–$(Ala)_8$–Mo	+4.2
Z–$(Ala)_9$–Mo	+2.1

acid–TFE. Thus, once there is enough acid present to destroy intramolecular folding, the sulfuric acid can apparently solvate the chain backbone, resulting in similar spectral patterns for all oligomers.

The studies in 25% sulfuric acid–TFE yield an additional interesting result. Table 3 compares the molar ellipticities of the various alanine oligomers in this solvent, at 215 nm. As can be seen, all of the oligopeptides with ethyl ester end groups have zero or negative ellipticities at this wavelength. The oligomers with morpholine amide end groups, however, all have positive ellipticities at the same wavelength. Our conformational analyses give evidence that in 25% sulfuric acid–TFE all oligomers are in a "random coil" conformation. Thus, the appearance of a positive band in the 215- to 225-nm region cannot be due to conformational differences. Apparently, the sulfuric acid interacts differently with the morpholine and ethyl ester blocking groups resulting in the positive band.

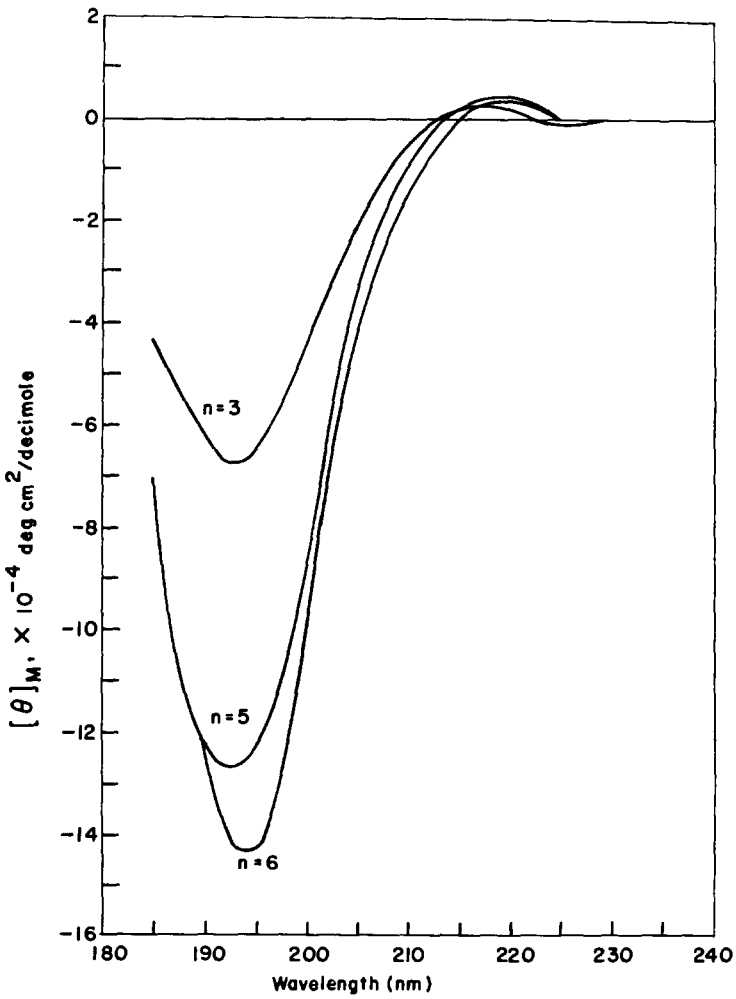

FIG. 11. Circular dichroism of MEEA–$(Ala)_n$–Mo in H_2O.

C. CD Studies in Water and TFE–Water Mixtures

One of the original goals of our synthesis was the preparation of water-soluble oligomers. Unfortunately, the higher oligopeptides in both series were insoluble in aqueous media. Nevertheless we did study the circular dichroism of those compounds which were water soluble. Figure 11 presents our results for the MEEA–$(Ala)_n$–Mo series. The spectral patterns are typical of those observed for "random coil" polypeptides. It should be noted how different these patterns are from those found for the same compound in TFE. Since it is likely that these trimers, pentamers, and hexamers

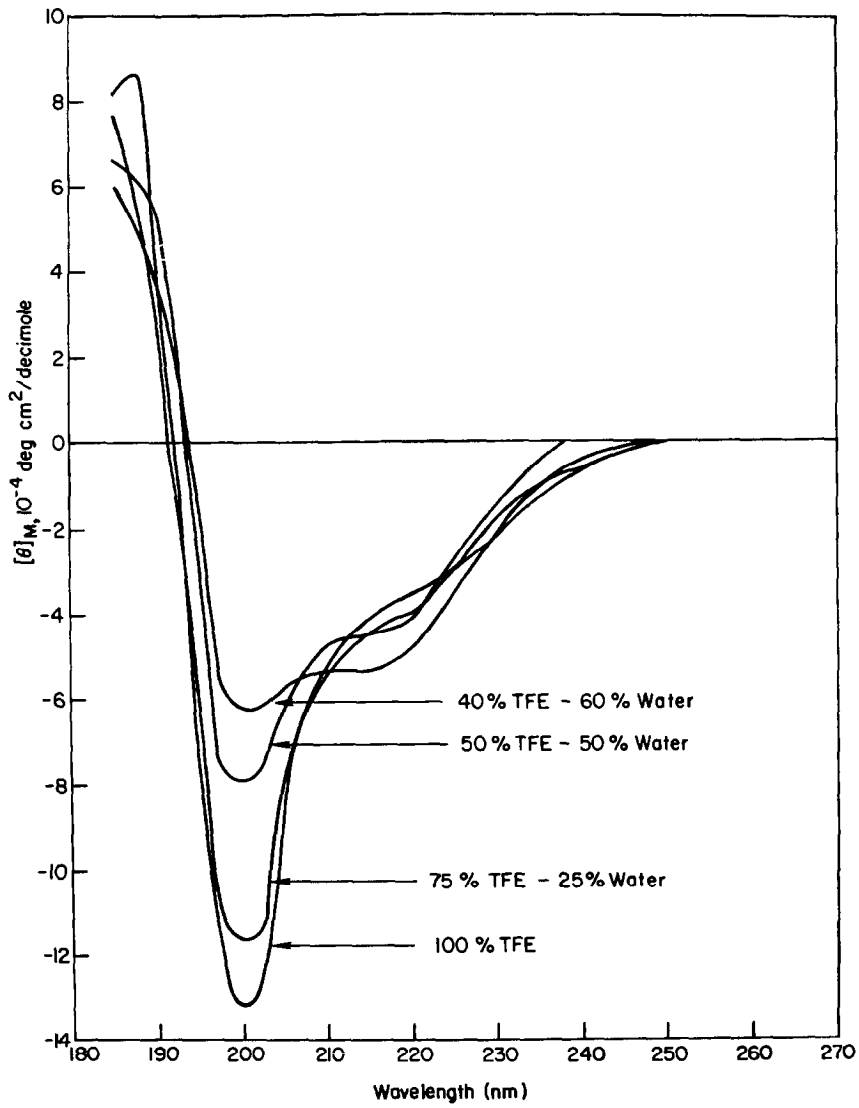

FIG. 12. Circular dichroism of MEEA–$(Ala)_8$–Mo in TFE–water mixtures (spectra measured 15 min after addition of water).

are disordered in both of these solvents, our results point to the important effect that solvation has in the determination of CD spectral patterns.

In order to determine the effect of water on a helical alanine oligomer, we also studied MEEA–$(Ala)_8$–Mo in different TFE–water mixtures (Figs. 12 and 13). The CD spectra were measured immediately after the addition of water and after standing 4 days in solution.

In the spectra taken immediately after the addition of water, we see two negative bands, one at 215–220 nm which we attribute to an $n \rightarrow \pi^*$ absorption and one at 200

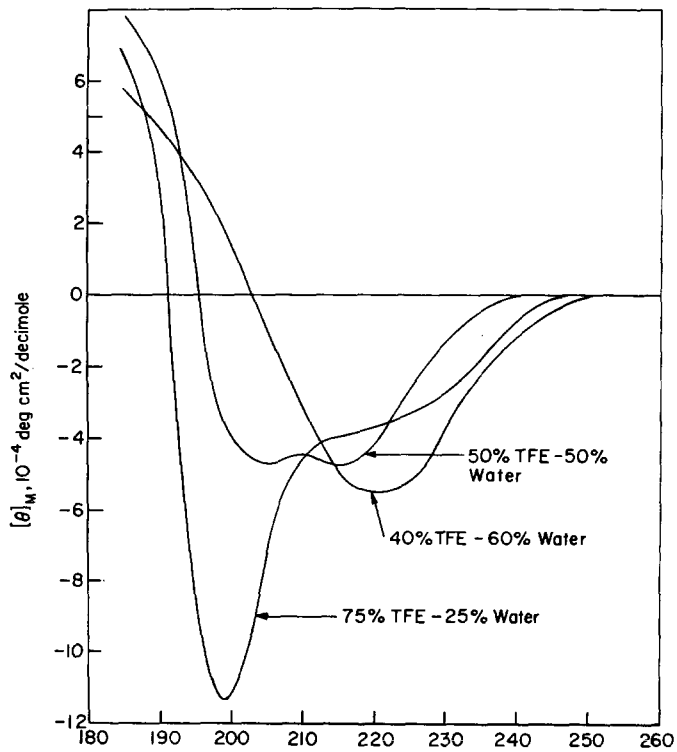

FIG. 13. Circular dichroism of MEEA–$(Ala)_8$–Mo in TFE–water mixtures (4 days in solution).

nm which we assign to a $\pi \rightarrow \pi^*$ transition. The 200-nm band decreases in intensity for solvent mixtures containing progressively larger amounts of water. The 215 to 220-nm band changes little in intensity with the percentage of water in the solvent mixture. In 60% water–40% TFE the two bands are almost equal in intensity. In solvent mixtures containing more than 60% water, the oligomer precipitates out upon the addition of the water.

The CD of these solutions were measured after storage at room temperature for 4 days in solution. The spectrum of the octamer in 75% TFE–25% water does not change from that obtained immediately after the addition of the water. The CD patterns of the solutions containing larger amounts of water change dramatically. In 50% TFE–50% water, negative bands of equal intensity were found at 205 and 215 nm. The CD pattern found in 40% TFE–60% water exhibited a single negative band at 220 nm and

an increasingly positive absorption below 204 nm. The changes in these CD patterns with time indicate that association is taking place. The spectrum observed for MEEA–$(Ala)_8$–Mo in 40% TFE–60% water, after 4 days in solution, is very similar to the CD pattern expected for a β-structure. These findings indicate that the β-associated structure is the preferred conformation for this oligomer in water.

CONCLUSIONS

The conformational analysis on L-alanine oligopeptides permits us to examine the effect of chain length upon conformation. Secondary structures of the β-conformation type were first observed at the heptamer in two series of alanine oligomers and at the nonamer in another. This leads us to believe that extended associated structures may have a critical chain length analogous to the critical size necessary for helix formation. A critical size for the formation of a β-conformation is also observed for isoleucine oligomers (*8*).

Our studies also suggest that (besides chain lengths) concentration, solvent, and blocking group are all important in determining the conformation of oligopeptides in solution. Thus, the fact that an oligomer is β at a concentration of 1 mg/ml does not preclude the formation of helical species at lower concentrations. Future studies should, therefore, deal with several concentration ranges before conclusions are drawn. The fact that the completely different structures which the same oligopeptide can have in different solvents once again points out how critical solute–solvent interactions are in determining secondary structure. Especially interesting are the results in mixed TFE–HFIP media which give evidence for the delicate balance between various conformational species. Finally, although the data are too sparse to be conclusive, we feel we have demonstrated, perhaps for the first time, that the blocking group definitely influences the conformation of oligopeptides in solution.

Poly-L-alanine has heretofore been observed to exist only in α-helical and random coil conformations in solution (*3*, *9–12*). Recently, Scheraga predicted that decaalanine should form a β-conformation in aqueous solution (*13*). An attempt experimentally to verify this prediction by preparing a water-soluble decaalanine sandwiched between poly DL lysine blocking groups resulted in a random oligopeptide (*4*). Our findings give the first experimental verification that Scheraga's calculations may be correct. Although we were unable to prepare a water-soluble decaalanine, the existence of an alanine nonapeptide in a β-conformation in TFE and an alanine octapeptide in TFE–water mixtures suggests that similar structures may also be formed in water. Studies in sulfuric acid–TFE mixtures indicate that small amounts of acid (1%) disrupt intermolecular association while stabilizing intramolecular helical forms. In the presence of larger amounts of acid, only disordered forms exist. Examination of the alanine oligomers in 1% sulfuric acid–TFE suggests that helical species first appear at the hexamer or heptamer. A critical chain length of seven has been reported for γ-ethyl-L-glutamate oligopeptides in trifluoroethanol (*5*).

We believe that future examination of these oligopeptides should include CD studies at different temperatures. Such investigations would yield thermodynamic information which may prove useful in determining the nature of the forces that stabilize the different secondary structures. We are also examining the nmr of our alanine oligopeptides at the same concentration employed in the CD analyses. Such a study will allow us to make a direct assignment of the population of each of the stereochemical forms in solution and to characterize the transitions among them.

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