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Linear Oligopeptides. 57.[1] A Circular Dichroism Study of α-Helix and β-Structure Formation in Solution by Homooligo-L-methionines

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ABSTRACT: The conformational properties of poly(ethylene glycol)-bound, monodispersed *N-tert*-butyloxycarbonylhomooligo-L-methionines to the pentadecapeptide were examined in solvents of high polarity (a variety of alcohols, haloalcohols, and water) using circular dichroism. The corresponding N-deblocked derivatives were also investigated. This study shows that the L-methionine peptides may exist in right-handed α-helical, β, and statistical coil conformation depending upon chain length, solvent polarity, temperature, concentration, pH, ionic strength, and presence of the N-blocking group.

The possibility of investigating the conformation of solubilized oligopeptides through attachment to a suitable polymer, e.g., poly(ethylene glycol) (PEG), has recently opened the door to a number of circular dichroism (CD) studies aiming to provide a deeper insight into the relation between conformation and chain length, primary sequence, protecting group, solvent, concentration, ionic strength and temperature.[3-10]

A CD investigation of PEG-bound ACTH peptides revealed a distinct tendency for ordered structures at the N-terminal part of the hormone.[10] A significant increase in helical structure was noted when the helix-promoting sequence L-Pro-L-Ala-L-Ala was inserted at the N terminus. Transitions from statistically unordered to partially helical structures at chain lengths of about eight to ten residues were observed in 2,2,2-trifluoroethanol (TFE) during the stepwise liquid-phase synthesis[11] of the myoglobin fragment 66–73,[4] the hormone substance P,[3-4] and model peptides for the antibiotic alamethicin.[9]

The formation of the α helix at acidic pH for PEG-bound homooligopeptides derived from L-Glu(OH) starts at $n = 7$, at $n = 14$ the helix content amounts to about 35%, and at $n = 20$ the helix content amounts to about 60%.[5,6] A helix $\rightarrow$ coil transition is induced by the ionization of the carboxyl groups of the lateral chains. TFE stabilizes the α helix more than water does; the addition of increasing amounts of trifluoroacetic acid resulted in the expected disruption of the helical structure. Also, the solvent-depended conformational properties were not changed by the anchor polymer. The homooligopeptide series derived from L-Glu(OBzl) exhibits in TFE higher helicities than the corresponding L-Glu(OH) analogues, although the onset of the α helix is still observed at $n = 7$.

In the context of the collaborative program which is underway between the Tübingen and Padova laboratories, the following series of PEG-bound *N-tert*-butyloxycarbonyl (*t*-Boc) apolar homooligopeptides have been synthesized by the liquid-phase method[4,10,12] and their conformational preferences investigated by CD:[4,5,7,8,10] *t*-Boc-(L-Ala)$_n$-Gly-OPEG ($n = 1$–8), *t*-Boc-(L-Ala)$_n$-OPEG ($n = 1$–10), *t*-Boc-(L-Val)$_n$-Gly-OPEG ($n = 1$–7), *t*-Boc-(L-Val)$_n$-Gly-OPEG-M ($n = 2$–8) (PEG-M, poly(ethylene glycol) monomethyl ether), and *t*-Boc-(L-Ile)$_n$-OPEG ($n = 1$–8).

In water the peptides from L-Ala and L-Val with $n = 6$, 7 adopt a β conformation. High concentration, temperature, ionic strength, and absence of the N-terminal ammonium group favor the formation of the ordered structure. The β structure of L-Val peptides is more stable than that of L-Ala peptides. In TFE only the L-Ala peptides assume an α-helical conformation (starting at about $n = 8$). More polar solvents, e.g., 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP), and high temperatures hamper the formation of the α-helical structure in the L-Ala peptides. The absence of the *N-t*-Boc group and the use of less polar solvents, e.g., methanol (MeOH), favor the formation of associated species (β structure). In general, the effect brought about the polymeric support, and its molecular weight, even if not negligible, should be considered as a minor one. In TFE the order of stability of the β structure of the apolar homooligopeptides appears to be L-Ile > L-Val > L-Ala.

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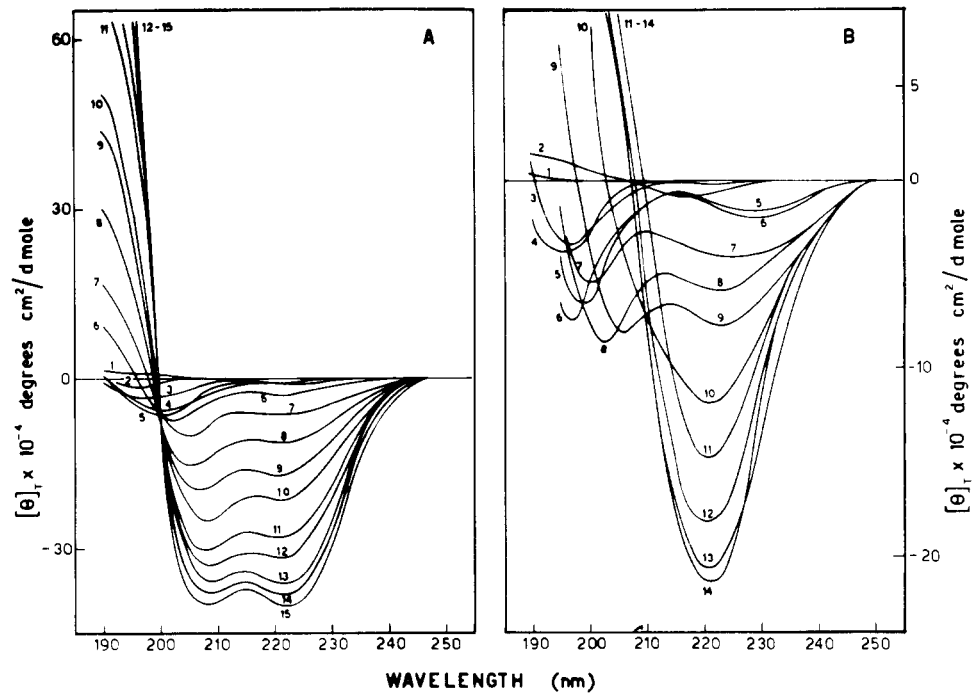

Figure 1. Circular dichroism spectra in TFE of (A) *t*-Boc-(L-Met)$_{1-15}$-NHPEG and (B) H_2^+-(L-Met)$_{1-14}$-NHPEG.

In this paper we wish to report the results of the conformational analysis, carried out using CD in solvents of high polarity (a variety of alcohols, haloalcohols, and water) of the monodispersed homooligopeptide series derived from L-Met, bound to the macromolecular bifunctional support H_2N-(CH_2-CH_2-O-)$_n$-CH_2-CH_2-NH_2 ("amino-PEG"),[13] having the general formula *t*-Boc-(L-Met)$_n$-NHPEG (n = 1–15). The N-deblocked analogues have also been examined. This series has been selected since it is well known that Met homopeptides are the most soluble among those derived from all the other apolar α-amino acid residues. This property in turn could allow us to examine homopeptides with a number of residues higher than those previously studied, and consequently to follow not only the common conformational transitions α helix $\rightleftharpoons$ random coil and β form $\rightleftharpoons$ random coil but also the transition between the two ordered structures, i.e., α helix $\rightleftharpoons$ β structure, never clearly observed before for low molecular weight peptide systems ($n \leq 20$).

This expectation was based on several CD data: (i) high molecular weight poly(L-Met) adopts a right-handed α-helical conformation in HFIP solution,[14] (ii) incorporation of L-Met into polymers from N^5-(3-hydroxypropyl)-L-glutamine increases their α-helix content at all temperatures in the range of 0–60 °C in water,[15] and (iii) *t*-Boc(L-Met)$_n$-OMe (OMe, methoxy) (n = 2–7, 9) exhibit the critical size for α-helix formation at n = 7 in TFE, whereas the β-associated structure appears at the pentapeptide stage in ethylene glycol and TFE–water (20:80, v/v).[16-21] Furthermore, in proteins Met is considered a strong α and β former (stronger as α former).[22]

Experimental Section

Synthesis of Peptides. The details of the synthesis of the monodispersed, chemically and optically pure series *t*-Boc-(L-Met)$_n$-NHPEG (n = 1–15) and H_2^+-(L-Met)$_n$-NHPEG (n = 1–14), using the liquid-phase method[11] are reported elsewhere.[23] The "amino-PEG", prepared by bifunctional PEG, molecular weight 6000 (Hoechst, Frankfurt), according to the procedure described by Mutter,[13] has been employed.

Circular Dichroism. Circular dichroic spectra were recorded using a Cary Model 61 circular dichroism spectrophotometer. The spectra were obtained using cylindrical fused quartz cells of 0.5, 1, and 10-mm path length. Dry prepurified nitrogen was employed to keep the instrument oxygen free during the experiments. A complete base line was recorded for each measurement using the same cell in which the sample solution had been replaced with pure solvent. To avoid kinetic effects, the solutions were prepared by placing the weighed peptide (stored in vacuo in the presence of phosphorus pentoxide) in a volumetric flask, adding the appropriate solvent, and keeping the resulting solution under magnetic stirring for a least 12 h. The circular dichroism data represent average values from a least three separate recordings. Unless stated otherwise, the concentrations were 2 mg/mL and the temperature 20 °C. The calibration was based upon $[\theta]_{290}$ = 7.840 deg cm^2 dmol^{-1} for a purified sample of camphorsulfonic-*10-d* acid (Fluka) in 0.1% aqueous solution.[24] The Lorentz refractive index correction was not applied. In calculating the total molecular ellipticity values $[\theta]_T$ the capacity of the polymer (6 mg of bifunctional "amino-PEG" are equivalent to 1.1 μmol of peptide, as determined by amino acid analysis) and its constancy throughout all the peptide series were taken into account. The percentages of right-handed α-helical conformation were calculated according to the relationships proposed by (i) Greenfield and Fasman[25] and (ii) Chen and Yang.[26]

The solvents used were double distilled water and MeOH (Merck), absolute ethanol (EtOH) (Erba), 2-chloroethanol (ClEtOH) (Fluka), 3-chloropropan-1-ol (ClPrOH) (Fluka), 4-chlorobutan-1-ol (ClBuOH) (Fluka), TFE (Fluka), HFIP (Eastman), and 1,1,1,3,3,3-hexafluoroacetone sesquihydrate (HFA) (Dupont). All solvents were of the highest purity commercially available and were employed without further purification.

Results and Discussion

The CD curves of the N-protected and N-deblocked homooligo-L-methionines bound to "amino-PEG" in TFE are illustrated in Figure 1. From an inspection of Figure 1A (3×10^{-4} M concentration) it turns out that the peptides to n = 6 exist predominantly as statistically unordered forms, characterized by a relatively intense negative maximum near 200 nm (amide $\pi \rightarrow \pi^*$ transition) accompanied by a weak negative maximum located at about 220 nm (amide n $\rightarrow \pi^*$ transition).[18] The CD spectrum indicative of the onset of the right-handed α-helical conformation is clearly seen at the heptapeptide level (intense negative maxima at 220–221 nn, amide n $\rightarrow$ π^* transition, and at 207–208 nm, parallel component of the exciton-split amide $\pi \rightarrow \pi^*$ transition; the cross-over

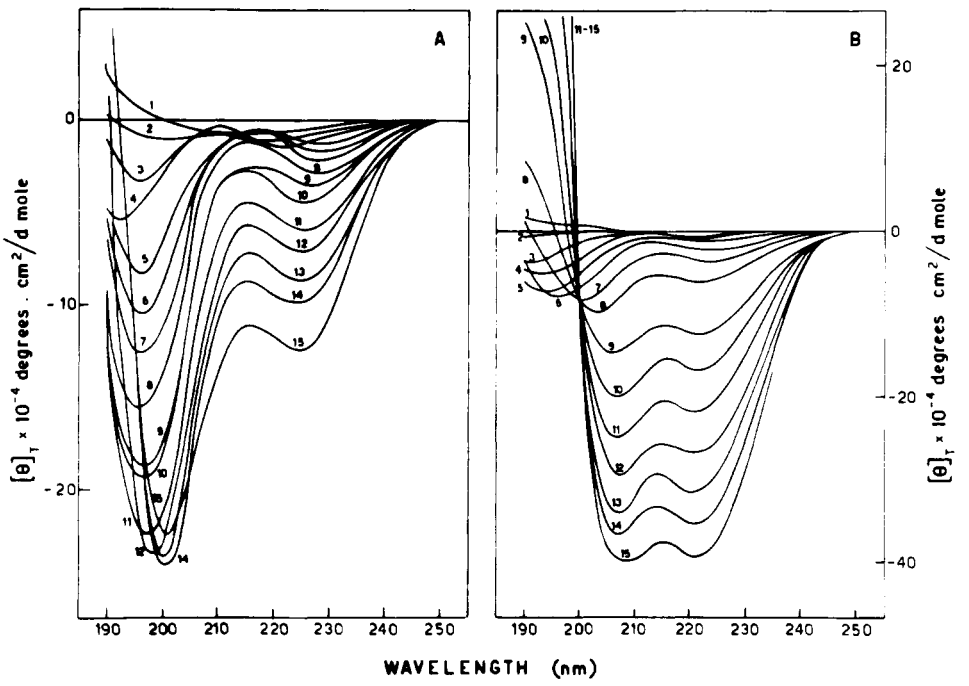

Figure 2. Circular dichroism spectra in HFA (A) and HFIP (B) of *t*-Boc-(L-Met)$_{1-15}$-NHPEG.

point is at 200–201 nm),[18,27-29] remaining substantially unaltered throughout all the series.

Dramatically different conformational preferences are shown by the highest molecular weight oligopeptides in the N-deblocked series under the same experimental conditions of solvent and concentration (Figure 1B). Already at the decapeptide level a CD spectrum suggesting partial onset of the β conformation (intense negative dichroic band at 220–221 nm, n $\rightarrow$ π^* amide transition; cross-over point at 204–207 nm; intense positive dichroic band centered below 197 nm, $\pi \rightarrow \pi^*$ amide transition) is observed.[18,27]

It is probable that the CD pattern of the oligopeptides with n = 7–9 would represent mixtures of β-type and unordered conformations (the latter adopted by the lowest oligopeptides, n = 1–6). From the above data it may be concluded that in TFE the absence of the bulky *t*-Boc-N-protecting group markedly favors the tendency of the peptides to form a β structure. When formation of the β structure is prevented the tendency of the higher L-Met homooligopeptides to adopt the right-handed α-helical structure becomes evident. It is interesting to note that this phenomenon (α-helix $\rightarrow$ β-structure conformational change following removal of the N-protecting group) does not take place in HFIP (not shown in the figure); in this fluoroalcohol the influence of the *N*-*t*-Boc group on the CD spectra, even from the quantitative standpoint, seems to be negligible.

The effect of the nature of the solvent on the conformation of N-protected homooligo-L-methionines is apparent from Figures 2–5 (compared with Figure 1A). We have examined a variety of alcohols of divergent polarity and water (at the same peptide concentration). Clearly, in solvents of high (or relatively high) tendency to solvate the peptide chain (as hydrogen-bonding donors), such as HFA,[30] HFIP,[14,19,20] TFE,[19] ClEtOH,[31] ClPrOH, and ClBuOH, the α-helical conformation is the preferred one by the highest oligopeptides, whereas in less polar alcohols, such as MeOH and EtOH, and in water the preferred one is the β structure.

The tendency to favor the α-helical structure relative to the unordered conformation decreases with increasing

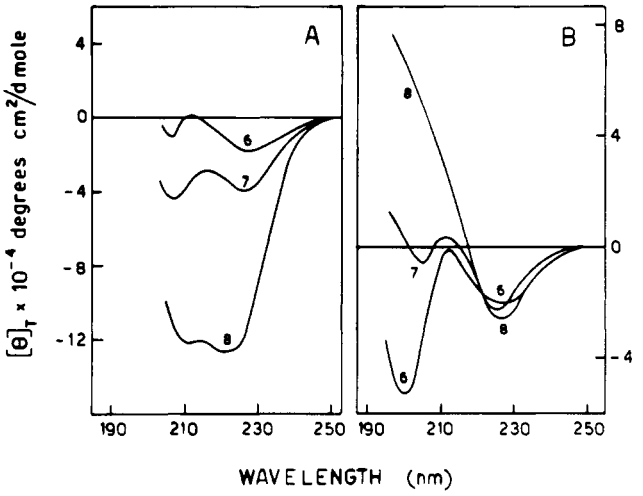

Figure 3. Circular dichroism spectra in ClEtOH (A) and EtOH (B) of *t*-Boc-(L-Met)$_{6-8}$-NHPEG.

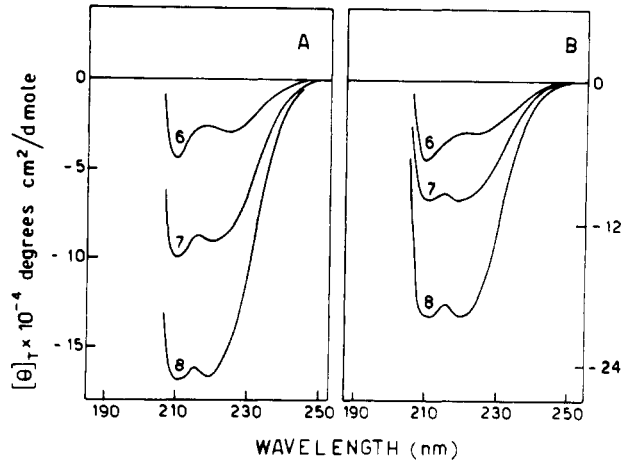

Figure 4. Circular dichroism spectra in ClPrOH (A) and ClBuOH (B) of *t*-Boc-(L-Met)$_{6-8}$-NHPEG.

solvent polarity: ClBuOH > ClPrOH > ClEtOH > TFE > HFIP > HFA. However, it should be stressed that in

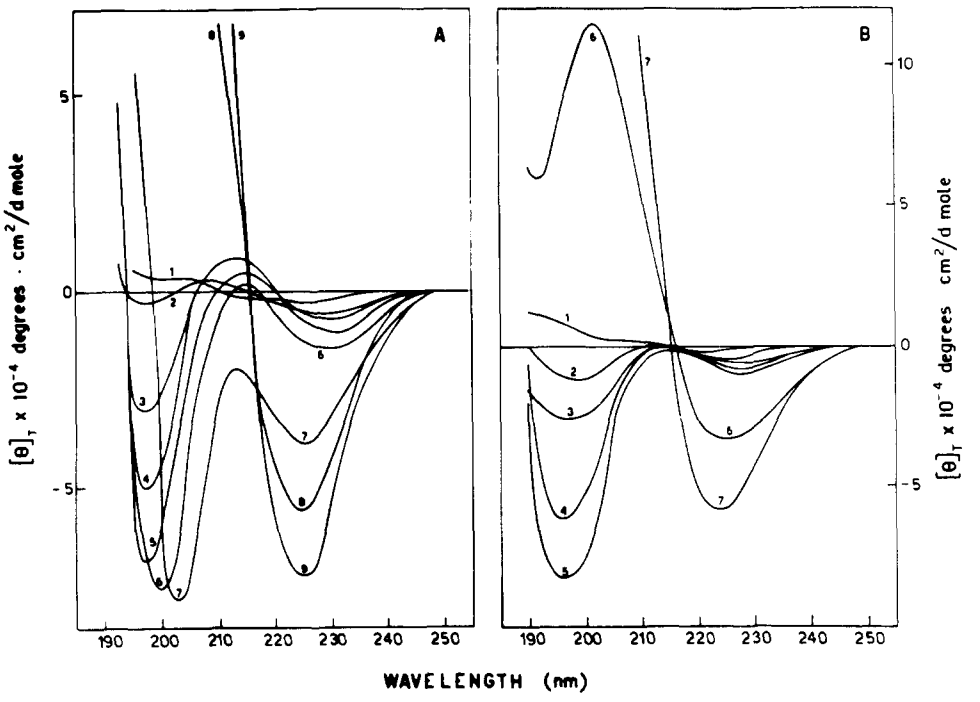

Figure 5. Circular dichroism spectra of *t*-Boc-(L-Met)$_{1-9}$-NHPEG in MeOH (A) and *t*-Boc-(L-Met)$_{1-7}$-NHPEG in H_2O (B).

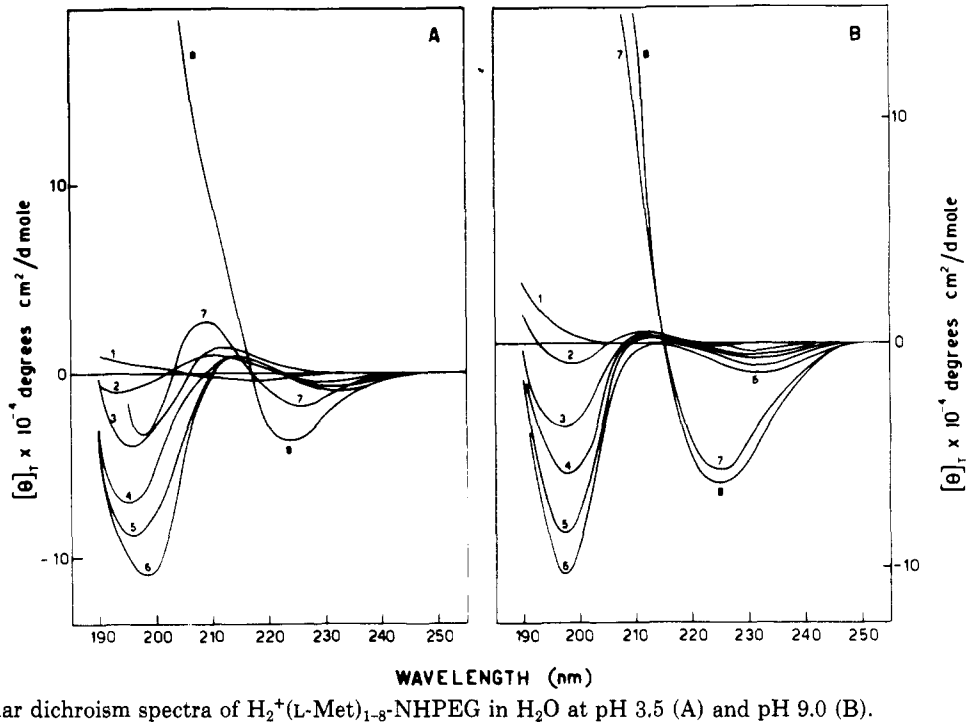

Figure 6. Circular dichroism spectra of H_2^+(L-Met)$_{1-8}$-NHPEG in H_2O at pH 3.5 (A) and pH 9.0 (B).

this paper for the first time we have described CD curves of oligopeptides ($n \leq 20$) partially in α-helical structure in solvents such as HFIP and HFA, generally considered as prototypes of destructive solvents for low molecular weight polypeptides.[8,16,17,19,20,22]

On the other hand, water supports the β structure of the L-Met oligopeptides better than EtOH does, and the latter, in turn, better than MeOH: in fact, the ordered secondary structure is already substantially formed at the hexapeptide stage in water, whereas at $n = 8$ in EtOH and MeOH (at the heptapeptide stage aggregation is more extensively developed in EtOH than in MeOH); furthermore, these peptides are soluble to $n = 7$ in water, whereas to $n = 8$ in EtOH and to $n = 9$ in MeOH (solubility represents a reliable parameter for detecting the onset of a large percentage of β structure). At the hexapeptide–octapeptide level in water, MeOH, and EtOH the formation of solvent–solute hydrogen bonds (solvation and hence absence of ordered secondary structure) is not enough strong to prevail over the formation of solute–solute hydrogen bonds (β-structure formation). The delicate balance of all these interactions makes in particular the three chloroalcohols and TFE, but also HFIP, and, although to a much lower extent, even HFA, suitable solvents for observing the appearance of the α-helical structure. The polarity of solvent, where the α-helix $\rightarrow$ β-structure conversion occurs, appears to be intermediate between those of ClBuOH and MeOH.

Figure 6, compared with Figure 5B, illustrates the effect of the presence of the hydrophobic *t*-Boc N-protecting

Table I
Percentages of Right-Handed α-Helical Conformation of *t*-Boc-(L-Met)$_n$-NHPEG in Various Fluoroalcohols Calculated According to the Relationships Proposed by Greenfield-Fasman[25] (G-F) and Chen-Yang[26] (C-Y)

	TFE		HFIP		HFA	
n	G-F	C-Y	G-F	C-Y	G-F	C-Y
6	12	9	2	2	0	0
7	30	25	9	9	0	0
8	49	44	18	17	2	2
9	61	55	45	44	8	5
10	69	64	50	48	10	8
11	80	76	63	57	14	9
12	81	79	70	66	18	12
13	82	80	76	72	19	14
14	81	82	78	74	20	16
15	80	80	82	81	23	19

group and the state of ionization of the N-terminal amino group on the conformation of homooligo-L-methionines in aqueous solution. From an inspection of the spectra of the oligopeptides with n = 6–8 it turns out that the propensity to form the β structure decreases in the order X = *t*-Boc > H > H_2^+ in X-(L-Met)$_n$-NHPEG (we could never obtain evidence of α-helix formation). It should be noted that the effect of the presence of the *N*-*t*-Boc group on the tendency to form the β structure in the homooligo-L-methionines is opposite in water with respect to TFE. Furthermore, from a comparison of Figures 1B and 6A, in particular from the curves of the octapeptide, it is evident the higher propensity of water with respect to TFE supports the β structure (under identical conditions of the N-terminal amino group).

The absence of isodichroic points in Figures 1–6, which show the CD spectra of the complete series, with the single exception of λ 201 nm in Figure 1A for the oligopeptides with n = 5–15, indicates that in general there is a simultaneous occurrence of a number of species in the conformational equilibrium mixtures of homooligo-L-methionines under the variety of experimental conditions considered. Distorted α-helical structures in HFIP[14] and various types of associated forms different from the classical β structure[32] probably contribute to the observed phenomenon.

The various percentages of right-handed α-helical conformation of *t*-Boc-(L-Met)$_n$-NHPEG (n = 6–15) in TFE, HFIP, and HFA, calculated according to the relationships proposed by Greenfield–Fasman[25] and Chen–Yang,[26] which make use of λ 208 and 222 nm, respectively, are listed in Table I.

In general, the Greenfield–Fasman relationship gives slightly higher values than the Chen–Yang relationship. The scale of solvents in supporting the α-helix formation is that qualitatively discussed above: TFE > HFIP ≫ HFA.

A comparison of the results described in this article on homooligo-L-methionines with those already published on the homologous series derived from L-Ala,[4,5,7,8] L-Val,[5,7,8] L-Ile,[5] L-Glu(OBzl),[6] and L-Glu(OH),[5,6] also covalently linked to the soluble C-protecting macromolecular polyether PEG, allows us to conclude that: (i) in TFE only the homopeptides derived from the L-Met, L-Ala, L-Glu(OBzl), and L-Glu(OH) adopt the α-helical conformation; the scale of stability of this ordered structure appears to be L-Met > L-Ala > L-Glu(OBzl); (ii) in water the homopeptides derived from the hydrophobic amino acid residues L-Met, L-Ala, and L-Val assume a β structure, whereas those from L-Glu(OH), which contains a highly polar group in the side chain, assume the α-helical conformation; the stability of the β structure decreases in the order of decreasing hydrophobicity: L-Met ≃ L-Val, L-Ala; and (iii) the influence of the presence of the *N*-*t*-Boc group in TFE and water, the ionization of the N-terminal α-amino group, and solvent polarity are qualitatively identical and quantitatively very close for the L-Met, L-Val, and L-Ala series.

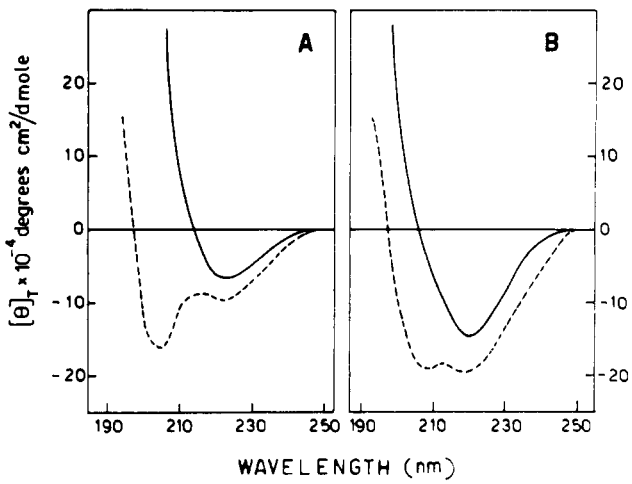

Figure 7. Circular dichroism spectra of *t*-Boc-(L-Met)$_8$-NHPEG at 20 °C (—) and 60 °C (---) in MeOH (A), and H_2^+-(L-Met)$_{10}$-NHPEG in TFE at 20 °C at 4 mg/mL concentration (—) and 0.2 mg/mL concentration (---) (B).

Previous chirospectroscopic studies in solution on monodispersed homooligo-L-methionines having the general formula *t*-Boc-(L-Met)$_n$-OMe (n = 2–7, 9) have suggested that: (i) in HFA to n = 9[16] and HFIP to n = 7[17] (the nonapeptide was not examined in this solvent) the peptides adopt essentially a statistical coil conformation; (ii) in TFE the right-handed α helix substantially develops at the heptapeptide stage, and at the nonapeptide stage it is already extensively formed;[16–18] and (iii) the addition of water to the TFE solutions of the peptides with n = 5–7 induces the onset of β-type structures in a large amount.[17,19]

These results are clearly in excellent agreement with those described throughout this paper and confirm[7,8] that the soluble polymeric support has an extremely limited influence in directing the conformational properties of the peptides to which it is covalently attached.

Finally, since the CD spectra reported in this paper are typical of statistical coil conformations[18] or right-handed α-helical[16,18,27–29] or β conformations,[18,27] the influence of the γ-thioether chromophore upon the chirospectroscopic properties of L-Met, its derivatives, and peptides is confirmed to be negligible.[33]

Various attempts have been conducted with the purpose of disrupting the α-helical structures of the homooligo-L-methionines in TFE and HFIP. In all cases examined the general features of the curves remain unaltered on passing from 20 to 60 °C, only a small decrease (~10–15%) being observed in the ellipticities (in the absolute value).

Figure 7 shows that the β structure of the homooligo-L-methionines can be destroyed by heating or dilution.[17,19] The latter finding strongly supports the view that the β structure involves hydrogen bonds between different peptide chains. It is probable that the complete disruption of the β forms would permit the partial development of the α-helical conformation in these highest homopeptides under these experimental conditions.

A further example of α ⇌ β transconformational change is apparent from Figure 8. The CD curve of *t*-Boc-(L-Met)$_8$-NHPEG, which is still indicative of a partial formation of the α-helical form in the TFE-H_2O 44.9%:51.1% (v/v) mixture, on the other hand clearly suggests the

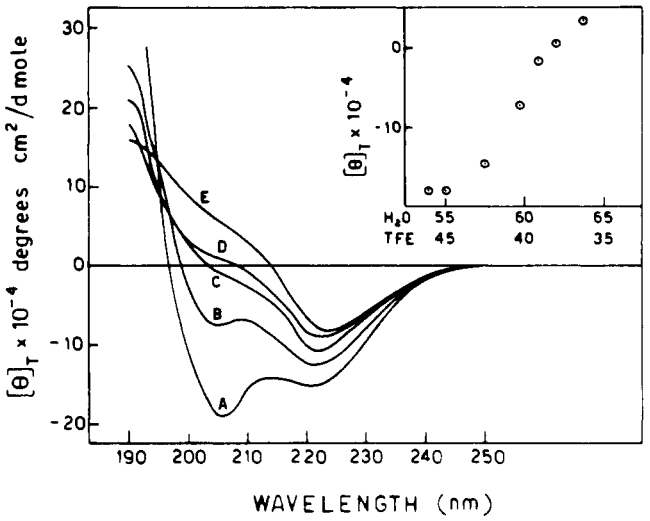

Figure 8. Circular dichroism spectra of *t*-Boc-(L-Met)$_8$-NHPEG in various TFE/H_2O mixtures (v/v): 55.1% H_2O (A), 59.8% H_2O (B), 60.8% H_2O (C), 62.1% H_2O (D), and 63.6% H_2O (E). In the insert is the plot of total molar ellipticity values at 208 nm of the octapeptide vs. percent of H_2O in the TFE solution.

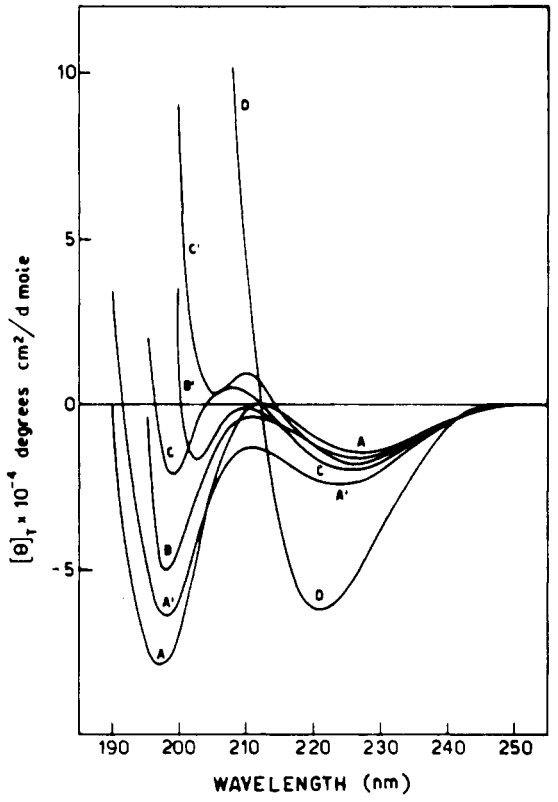

Figure 9. Circular dichroism spectra of *t*-Boc-(L-Met)$_5$-NHPEG in H_2O at 20 °C (A) and 80 °C (A′): in 0.6 M NaCl at 20 °C (B) and at 80 °C (B′), in 0.9 M NaCl at 20 °C (C) and at 80 °C (C′), and in 2.1 M NaCl at 20 °C (D).

partial formation of the β form in the TFE–H_2O 36.4%:63.6% (v/v) mixture (at a percentage of water in TFE higher than 63.6% (v/v) the octapeptide precipitates out of the solution). The conformational transition as a function of the percentage of water in TFE does not reveal any marked cooperative effect (see insert in Figure 8), as expected on the basis of the relatively short chain length of this peptide.

Finally, it is possible to increase the tendency of homooligo-L-methionines to form the β structure in water either by heating the solution or by increasing the ionic strength of the medium (Figure 9). These findings illustrate the important role of hydrophobic interactions on the stability of the associated species and consequently indicate that the critical chain length for the β-structure formation in water depends inter alia upon the temperature and ionic strength of the solution.[7]

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