Linear oligopeptides: 53. Conformations of poly(ethylene glycol)-bound alanine and valine homo-L-oligopeptides in alcoholic solution

G. M. Bonora and C. Toniolo
Biopolymer Research Centre, CNR, Institute of Organic Chemistry, University of Padua, 35100 Padua, Italy
and M. Mutter
Institüt fur Organische Chemie der Universität, D-7400 Tübingen, West Germany
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The conformational properties of poly(ethylene glycol)-bound *N*-t-butyloxycarbonyl-homo-oligo-L-alanines and L-valines to Ala_8 and Val_8 have been examined in alcoholic solution using circular dichroism. This study demonstrates that the L-alanine peptides may exist in right-handed α helical, β and statistical coil conformations depending upon chain length, solvent polarity, temperature and presence of the *N*-blocking group. In addition, the β structures formed by the oligo-L-valines are more stable than those formed by the corresponding L-alanine oligopeptides. The effect of mono- and bifunctional poly(ethylene glycol) upon oligopeptide conformation is also discussed. It was concluded that poly(ethylene glycol)-bound peptides represent a valuable tool for delineating the intrinsic conformational preferences of oligopeptides in alcoholic solution.

INTRODUCTION

Over the past decade there has been a major effort to understand the conformational preferences of linear homo-oligopeptides in alcoholic solution[2-5]. These studies have indicated that side chain, molecular weight, solvent polarity, temperature and nature of blocking groups are all important factors in determining peptide conformation. In particular, it has been shown that L-alanine peptides may exist in right-handed α helical, β or statistical coil conformations[3,4,6-10], while L-valine peptides tend to adopt either a statistical coil or a β conformation of very high stability[3,4,9,11].

In addition, recent studies have been devoted to clarifying the conformation and conformational stability of biologically active polypeptide molecules when covalently linked to a polymeric support[12-14]. This paper is strictly related to the aforementioned investigations since it reports circular dichroism (c.d.) data as a function of temperature and solvent polarity, i.e. in 2,2,2-trifluoroethanol (TFE), 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP) and methanol (MeOH), and in mixed solvents TFE–HFIP and MeOH–HFIP, of the following three complete, monodispersed, poly(ethylene glycol) bound homo-oligopeptide series: t-Boc-(L-Ala-)$_{1-8}$Gly–OPEG, t-Boc-(L-Val-)$_{1-7}$Gly–OPEG and t-Boc-(L-Val)$_{2-8}$Gly–OPEG–M [t-Boc, tert-butyloxycarbonyl; Ala, alanine; Gly, glycine; Val, valine; PEG, poly(ethylene glycol); PEG–M, poly(ethylene glycol)–monomethyl ether]. Their *N*-deblocked derivatives have also been examined. In each case series a single glycyl residue has been incorporated at the C-terminal end of the peptide chain as an internal standard in the amino-acid analyses[15]. The conformational preferences of t-Boc-(L-Ala-)$_{9,10}$OPEG and their *N*-deblocked derivatives in TFE have been reported by Mutter *et al.*[16].

EXPERIMENTAL

Preparation of compounds

The synthesis of t-Boc-(LAla-)$_{1-8}$Gly–OPEG, t-Boc-(L-Val-)$_{1-7}$Gly–OPEG, t-Boc-(L-Val-)$_{2-8}$Gly–OPEG–M has been described elsewhere[15,17]. All compounds were chromatographically and analytically pure, with the single exception of t-Boc-(L-Val-)$_7$Gly–OPEG which was shown to contain $\simeq$ 10% of its lower homologue in the *N*-deblocked form[15]. PEG and PEG–M, molecular weight 10 000, were Hoechst (Frankfurt) products.

Methods

Circular dichroism spectra were recorded using a Cary 61 dichrograph, and 0.5 mm, 1 mm and 1 cm path length cells. Dry prepurified nitrogen was employed to purge the instrument before and during the experiments. Temperature was controlled by means of a hollow-walled, brass cell holder through which water was circulated. The temperature in the cell was determined using a Philips thermistor. No thermal deblocking of the *N*-terminal protecting group occurred, as shown by the absence of glycine in the amino-acid chromatograms from a samples of t-Boc–Gly–OMe which were heated at 65°C for 30 min in MeOH and TFE solutions and subsequently hydrolysed in 1 N NaOH solution. The absence of thermal degradation of the samples was confirmed by regeneration of the original c.d. spectra upon cooling.

The values are expressed in terms of $[\theta]_T$, total molar ellipticity of the peptide moiety. The Lorentz refractive index correction was not applied. The calibration was based upon $[\theta]_{290} = 7.840$ degrees cm^2/dmol for a purified sample of *d*-10-camphorsulphonic acid (Fluka) in 0.1%

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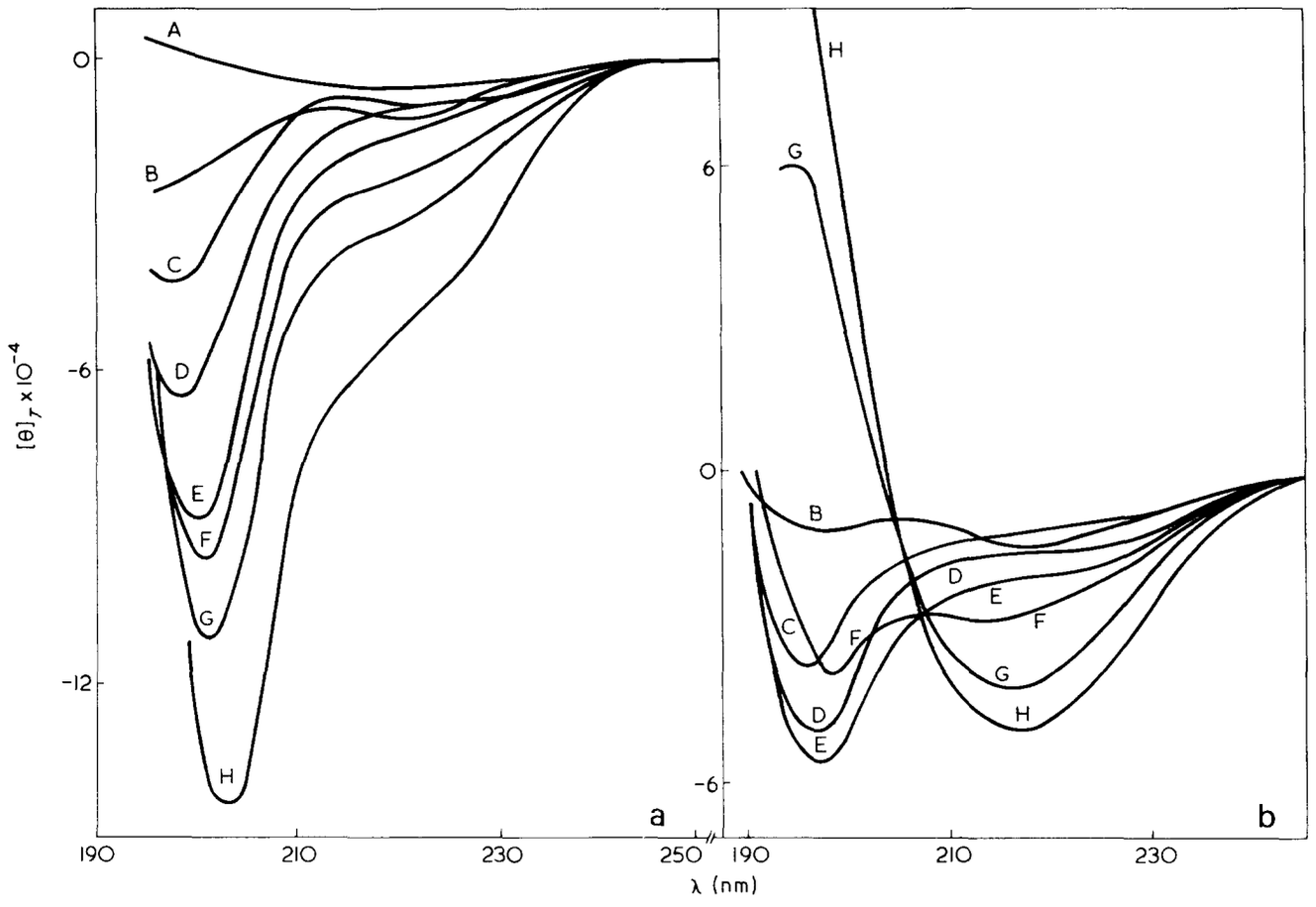

Figure 1 Circular dichroism spectra in TFE of (a) t-Boc-(Boc-L- Ala)$_{1-8}$ Gly–OPEG and (b) $^{+}H_2$-(L-Ala)$_{1-8}$Gly–OPEG; concentration 3 x 10^{-4} M; temperature 20° C; A, n = 1; H, n = 8

aqueous solution. The c.d. data represent average values from at least three separate recordings.

The solvents used for the c.d. measurements were TFE (Fluka, Büchs), HFIP (Eastman, Rochester, New York) and MeOH (Merck, Darmstadt). All solvents were of the highest purity commercially available and were used without further purification.

RESULTS AND DISCUSSION

Effect of chain length and presence of the N*-protecting group*

The c.d. spectra of the *N*-protected and *N*-deblocked alanine and valine homo-oligopeptide series bound to bifunctional and monofunctional PEG, respectively, in TFE are illustrated in *Figures 1* and *2*. In *Figure 1a* it is evident that all *N*-protected alanine peptides exist predominantly in the statistical coil conformation, characterized by an intense negative Cotton effect near 200 nm and a weak negative Cotton effect at 220–225 nm, at 3 x 10^{-4} M concentration[3,9,18]. There are no abrupt spectra variations in going from Ala_3 to Ala_8. However, the red shift of the band near 200 nm (it is observed at 203 nm in Ala_8) with the concomitant increase in intensity of the band at about 220 nm may be indicative of the onset of a right-handed α helical conformation or a β structure in the higher homologues, although to a rather low extent[3,18–21]. The former hypothesis is strongly supported by the recent c.d. results reported by Mutter *et al.*[16] which unambiguously show that t-Boc-(L-Ala)$_{10}$OPEG assumes the right-handed α helical conformation in a substantial amount in TFE. No appreciable changes in the c.d. spectra of our oligoalanines are apparent when the concentration is increased to 1.5 x 10^{-3} M.

In going from $n = 2$ to $n = 8$ in the *N*-protected valine series the c.d. bands in both the 210–220 nm and 195–205 nm regions increase gradually in intensity (*Figure 2a*). The dichroic band associated with the $\pi \rightarrow \pi^*$ transition of the peptide chromophore also exhibits a red shift (from 197 to 205 nm). As above, the c.d. curves of Val_7 and Val_8 are reminiscent of those due to mixtures of a predominantly statistical coil conformation with a minor amount of either the right-handed α helical or a β conformation[3,18–22]. Since, on the basis of a large body of experimental data, valyl residues are commonly considered as β-formers[3,4,11,23–32], we tentatively assign the ordered secondary structure formed in the highest homologues of the valine series as the β conformation.

From a comparison of *Figures 1a* and *2a* with *Figures 1b* and *2b*, respectively, the effect of the presence of the *N*-protecting t-Boc group in both the alanine and valine series stands out clearly. The c.d. patterns of the *N*-deblocked $Ala_{7,8}$ and $Val_{7,8}$ characterized by a strong negative maximum at 215–216 nm and a strong positive maximum in the region of 195 nm[18–21], suggest the onset of a β conformation in considerable amount (the dichroic curves of Ala_6 and Val_6 also indicate the presence of the β conformation, although to a lower extent).

The c.d. spectra of the *N*-protected and *N*-deblocked bifunctional PEG bound valine peptides (not shown) are substantially similar to those reported in *Figure 2*.

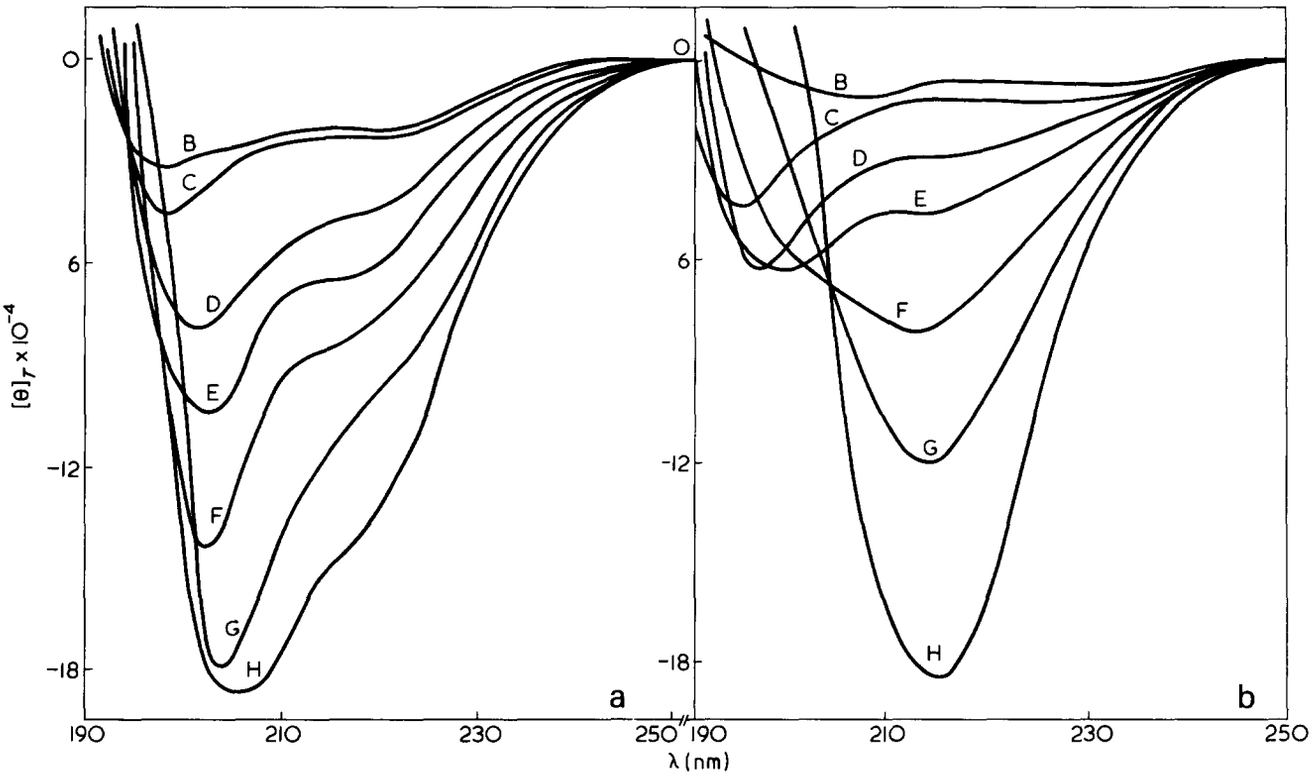

Figure 2 Circular dichroism spectra in TFE of (a) t-Boc{L-Val}$_{2-8}$Gly–OPEG–M and (b) $^{+}H_2$–{L-Val}$_{2-8}$Gly–OPEG–M; concentration 10^{-4} M; temperature, 20°C; A, n = 1; H, n = 8

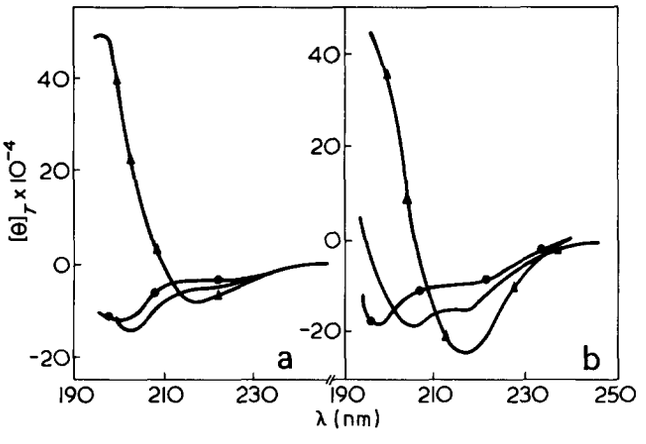

Figure 3 (a) Circular dichroism spectra in TFE (——), HFIP((—●—), and MeOH (–▲–). (a) t-Boc{ L-Ala}$_8$Gly–OPEG, concentration 3 x 10^{-4} M and (b) t-Boc{L-Val}$_8$Gly-OPEG-M, concentration 10^{-4} M; temperature 20°C

From the above data one may conclude that in TFE the absence of the bulky t-Boc group dramatically favours the tendency of the peptides to form a β structure, the onset of which is observed at the $n = 6–7$ stage in all the series examined. When association is prevented, the true tendency of the L-alanine homopeptides to adopt the right-handed α-helical structure becomes evident: the critical chain length for the formation of the α helix is confirmed[3,5,7] to be about seven L-alanine residues.

Effect of solvent polarity

Figure 3 illustrates the influence of solvent on t-Boc{L-Ala}$_8$Gly–OPEG and t-Boc{L-Val}$_8$Gly–OPEG–M conformations. Three alcohols of different polarity have been examined: HFIP > TFE > MeOH[8,9]. Clearly, if the tendency to support the β structure is considered, the scale appears to be MeOH > TFE > HFIP, whereas, if the tendency to support the α helical structure is considered, the scale is as follows: TFE > HFIP.

These results demonstrate that HFIP, which is considerably more acidic than TFE and MeOH[9], requires longer chain lengths for the onset of ordered secondary structures[7,8,11] (according to our analysis the same conclusions apply also to the *N*-deblocked series – now shown). In contrast, in MeOH solvent–solute hydrogen bonding (solvation) is not strong enough to prevail over solute–solute hydrogen bonding (β structure formation) in the case of Ala_8 and Val_8; a detailed conformational analysis of the three *N*-protected and *N*-deblocked series (not shown) suggests that in MeOH the onset of the β structures also takes place at the $n = 6–7$ stage[8]. The delicate balance of the various interactions makes TFE a favourable solvent for observing the appearance of the α helical form[3] (in the alanine series).

Effect of side chain and temperature

A first example of the influence of side chain in determining the conformation of alanine and valine peptides has been already discussed (compare *Figures 1a* and *2a*). Less ambiguous examples are shown in *Figures 4* and *5*. The addition of 30% HFIP (v/v) to a solution of t-Boc{L-Ala}$_7$Gly–OPEG in MeOH induces a dramatic conformational variation from a β to a statistical coil structure (*Figure 4a*). In contrast, a more gradual change is apparent in the case of the valine analogue, the transition midpoint being observed at about 45% HFIP (v/v) (*Figure 4b*). It is possible that the occurence of a small amount of the *N*-deblocked Val_6 in the Val_7 sample (see Experimental) would be responsible, at least in part of this experimental finding; however, this should not be in the direction of altering the observed scale of stability

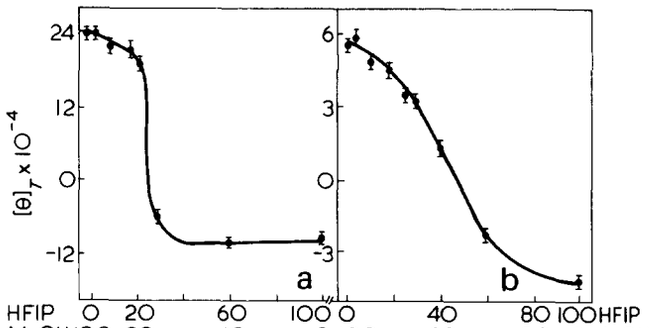

Figure 4 Plots of total molar ellipticity values at 200 nm in various MeOH–HFIP mixtures at 20°C (concentration 3 x 10^{-4} M) of (a) t-Boc{L-Ala}$_7$Gly–OPEG and (b) t-Boc{L-Val}$_7$Gly–OPEG

of the β associated structures which is as follows: Val_7 > Ala_7.

Our c.d. study also showed that the β structures taken by Ala_6 in MeOH can be completely destroyed by increasing the temperature (*Figure 5*). Again, the stability of the β form of valine peptides appears to be higher than that of the alanine analogues.

As far as the effect of side chain on peptide conformation is concerned, it is possible to conclude that β branching, as in valyl residues, induces the onset of rather stable β structures in solution. The above findings are in line with literature data on this subject[3,4,11,26]. In addition, it was also previously reported that β structures of homo-oligopeptides in alcoholic solution can be disrupted by heating[4,8,11].

Effect of mono- and bifunctional PEG

A thorough analysis of all the c.d. data accumulated in the present study of PEG (and PEG–M)-bound alanine and valine homo-oligopeptides (to Ala_8 and Val_8), in comparison with those previously reported on the analogous compounds containing non-polymeric C-terminal protecting groups[3,4,7–11], first indicate that the effect of PEG and PEG–M upon peptide conformation is dependent upon the polarity of the alcoholic solution.

In the acidic alcohol HFIP all alanine and valine homopeptides exist in a statistical coil conformation which is independent of the presence and nature of the blocking groups (see *Figures 3* and *4* and refs 4, 7–9 and 11).

In TFE, an alcohol of intermediate polarity, t-Boc{L-Ala}$_7$OMe exists essentially in the β conformation at a concentration of 1.2 x 10^{-3} M[4], whereas t-Boc{L-Ala}$_7$Gly–OPEG exibits a low amount of α helical form, as the only ordered secondary structure, under the same experimental conditions (see above). Dilution of the t-Boc{L-Ala}$_7$OMe solution ultimately causes a disruption of the associated structure, resulting in a spectral pattern similar to that observed for partly developed α helical forms, e.g. that of t-Boc{L-Ala}$_7$Gly–OPEG[4,8]. Thus, it appears that the competition between β associated and unassociated (either α helical or statistical coil) forms would depend on the C-blocking group employed. The present conclusions complement the observation made by Goodman and coworkers[7] that the nature of the *N*-blocking group definitely influences the tendency to form associated structures for homo-oligoalanines in TFE solution. It is of interest to note, however, that the nature of *N*- and C-blocking groups does not seem to have any influence on the critical chain length for α helix formation (about seven residues, i.e. two turns of the helix). Further support for the above conclusions derives from a comparison of the c.d. properties of the t-Boc{L-Val}$_7$OMe with those of t-Boc{L-Val}$_7$Gly–OPEG–M and t-Boc{L-Val}$_{6,7}$Gly–OPEG. At comparable concentrations (≃ 1–3 x 10^{-1} M) a stable β-associated form is adopted by the former oligopeptide[4], while an essentially statistical coil conformation (most probably also containing a minor amount of β structure) in the PEG (or PEG–M)-bound oligopeptides.

In MeOH, the least powerful of the three solvents examined, t-Boc{L-Ala}$_{5-7}$OMe[8] and t-Boc{L-Ala}$_{6-8}$Gly–OPEG (see above) assume predominantly β associated structures at similar concentrations (≃ 3 x 10^{-4} M). In the two Ala_7 derivatives disruption of the ordered secondary structure is achieved by addition of HFIP: 30% (v/v) HFIP for t-Boc{L-Ala}$_7$Gly–OPEG (*Figure 4a*), but more than 60% (v/v) HFIP for t-Boc{L-Ala}$_7$OMe[33]. Hence, in MeOH solution the influence of PEG upon the association of peptide chains is also apparent, although less critical than in TFE.

Finally, if the effects of mono- and bifunctional PEG are compared (in the valine series), no remarkable difference could be detected in any of the alcohols considered.

To summarize, differences were found between the c.d. spectra of PEG- and MeO-protected homo-oligopeptides in cases where the stability of the ordered structure is strongly dependent upon solvent polarity and concentration, e.g. in the transition region of the statistical coil and β-like structures. PEG enhances the solvation of the peptide chains by preventing their aggregation in poor solvents. Specific interactions between PEG and peptide, e.g. through formation of stable hydrogen bonds, appear to be absent. This finding is in harmony with the conformational characteristics of PEG, which exhibits a flexible statistical coil with very low density under these conditions. Yet, the alignment of several peptide chains to form hydrogen-bonded aggregates may induce entropically and sterically unfavourable effects in the polymeric ester moiety. Only in cases where strong lyophobic interactions between the side chains of the peptide part are compensating these effects, the peptide chain also tends to associate in the form of its PEG ester; this phenomenon is observed when the peptide grows longer, when the concentration is increased or when the solvent is incapable of affo.ding sufficient solvation energy[34]. In particular, when the ordered structure is stabilized by intrapeptide chain hydrogen bonds, as in the α helix, no influence of PEG upon the stability of the ordered structure could be detected[35].

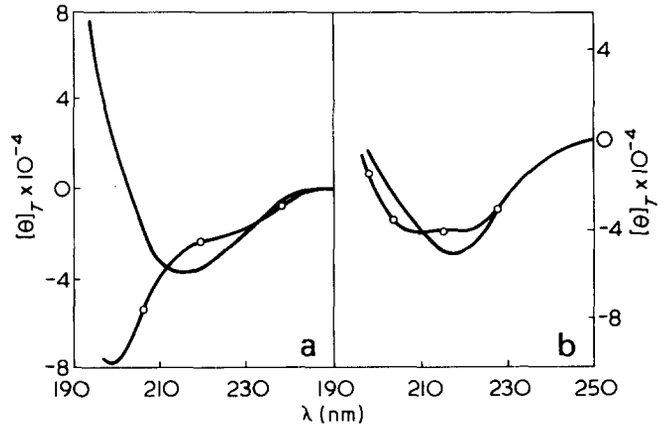

Figure 5 Circular dichroism spectra in MeOH at 20°C (——) and 60°C (—o—) of (a) t-Boc{L-Ala}$_6$Gly–OPEG and (b) t-Boc{L-Val}$_6$Gly–OPEG; (concentration 3 x 10^{-4}M)

Because the aggregation of PEG-bound peptides is hindered, if not prevented, the true tendency of the oligopeptides for the formation of the ordered structures becomes more evident. Consequently, PEG bound peptides represent a valuable tool for delineating the intrinsic conformational preferences of oligopeptides in solution.

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