CIRCULAR DICHROISM AND INFRARED STUDY OF β-TURN FORMATION IN REPEAT PEPTIDES OF ELASTIN

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The conformation of elastin-like peptides Boc-Ala-Pro-Gly-Val-APEGM, Boc-Ala-Pro-Gly-Val-Gly-Val-APEGM, Boc-Ala-Pro-Gly-Val-Ala-Pro-Gly-Val-Gly-Val-APEGM, Boc-Ala-Pro-Gly-Val-Gly-Val-Ala-Pro-Gly-Val-Gly-Val-APEGM were examined in solution using circular dichroism at 30°C, 50°C, and 70°C and in solid state by IR at room temperature. The studies show that the β-turn is a significant conformational feature for peptides under investigation in solution at 30°C and 50°C, but at 70°C the tetra, hexa, and decappetides show the CD feature characteristic of the β-structure while the dodecapeptide spectra show the presence of β-turn which indicates the stability of the β-turn at this chain length. The IR spectra show that in the solid state at room temperature all investigated peptides assume essentially a β-turn except the tetrapeptide which present evidence of antiparallel β-structure. The β-turn contribution in the IR spectra increases with the increase of the chain length of the peptide.

In the present contribution the conformation of elastin-like peptides *I*–*IV* is examined in the solution using circular dichroism measurements and in the solid state using IR spectroscopy. The synthesis of peptides *I*–*IV* was described in the previous paper[1]. The peptides are covalently bounded to the poly(ethylene glycol) monomethyl ether which increases the solubility of the attached peptide moiety and has no influence on its conformation. As anchoring group for the peptide attachment a photosensitive 3-nitro-4-bromomethylbenzoyl handle, denoted as A in formulae, was applied.

I	Boc-Ala-Pro-Gly-Val-APEGM
II	Boc-Ala-Pro-Gly-Val-Gly-Val-APEGM
III	Boc-Ala-Pro-Gly-Val-Ala-Pro-Gly-Val-Gly-Val-APEGM
IV	Boc-Ala-Pro-Gly-Val-Gly-Val-Ala-Pro-Gly-Val-Gly-Val-APEGM

EXPERIMENTAL

The synthesis of monodispersed, chemically and optically pure peptides mentioned above using the liquid-phase method[2] are reported elsewhere[1]. Circular dichroic spectra were recorded

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using a Jouan Model CD 185 spectrophotometer and 0·5 mm, 1 mm path length quartz cells. Dry prepurified nitrogen was used to keep the instrument oxygen free during the experiments. The values are expressed in terms of $[\Theta]_T$, total molar ellipticity. The calibration was based upon $[\Theta]_{304} = +10{\cdot}870$ deg cm^2 dmol^{-1} for epiandrosterone (Roussel-Jouan, Paris) in dioxane. Spectra obtained at 30, 50, and 70°C. The solutions were prepared as 1 mg of peptide *per* 1 ml trifluoroethanol. IR absorption spectra were recorded on Perkin–Elmer Infracord. The samples were prepared in the form of KBr disc containing 1·5 mg of peptide in 300 mg KBr.

RESULTS AND DISCUSSION

In the region of characteristic electronic absorption bands for amide groups between 200–240 nm the CD spectra have been measured. The experimental data are reported in Figs 1–4 for solutions in trifluoroethanol of equal concentration. The ellipticity *per* each residue is then calculated, by $\Theta = \Delta\varepsilon \times 3\,300$ (deg cm^2 dmol^{-1}).

Fig. 1 shows the CD spectra of the tetrapeptide Boc-Ala-Pro-Gly-Val-APEGM (*I*) at 30°C, 50°C, and 70°C. The spectral patterns at 30°C exhibits a negative maximum at 225 nm, a zero crossing point at 210 nm, and a positive maximum at 205 nm. At 50°C the CD curve *2* shows a negative Cotton effect at 222·5 nm, zero crossing point at 210 nm, and positive maximum at 205 nm. The curve *3* in Fig. 1 represents the CD spectra of the tetrapeptide *I* at 70°C. It shows a negative Cotton effect at 220 nm, a zero crossing point at 209·3 nm, and a positive Cotton effect at 202·5 nm. The shape of curves *1* and *2* is similar to that described by Woody *et al.*[3] for β-turn

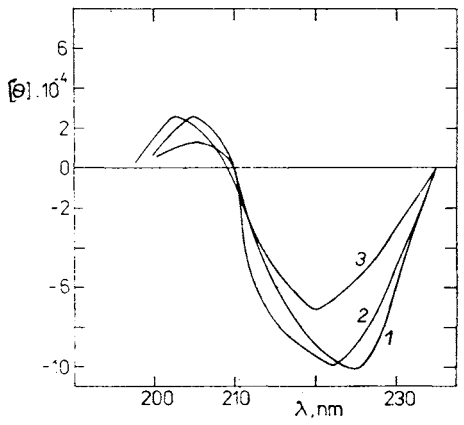

FIG. 1

CD spectra of the tetrapeptide *I* in trifluoroethanol at 30°C (*1*), 50°C (*2*), and 70°C (*3*)

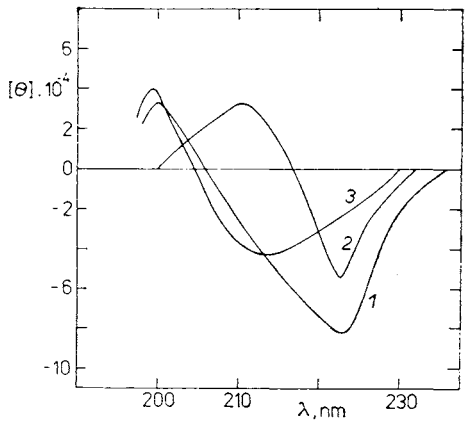

FIG. 2

CD spectra of the hexapeptide *II* in trifluoroethanol at 30°C (*1*), 50°C (*2*), and 70°C (*3*)

conformation while in curve 3 the negative maximum is shifted towards shorter wavelength and the general shape of the spectra is similar to that of the β-structure[4].

Fig. 2 shows the CD spectra of the hexapeptide Boc-Ala-Pro-Gly-Val-Gly-Val--APEGM (*II*) at 30°C (*1*), 50°C (*2*), and 70°C (*3*). The spectra of this peptide at 30°C and 50°C indicate the presence of the β-turn (negative Cotton effect at 223·5 (*1*), 222·5 (*2*), which is assigned to $n-\pi^*$ transition, the $\pi-\pi^*$ transition appears between 200–210 nm as a positive Cotton effect and the zero crossing point shines between 205·75–216·5 nm). The spectra at 70°C show no more the existence of the β-turn. The spectra in this case (negative Cotton effect at 215 nm and positive Cotton effect at 199·5 nm) look like that described by Fasman *et al.* for the β-structure[4].

Fig. 3 presents the CD spectra of the decapeptide Boc-Ala-Pro-Gly-Val-Ala-Pro--Gly-Val-Gly-Val-APEGM (*III*). In this case the spectral pattern is similar to that of the hexapeptide at 30°C and 50°C. The spectrum is in agreement with that described by Woody *et al.*[3] for β-turn conformation (negative Cotton effect in the range 222·5–224 nm, positive Cotton effect at 205 nm, and zero crossing point between 212·5 and 214·5 nm). At 70°C the conformation of the peptide seems to be β-structure (positive maximum at 210 nm, negative maximum at 220 nm, and zero crossing point at 213·5 nm).

Fig. 4 represents the CD spectra of the dodecapeptide Boc-Ala-Pro-Gly-Val-Gly--Val-Ala-Pro-Gly-Val-Gly-Val-APEGM (*IV*) at 30°C, 50°C, and 70°C. The spectra exhibits a negative Cotton effect in the range of 225–227·5 nm and a positive Cotton

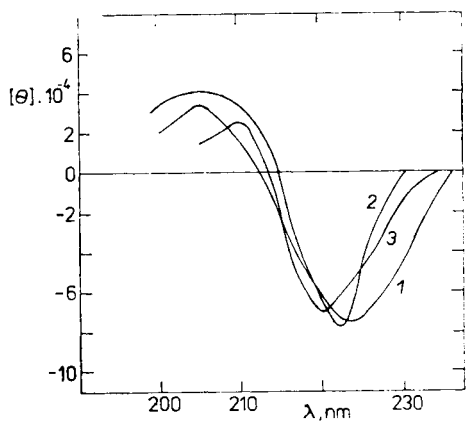

FIG. 3

CD spectra of the decapeptide *III* in trifluoroethanol at 30°C (*1*), 50°C (*2*), and 70°C (*3*)

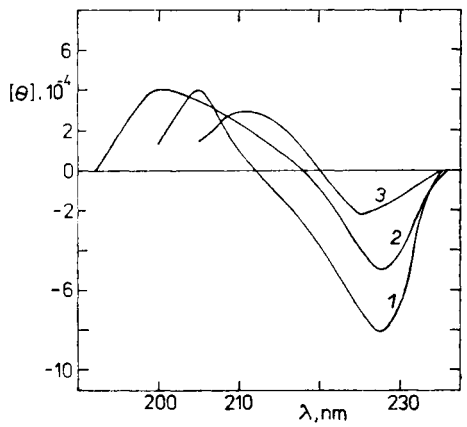

FIG. 4

CD spectra of the dodecapeptide *IV* in trifluoroethanol at 30°C (*1*), 50°C (*2*), and 70°C (*3*)

effect in the range 200–210 nm. The zero crossing point is between 212–220 nm which indicates the presence of the β-turn conformation at all temperatures.

The CD analysis of the tetra, hexa, deca, and dodecapeptides *I–IV* suggests that these peptides form a β-turn conformation in trifluoroethanol at 30 and 50°C. At 70°C the tetra, hexa, and decapeptides *I–III* show the CD feature characteristic of the β-structure while the dodecapeptide spectrum at this temperature indicates the presence of β-turn[5]. Increasing temperature causes no detectable change in the CD spectrum of the dodecapeptide *IV* which shows that the β-turn is more stable at this chain length.

From the above results, the CD studies indicate that β-turn is a significant conformational feature for the peptides *I–IV* which is in good agreement with the NMR studies[6]. This indicates the existence of the β-turn in the oligopeptides of elastin and the occurrence of it in repealing sequences. These results confirm the postulation of Ohnishi *et al.*[7] that the presence of large percentage of glycine residues would, when in appropriate sequence with bulky and hydrophobic L-amino acids, result in the formation of the β-turn conformation. The proposed β-turn for the investigated peptides is hown in Fig. 5.

The solid-state conformational preferences of the poly(ethylene glycol) bound peptides were examined by the IR absorption. The characteristic absorption bands of poly(ethylene glycol) do not interfere with the spectral regions of interest (Amide A 3 200–3 450 cm^{-1}, Amide I 1 600–1 750 cm^{-1}, and Amide V 600–750 cm^{-1}). It was reported by Toniolo *et al.*[8] that Amide I and Amide V regions are the most sensitive to conformational changes of peptide main chain[9,10]. Consequently, they were used as the principal, although not exclusive, diagnostic tools. At this point it should be recalled that unordered conformation of polypeptides show the Amide I and Amide V bands near 1 655 cm^{-1} and 650 cm^{-1}, respectively, the various types of β-structure have strong bands near 1 635 cm^{-1} and 710 cm^{-1}, whereas absorption at about 1 650 cm^{-1} and 615 cm^{-1} are associated with α-helix[9]. It is important to point out that the carbamate chromophore of the tert-butyloxycarbonyl group also exhibits vibrational bands[11] in the aforementioned two spectral regions. Obviously,

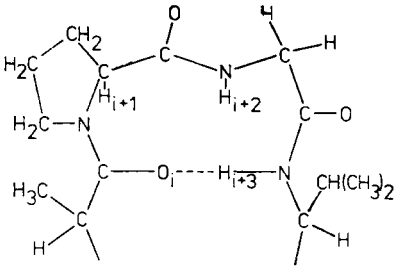

FIG. 5
Proposed β-turn for peptides *I–IV*

however, their relative weight decreases with increasing number of the peptide bond in the chain.

The results of the IR studies are shown in Table I and Fig. 6. The spectrum of the tetrapeptide *I* exhibits a strong absorption band at 740 cm^{-1} and very strong band at 1 690 cm^{-1} (Amide I). It has also a shoulder at 1 720 cm^{-1} and a sensitive band at 3 420 cm^{-1} (Amide A). The band at 1 690 cm^{-1} is usually assigned to the parallel components of the splitted Amide I of polypeptides in the antiparallel β-form. However in the present case, a conformational assignment would be ambiguous since the carbamate chromophore of the tert-butyloxycarbonyl group also exhibits an absorption at 1 685 – 1 695 cm^{-1}.

The IR spectra of hexa, deca, and dodecapeptides *II*–*IV* (Table I) compared to the spectra of unordered conformation, β-structure, and the α-helix show significant differences which indicate the presence of a new conformer characterized by 15 to

TABLE I

IR absorption frequencies (wavenumbers in cm^{-1}) of the poly(ethylene glycol) bound peptides

Peptide	Amide V	Amide I	Amide A
I	740	1 690, 1 720 sh	3 420
II	740	1 675, 1 725 sh	3 420
III	740	1 670, 1 725 sh	3 420
IV	740	1 660, 1 725 sh	3 420

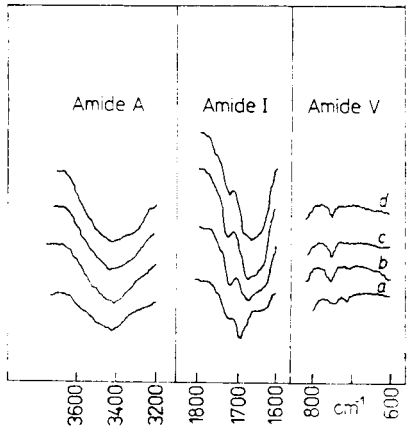

FIG. 6

IR absorption spectra of tetrapeptide *I* (*a*), hexapeptide *II* (*b*), decapeptide *III* (*c*), and dodecapeptide *IV* (*d*) in Amide A, Amide I, and Amide V region

30 cm^{-1} shift to lower wavenumbers of the band attributed to the carbonyl group (Amide I). These spectra are in agreement with those obtained by Deber[12] who referred them to the presence of the β-turn, the shift to lower wavenumbers of the Amide I band in hexa, deca, and dodecapeptides *II–IV* may reflect an increase of the double bond character of the C—N bond of the peptide group, but weakness the C=O bond.

Fig. 6 shows that the β-turn contribution to the spectra is increasing with the increase of the chain length of the peptide. It was also observed that all the investigated peptides *I–IV* have a well defined band at 740 cm^{-1} (Amide V) and another strong one at 3 420 cm^{-1} (Amide A).

The IR studies indicate that in the solid state all peptides *I–IV* assume essentially a β-turn conformation except the tetrapeptide *I* which presents evidence of antiparallel β-structure. The presence of the β-turn in hexa, deca, and dodecapeptides *II–IV* in solid state and the increase of its contribution with the increase of the chain length is in agreement with the results obtained by the CD analysis in solution.

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