Spectroscopic Study of Environment-Dependent Changes in the Conformation of the Isolated Carboxy-Terminal Telopeptide of Type I Collagen†

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ABSTRACT: The C-terminal telopeptide of the α1 chain of type I collagen from bovine skin was isolated from a bacterial collagenase digest. Two forms of the telopeptide were obtained, one with two and the other with three residues of tyrosine. In both of these, the single lysyl residue had been oxidized to α-aminoadipic δ-semialdehyde. Circular dichroism spectra of the telopeptide in aqueous solution at neutral pH were interpreted as indicating the presence of little regular secondary structure. However, sodium dodecyl sulfate at a concentration of 40 mM induced some α helix, as predicted from the sequence, and trifluoroethanol also induced secondary structure, probably a mixture of α helix and β sheet. A major feature of the circular dichroism spectra of the telopeptide in sodium dodecyl sulfate, in denaturing agents, and in sodium phosphate buffer at low temperature was a positive band at 227 nm due to tyrosine side-chain chromophores. The disappearance of this band on heating and at high pH was ascribed to the adoption by the telopeptide of a specific tertiary structure. Poly(ethylene glycol) 1000 used as a perturbant in UV difference spectroscopy caused conformational changes resulting in decreased accessibility of tyrosine side chains and transfer of these to a less polar environment. A structural model in which the four aromatic side chains of the telopeptide are arranged in two pairs with the rings antiparallel is proposed to account for these results.

Type I collagen makes up the bulk of the fibers which give mechanical strength to many connective tissues including tendon, skin, bone, and dentin. Like other collagens, it consists of molecules composed of three polypeptides (α chains) each over 1000 amino acid residues long. Two of these chains are identical and are termed α1(I), and one is distinct and termed α2(I). Each α chain is made up of 338 contiguous Gly-X-Y tripeptides, together with short N- and C-terminal sequences, called telopeptides, which do not have glycine in every third position and therefore cannot adopt the characteristic collagen triple-helical structure (Glanville & Kuhn, 1979). The α1(I) chain N-telopeptide consists of 16 residues and the C-telopeptide 25 residues. The corresponding regions of the α2(I) chain are 9 and 6 residues long.

One of the most striking properties of type I collagen in solution is its ability to form native D-periodic fibrils under physiological conditions of temperature and pH. Since originally described (Gross et al., 1955; Jackson & Fessler, 1955), this phenomenon has been studied intensively with a view to defining a mechanism of fibrillogenesis which could operate both in vivo and in vitro. During early work on collagen structure, it was found that treatment with proteinases such as pepsin (Rubin et al., 1963) and Pronase (Drake et al., 1966), which are unable to attack the collagen triple helix, severely impairs the capacity to form fibrils. This effect was attributed to removal of the telopeptides, a conclusion which has since been amply confirmed. More recent studies have added details concerning the roles of the telopeptides in fibrillogenesis; the N-telopeptide appears to be important during the nucleation phase and in elongation, while the C-telopeptide promotes lateral assembly as well as linear growth (Leibovich & Weiss, 1970; Helseth & Veis, 1981a; Capaldi & Chapman, 1982). It is likely that these functions depend on the adoption of specific conformations by the telopeptides. Indeed, it has been suggested that conformational changes occur during the initial temperature-dependent, concentration-independent lag phase of fibrillogenesis (Gelman et al., 1979), and data obtained by using fluorescent probes (Crabtree & Fujimori, 1980; Fujimori & Shambaugh, 1981) support this idea.

Helseth and co-workers have applied the Chou–Fasman predictive criteria to the known sequences of the N- (Helseth et al., 1979) and C-telopeptides (Helseth & Veis, 1981b). However, there appears to be only one published physical study of telopeptide conformation in solution—that of Chandrakasan et al. (1976), who interpreted the proton nuclear magnetic resonance (NMR)[1] spectrum of collagen in 0.5 N acetic acid to indicate that the telopeptides were unstructured under these conditions. The present paper reports what may be the first attempt to define some conformational properties of the isolated α1(I)-chain C-telopeptide in solution under a variety of conditions.

Experimental Procedures

Purification of α1CB6. Type I collagen was extracted from fetal calf skin with acetic acid and purified by repeated precipitation with NaCl as described by Volpin and Veis (1971). Portions (0.6 g) of this collagen were dissolved in 75 mL of 70% (w/v) formic acid which had been previously flushed with N_2 for 15 min. Cyanogen bromide (0.9 g) was then added and the solution incubated at 27 °C under N_2 for 4 h. The digest was diluted 20-fold with water, lyophilized, then redissolved in water, and relyophilized. The efficiency of digestion was checked by examining the peptides by electrophoresis on SDS–polyacrylamide gels (12% T, 0.4% C) by the method of Laemmli (1970). The products from two digestions (1.2 g) were combined for the isolation of α1CB6 [the C-terminal peptide of the α1(I) chain] as follows. Portions (0.3

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[1] Abbreviations: NMR, nuclear magnetic resonance; HPLC, high-performance liquid chromatography; Gdn·HCl, guanidine hydrochloride; SDS, sodium dodecyl sulfate; TFA, trifluoroacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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g) of the digest were dissolved in 20 mL of 0.02 M sodium citrate, pH 3.6, containing 4 M urea (buffer 1) and applied to a 2.5 cm × 31 cm column of Sephadex G50 Fine (Pharmacia Fine Chemicals Ltd.) equilibrated and eluted with buffer 1. The excluded fraction (60 mL) was then applied to a 2.5 cm × 12 cm jacketed column of carboxymethylcellulose (Whatman CM 52) at 42 °C and eluted with a linear gradient of 0–0.14 M NaCl in buffer 1 (1 L total volume). Fractions were desalted on a 3.5 cm × 40 cm column of Bio-Gel P2 (100–200 mesh, Bio-Rad Labs Ltd.), eluted with 0.1 M acetic acid, lyophilized, and examined by gel electrophoresis. This step yielded a mixture of α1CB6 and α1CB7 which was then resolved by chromatography on CM 52 in 0.03 M sodium acetate, pH 4.8, and 4 M urea by using a linear gradient (800 mL total volume) from 0 to 0.12 M NaCl. After being desalted, gel electrophoresis and amino acid analysis were used to select the fraction containing pure α1CB6 with the highest proportion of intact C-terminal telopeptide (see later).

Isolation and Purification of Telopeptides. A portion (20 mg) of pure α1CB6 was dissolved in 2 mL of 0.025 M Tris-HCl, pH 7.5, and 0.01 M $CaCl_2$, heated to 95 °C for 2 min, and then cooled to room temperature. One vial (2500 units) of purified bacterial collagenase (Advanced Biofactures type III) was dissolved in 1 mL of the same buffer. Digestion of α1CB6 was carried out at 37 °C over 3 days by adding a fresh aliquot (100 μL) of collagenase solution each day after the digest was neutralized with 0.1 M NaOH. The progress of digestion was monitored by removing portions of the digest and examining these by chromatography on a column of Sephadex G50 Superfine (see figure legends for details). After 3 days, when there was no further change in the elution profile, the whole digest was applied to G50. The fraction containing the telopeptides (as determined by amino acid analysis) was rechromatographed on the same column, lyophilized, and then dissolved in 0.1% (v/v) trifluoroacetic acid and chromatographed on a reverse-phase HPLC column (Vydac 201TP104, Separations Group Inc.) eluted with a linear gradient of acetonitrile in trifluoroacetic acid. The final pure telopeptide preparations were stored dry at 4 °C.

Preparation of N-Acetyl-L-tyrosyl-L-tyrosine. L-Tyrosyl-L-tyrosine (Sigma Chemical Co.) was dissolved in 1 mL of half-saturated sodium acetate. Acetic anhydride (0.1 mL) was then added in five equal portions over 1 h to the stirred solution at 0 °C. A portion of the reaction mixture was examined by reverse-phase HPLC, as above. The relatively complex elution profile obtained was interpreted as evidence of O-acetylation. This was reversed by raising the pH to 11.4 by addition of NaOH and incubating at 40 °C for 2 h. One major component was then seen on HPLC. To confirm that N-acetylation had occurred, the electrophoretic mobility of this sample was compared at low pH [electrophoresis buffer was 15% (v/v) acetic acid/5% (v/v) formic acid] with that of unreacted tyrosyltyrosine on a 10 cm × 10 cm cellulose thin-layer plate (Chromagram, Eastman Chemical Co.) which was then examined under fluorescent light and after being sprayed with ninhydrin reagent (Stahl, 1965).

Amino Acid Analyses. Samples were hydrolyzed at 105 °C for 22 h in redistilled constant-boiling HCl containing phenol (0.2% w/v) and analyzed in duplicate on a Beckman 121MB amino acid analyzer. A sample of each telopeptide (20 μg) (see later) was dissolved in 0.2 mL of 0.05 M sodium phosphate, pH 7.05, and reduced by addition of 100 μg of tritiated sodium borohydride (200 mCi/mmol; Amersham-Searle) at 0 °C for 2 min. Reaction was terminated by addition of glacial acetic acid and lyophilization. The reduced peptides were reisolated by HPLC, hydrolyzed, and analyzed as above but with the effluent from the colorimeter of the analyzer being collected in 1-mL (2-min) fractions and counted in a liquid scintillation counter (Scott & Goldberg, 1983).

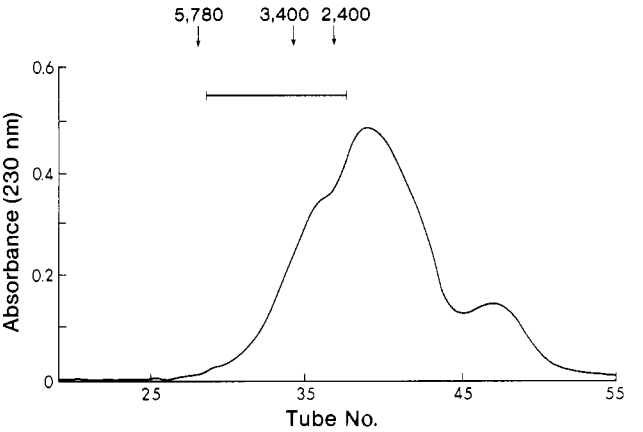

FIGURE 1: Chromatography of collagenase-digested α1CB6 on Sephadex G50 Superfine. Approximately 20 mg in 2.4 mL was applied to a 1 cm × 65 cm column eluted with 0.1 M acetic acid at a flow rate of 5 mL/h. Fraction size was 1.4 mL. Bars show fractions which were pooled, lyophilized, and rerun on the same column. Arrows show elution positions of insulin (M_r 5780), insulin A chain (M_r 3400), and insulin B chain (M_r 2400) used to calibrate the column.

Circular Dichroism Spectroscopy. Samples of telopeptide were dissolved in water at a concentration of approximately 1.25 mg/mL. Portions of this stock solution were taken and diluted to a final concentration of about 0.25 mg/mL in the appropriate solvent or buffer. The actual concentrations were determined by amino acid analysis, as were the mean residue weights. Acetylated tyrosyltyrosine was examined at a concentration of 0.06 mg/mL. Spectra were recorded over the range 250 or 240–200 nm in a Jasco J-20A spectropolarimeter in jacketed thermostated cells of 1- or 0.1-mm path length. The instrument was calibrated with *d*-10-camphorsulfonic acid (Eastman) which had been twice recrystallized from acetic acid and dried over P_2O_5 (DeTar, 1969). Other details are given in the figure legends.

UV Difference Spectroscopy. UV absorption spectra were determined with a Gilford 250 spectrophotometer on solutions of tyrosine (1 mM) in 0.05 M sodium phosphate buffer, pH 7.05, and on C-terminal telopeptide (0.13 mM, pH 6.0) in water at room temperature. A sufficient volume of a 66% (w/v) aqueous solution of poly(ethylene glycol) 1000 (Sigma) to give 11% (w/v) was then added to sample and reference cells and the spectrum read again. This was repeated with a second addition of the poly(ethylene glycol) solution to give 18.9% (w/v) concentration of perturbant. Difference spectra were calculated by manual subtraction after correction for dilution by poly(ethylene glycol) solution. For calculation of molar difference extinction coefficients, the concentration of peptide was determined from the absorbance at 276 nm in the absence of perturbant.

RESULTS

Purification of Telopeptides. Amino acid analysis demonstrated that the material eluting earliest on gel chromatography of the collagenase digest of purified α1CB6 on G50S (see Figure 1) contained tyrosine and histidine which are characteristic of the telopeptide. This material was rechromatographed on the same column to yield a fraction which was about 80% pure telopeptide, as judged from the amino acid composition. The molecular weight estimated by gel filtration was 2800 ± 400, corresponding to about 26 residues.

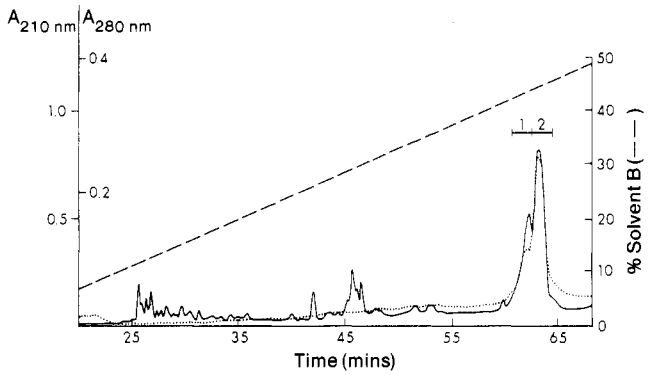

FIGURE 2: Final purification of telopeptides by HPLC. The telopeptide-containing fraction isolated by gel chromatography was dissolved in 0.2 mL of 0.1% (v/v) trifluoroacetic acid and applied to a 0.45 cm × 25 cm C_{18} reverse-phase column (Vydac 201TP104). Solvent A was 0.1% TFA and solvent B 100% CH_3CN. The effluent was monitored at 210 (—) and 280 nm (···). Fractions of 1 mL each were collected at a flow rate of 1 mL/min and pooled as indicated by bars.

Table I: Amino Acid Compositions (Residues per Peptide) of Purified C-Telopeptides[a]

amino acid	peptide 1[b]	peptide 2[b]	1^C–25^C[c]
hydroxyproline	*d*		
aspartic acid	1.9	2.0	2
threonine			
serine	2.0	2.1	2
homoserine			
glutamic acid	3.1	3.1	4
proline	4.7	4.7	3
glycine	4.6	4.7	4
alanine	1.1	1.1	1
cysteine			
valine			
methionine			
isoleucine			
leucine	2.0	2.1	2
tyrosine	2.3	2.8	3
phenylalanine	1.0	1.0	1
hydroxylysine			
lysine	0.3		1
histidine	0.8	1.0	1
arginine	0.9	1.0	1
	total 24.6	25.6	25

[a] Calculated by assuming 1 mol of Phe/mol of peptide. [b] HPLC fractions 1 and 2; see Figure 2. [c] From Rauterberg et al. (1972). [d] Denotes less than 0.05 residue/peptide.

Final purification was achieved by reverse-phase HPLC (see Figure 2), which resolved a number of minor components exhibiting absorption at 210 nm but not at 280 nm from two major components (1 and 2) absorbing at both wavelengths. The amino acid compositions (Table I) confirmed the identity of 1 and 2 as forms of the α1-chain C-terminal telopeptide, with the addition of the N-terminal glycylprolylprolyl tripeptide expected from the known specificity of clostridial collagenase (Mandi, 1961). The yield of fraction 1 peptide was 0.116 mg and that of fraction 2 peptide 0.267 mg. Virtually identical results were obtained from a second preparation. The molecular sizes calculated by assuming a content of 1 mol of phenylalanine/mol of peptide are very close to that determined by gel filtration for the unresolved mixture. The tyrosine content of fraction 2 was higher than that in fraction 1, consistent with both the order of elution from the reverse-phase column and the ratios of absorbances at 280 and 210 nm (Figure 2). In view of the well-known lability of the terminal tyrosine residues in the α1 chain (Rauterberg et al., 1972), it seems probable that the telopeptide in fraction 1 lost one of these either before or during isolation. Both fractions

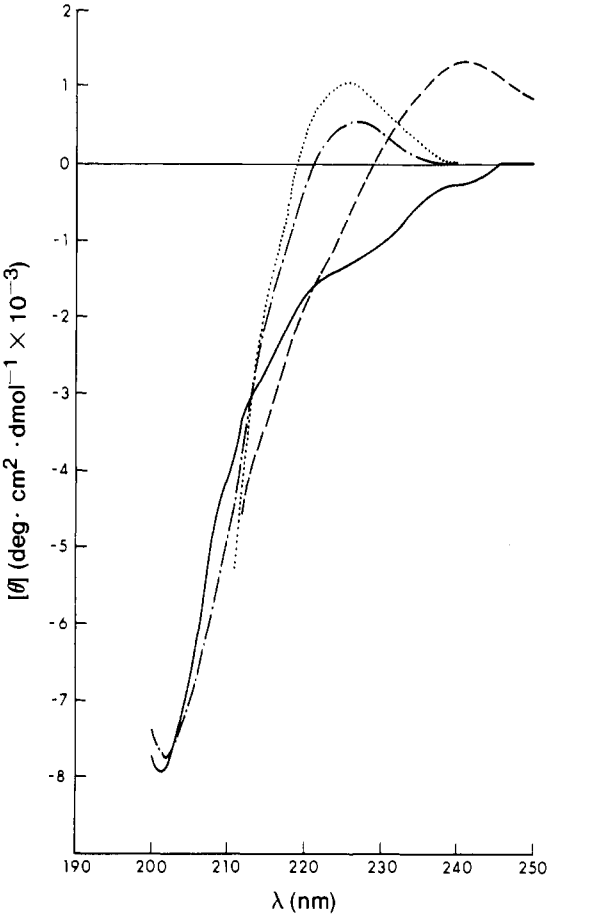

FIGURE 3: Circular dichroism spectra of telopeptide (fraction 2, see text) at 25 °C: 0.05 M sodium phosphate, pH 7.05 (—·—); 4.3 M urea, pH 7.05 (···); pH 11.3, no urea (—); pH 11.3, 4.3 M urea (- - -). Each spectrum shown is the average of four, replotted after subtraction of the buffer base line.

were virtually devoid of lysine but were found to contain tritiated hydroxynorleucine after reduction with sodium borohydride and analysis as described previously (Scott & Goldberg, 1983). This demonstrated that the lysine had undergone oxidative deamination by lysyl oxidase in preparation for interchain cross-linking.

A second difference from the analysis predicted from the published sequence (Rauterberg et al., 1972) is the presence of three rather than four residues of glutamic acid. An analytical error appears to be ruled out by the large number of analyses carried out (12 altogether on two independent preparations of telopeptides) and the finding of 15 residues, rather than the 16 anticipated, in the pure α1CB6 which was digested with collagenase. Possible explanations include genetic variation, reflecting differences in the sources of the calf skin collagen, or an error in the published sequence. Technical problems were apparently experienced around residue 16^C [reported as Glx by Rauterberg et al. (1972)]. Interestingly, the corresponding sequences of both the rabbit (Becker et al., 1975) and chick (Fuller & Boedtker, 1981) type I collagen α1 chains contain only two glutamines and one glutamic acid and would therefore give only three residues of glutamic acid on analysis.

Circular Dichroism. At 25 °C and neutral pH, both peptides gave essentially similar spectra with only two clearly resolved features, a positive feature at 227 nm and a negative feature near 220 nm (Figure 3). The intensity of the 227-nm band was about twice as great in the intact telopeptide (peptide 2). Because relatively less of peptide 1 was available and this

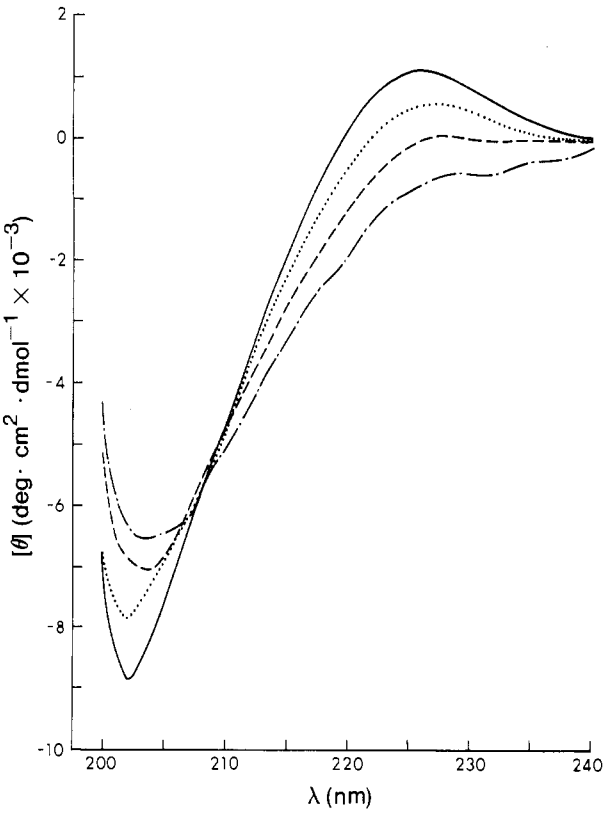

FIGURE 4: Effect of temperature on the circular dichroism spectrum of telopeptide 2 in 0.05 M sodium phosphate, pH 7.05: (—) 3 °C; (···) 15 °C; (- - -) 43 °C; (-·-) 66 °C.

was a partially degraded form of the telopeptide, its spectroscopic properties were not investigated in detail, except as indicated below.

High pH (11.3), in the presence of 4.3 M urea, induced a 15-nm red shift of the positive feature at 227 nm (Figure 3), strongly suggesting that this band should be assigned to the tyrosine side-chain chromophore (Adler et al., 1973). This would be consistent with the greater ellipticity at 227 nm for peptide 2, which had the higher tyrosine content. The red shift could not be discerned in the absence of urea; high pH alone (Figure 3) caused conformational changes resulting in masking of this band.

Increasing temperature caused both spectral features to decrease in magnitude in an approximately linear fashion (Figure 4), with reversal in sign at 227 nm occurring at about 45 °C. These changes were completely reversible on cooling. The relationship between ellipticity and temperature was not influenced by variations in peptide concentration over the range 0.15–0.3 mg/mL, indicating that this effect is not mediated through intermolecular interactions (aggregation or disaggregation).

Because of the apparent importance of the tyrosine side-chain chromophore in the telopeptide spectra and the fact that tyrosine constitutes a high proportion (3/27) of the amino acids, it was decided to investigate the effects of temperature and other environmental variables on the model dipeptide *N*-acetyltyrosyltyrosine. The spectrum of this compound at neutral pH (Figure 5) is virtually identical with that reported for *N*-acetyltyrosine amide (Shiraki, 1969). At high pH and in the presence of organic solvents or SDS, the expected red shifts were seen. In contrast to the observations with the telopeptides, the spectrum of *N*-acetyltyrosyltyrosine was virtually unaffected by increasing temperature—the ellipticity at 227 nm decreased by only 15% on heating from 5 to 45 °C.

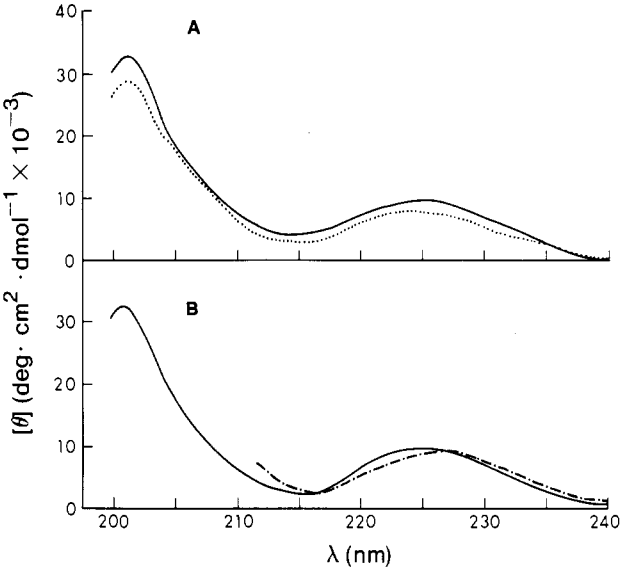

FIGURE 5: Circular dichroism spectra of *N*-acetyl-L-tyrosyl-L-tyrosine in 0.05 M sodium phosphate, pH 7.05. (A) Effect of temperature: (—) 5 °C; (···) 45 °C. (B) Effect of urea at 25 °C, pH 7.05: (—) no urea; (-·-) 7.2 M urea.

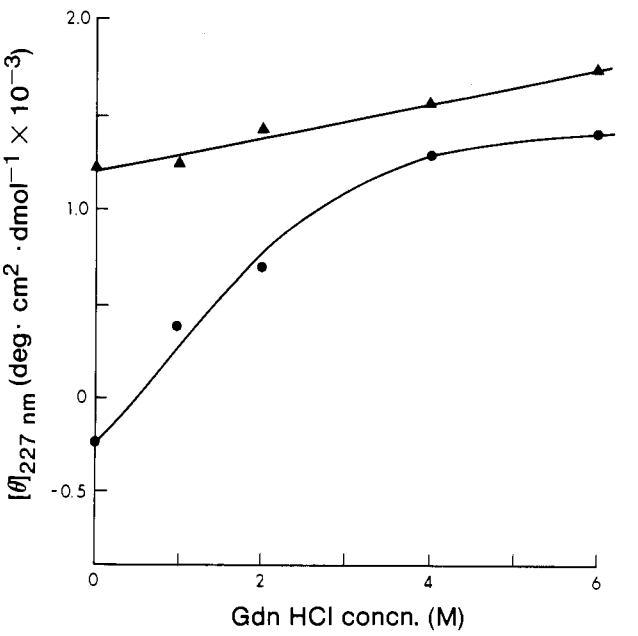

FIGURE 6: Effect of Gdn·HCl concentration on ellipticity at 227 nm in circular dichroism spectra of telopeptide 2 (●) and *N*-acetyl-L-tyrosyl-L-tyrosine (▲). Each point is the average of duplicate determinations at 25 °C. Values for the dipeptide are scaled down by a factor of 9 to permit comparison with telopeptide 2 at the same concentration of tyrosine.

Consequently, the more dramatic effects of temperature on the spectrum of the telopeptide must reflect conformational changes.

Both urea (Figure 3) and Gdn·HCl caused increases in the ellipticity at 227 nm in the telopeptide spectra at 25 °C. This denaturation effect appeared to be complete at about 4 M Gdn·HCl (Figure 6), at which point the measured mean residue ellipticity at 227 nm (1.20×10^3 deg·cm²·dmol⁻¹) was quite close to that at 5 °C in the absence of denaturant (1.00×10^3 deg·cm²·dmol⁻¹). Gdn·HCl caused a slight and continuous intensification of the spectrum of *N*-acetyltyrosyltyrosine.

The effects of SDS at a concentration of 40 mM are shown in Figure 7. The strengths of both bands are increased

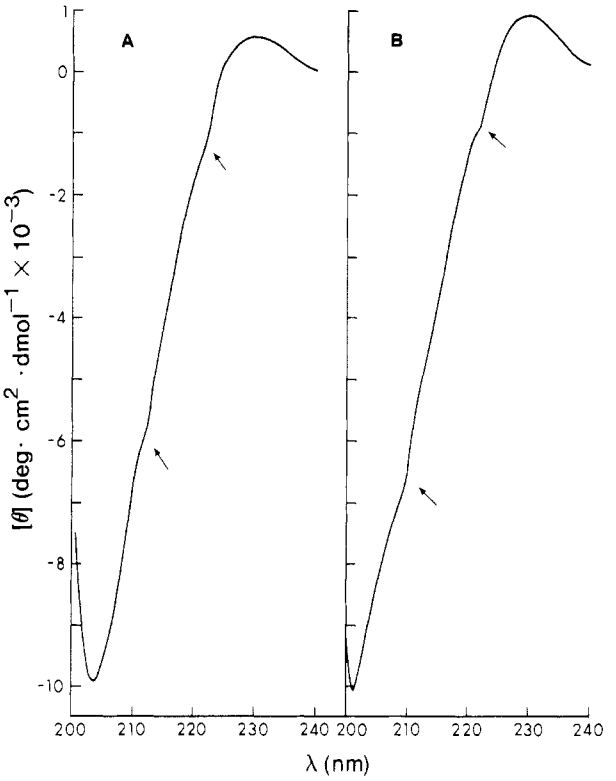

FIGURE 7: Effect of SDS (40 mM) on the spectrum of telopeptide 1 (A) and telopeptide 2 (B) at 25 °C in 0.05 M sodium phosphate, pH 7.05. Each spectrum is the average of four, replotted after subtraction of the buffer base line. Note the inflections (arrows) at 210 and 222 nm.

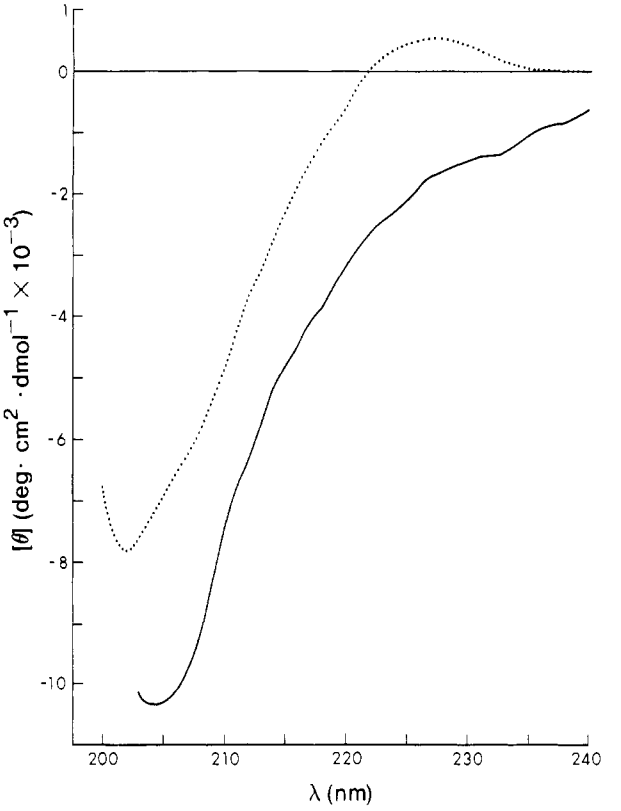

FIGURE 8: Effect of trifluoroethanol (80% v/v) on the spectrum of telopeptide 2 at 25 °C. The spectrum in 0.05 M sodium phosphate, pH 7.05 (···), is shown for comparison.

somewhat (compare with Figure 3) with the positive red-shifted by about 3 nm. In addition, weak but definite inflections appeared at 210 and 222 nm, indicating the induction of some α helix.

Fraction 2 telopeptide dissolved in 80% (v/v) trifluoroethanol gave a spectrum (Figure 8) which showed increased negative ellipticity throughout and lacked the 227-nm positive band.

UV Difference Spectra. The difference spectra for the telopeptide in 11% and 18.9% poly(ethylene glycol) 1000 are shown in Figure 9 with those for tyrosine under the same conditions. The most striking feature of these results is the change in the telopeptide spectrum on addition of the second aliquot of perturbant solution. Instead of an increase in the difference extinction coefficient, as seen with free tyrosine, a simultaneous red shift and decrease in intensity were found. In addition, the absorbance monitored at fixed wavelength (256 nm) was observed to decrease over several minutes after sample and perturbant solutions were mixed (data not shown). This effect was not seen with tyrosine. We conclude that poly(ethylene glycol) induces a conformational change which has the effect of decreasing the accessibility of tyrosine side-chain chromophores to this perturbant by transferring them into a less polar environment.

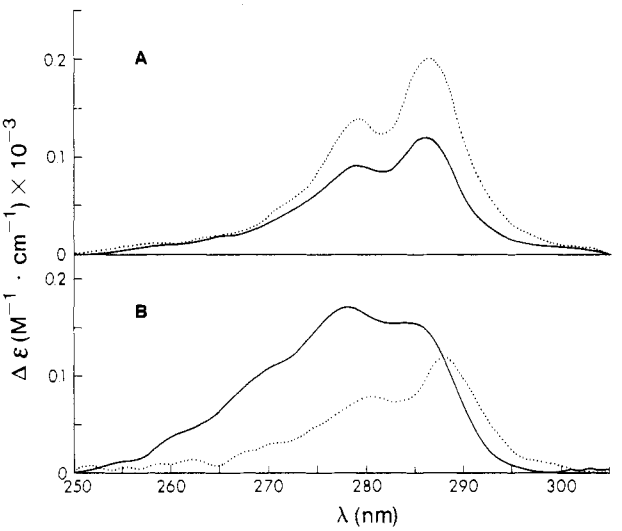

FIGURE 9: UV difference spectra obtained for tyrosine (A) and telopeptide 2 (B) in 11% (w/v) (—) and 18.9% (w/v) (···) poly(ethylene glycol) 1000.

Discussion

Circular dichroism spectra obtained under a variety of conditions clearly demonstrate that the conformation of the α1-chain C-telopeptide is sensitive to such environmental variables as temperature and pH and the presence of perturbants such as urea, Gdn·HCl, SDS, and trifluoroethanol. The interpretation of CD spectra in terms of relative proportions of the different forms of regular secondary structure is generally considered to be only partly successful for proteins and is even more difficult for small peptides, which may have rather flexible but nevertheless nonrandom structures in solution (Craig et al., 1975). We have therefore opted for a qualitative and largely phenomenological treatment of these data.

Visual comparisons of spectra obtained at 25 °C in phosphate buffer (Figure 3) with reference spectra of polypeptides (Greenfield & Fasman, 1969) or proteins (Chang et al., 1978) suggest that the telopeptides are predominantly in a random-coil structure, possibly with a few residues in β-sheet and/or β-turn conformations. While the spectra were sensitive to temperature and chemical denaturants, suggesting some restrictions on chain flexibility, the observed changes appear to

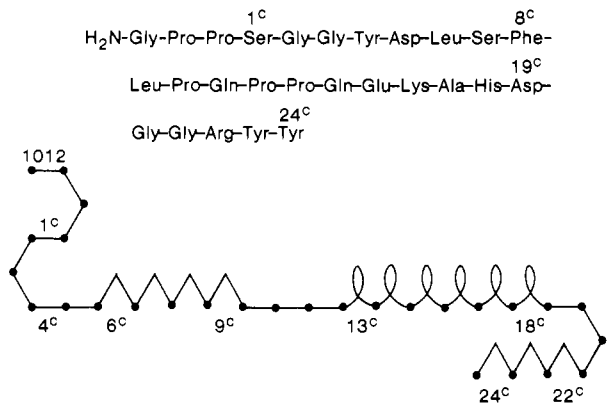

FIGURE 10: Sequence and predicted secondary structure of telopeptide 2. Lys-16^C was present in isolated material as α-aminoadipic δ-semialdehyde. This was modeled as Met or Gln in the calculations, with the same result as when Lys was used.

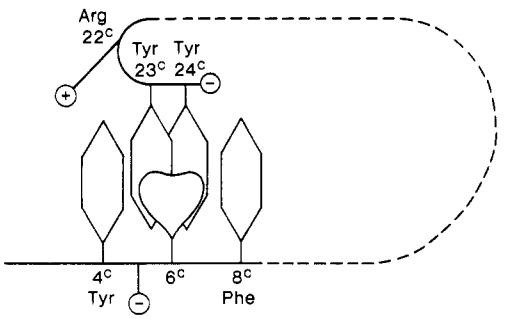

FIGURE 11: Tentative model of the C-telopeptide proposed to account for spectroscopic data. The antiparallel alignment of aromatic rings in the two pairs could reduce the contribution of tyrosine side-chain chromophores to circular dichroism spectra. A charge–charge interaction between Arg-22^C and the phenoxide of Tyr-4^C could account for the apparent structuring effect of high pH (Figure 4). The reverse turn at Arg-22^C is suggested by the Chou–Fasman prediction (Figure 10), but a similar arrangement of aromatic side chains is possible without this turn. No specific secondary structure is assumed for the rest of the peptide.

reflect formation or disruption of local interactions rather than cooperative conformational transitions.

Figure 10 shows the secondary structure predicted for the C-telopeptide by the Chou–Fasman method (Chou & Fasman, 1978). This differs in two relatively minor respects from the earlier prediction of Helseth and co-workers (Helseth & Veis, 1981b). The latter included a β turn for the central region, which we do not predict since the calculated turn probabilities do not rise above the lower cutoff (0.75×10^{-4}), and did not include the initial double turn (Figure 10) incorporating the Gly-Pro-Pro tripeptide which is present in our isolated material but not part of the telopeptide itself. The α helix predicted for residues 13^C–18^C is presumably that which is induced by SDS; helix-forming potential is low throughout the rest of the sequence. Trifluoroethanol also induced order in the peptide, and this structure probably included both β sheet and α helix. However, in relation to the telopeptide as it exists in aqueous solution in the absence of perturbants, the Chou–Fasman method grossly overestimates the proportion of regular secondary structure.

The positive band at 227 nm, which was observed at temperatures below about 45 °C and in the presence of denaturing agents and SDS, can be assigned with reasonable certainty to the tyrosine side-chain chromophore. Three lines of evidence support this assignment: (1) the large red shift seen in urea at a pH value (11.3) above the usual pK_{a_2} of tyrosine; (2) the approximate proportionality between ellipticity at this wavelength and tyrosine content in the two telopeptide preparations; and (3) the fact that in 4 or 6 M Gdn·HCl the ellipticity is almost exactly that observed for the model peptide *N*-acetyltyrosyltyrosine at an equivalent concentration (Figure 6). Spectra of this dipeptide, as anticipated, showed only minor changes in band position and intensity on heating or in the presence of the various perturbants.

While the 227-nm band should be sensitive to changes in the environment(s) of the tyrosine side chains, reflecting alterations in tertiary structure, the weak optical activity of the peptide bond chromophore near 230 nm (Cortijo et al., 1973) complicates interpretation. Nevertheless, decreases on heating at high pH and in trifluoroethanol are probably due in large part to a reduced positive contribution by tyrosine side-chain chromophores. Circular dichroism spectra in the near-UV region, which may have provided information on changes affecting the tyrosines without the complication of overlapping chromophores, could not be obtained because of the small amounts of material available.

The UV difference spectra for the telopeptide in poly(ethylene glycol) demonstrate that this perturbant causes a conformational change resulting in decreased accessibility of tyrosine side-chain chromophores and transfer of these to a less polar environment. This particular high molecular weight polyhydroxy compound was selected because the telopeptide is expected to retain a significant degree of chain flexibility in solution, even if it adopts a nonrandom conformation. Accessibility to a smaller probe such as ethylene glycol might not be sufficiently restricted under these conditions to give useful difference spectra. While it cannot be directly correlated with the circular dichroism data discussed above, this result confirms that the C-telopeptide is capable of undergoing environmentally induced conformational changes involving the tyrosines.

A tentative structural model to account for the observed changes in optical activity of telopeptide tyrosine side chains is given in Figure 11. The terminal tyrosyltyrosine could fit between the two other benzene rings in the sequence—those of Tyr-4^C and Phe-8^C. The opposite orientations of the rings in the pairs Tyr-4^C/Tyr-23^C and Phe-8^C/Tyr-24^C would be expected to significantly reduce the optical activity of these chromophores. Space-filling models confirm the feasibility of this arrangement, which could be stabilized by hydrophobic interactions and by hydrogen bonding between tyrosyl hydroxyls and peptide bonds. This model could account for the effects of high pH (Figure 3), since pH 11.3 is above the usual pK_a of the tyrosine hydroxyl but below that expected for the guanidino group of Arg-22^C which would be adjacent to Tyr-4^C in this particular arrangement.

On the basis of electron–optical analysis of the banding patterns of type I collagen fibrils and SLS (segment long spacing) dimers, two similar models have been proposed for the C-telopeptide (Capaldi & Chapman, 1982; Bender et al., 1983), both of which would be consistent with the evidence from X-ray (Hulmes et al., 1977) and neutron diffraction (Hulmes et al., 1980) analyses that the average rise per residue through the telopeptides is about 70% of that for the helix (2.86 Å). Capaldi and Chapman (1982) proposed that the C-telopeptide adopts a "condensed" conformation between Ser-1 and Pro-13 with the rest of the peptide extended along the helix of an adjacent D-staggered collagen monomer. The model of Bender et al. (1983) incorporates an "S fold" in the first 15 residues. Both models take into account the need to align the telopeptide lysine and Hyl-87 in an adjacent molecule to permit an intermolecular cross-link to form (Piez, 1976). This dictates that 16 or 17 telopeptide residues must be accommodated in an axial length of about 27 Å. The arrangement in Figure

11 would satisfy these requirements, although it was constructed solely to explain the spectroscopic data without consideration of other constraints.

It would be premature to relate the present results to the mechanism of collagen fibrillogenesis. Nevertheless, it is likely that a more detailed knowledge of telopeptide conformation will eventually lead to a better understanding of this important biological process.

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