Model Polytripeptides for Collagen

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Summary

Poly[ala-glu(OEt)-gly], poly[glu(OEt)-gly-gly], and poly [ala-gly-gly] have been prepared from their respective tripeptide activated esters. Preliminary characterization by X-ray diffraction, infrared, circular dichroism, and optical rotatory dispersion spectroscopy indicate that poly[ala-glu(OEt)-gly], as obtained from an aqueous suspension or film cast from formic acid, is in an anti-parallel β sheet while the other two polymers are obtained in a random conformation from their aqueous solutions.

Introduction

Collagen has been described as having a primary structure composed of polar, $[gly\text{-}X\text{-}Y]_n$, and non-polar, $[gly\text{-}pro\text{-}Z']_n$, glycine-led triads where X and Y are usually polar amino acids and Z' can be any amino acid(1). The crystallinity of the tropocollagen molecule has been ascribed to the non-polar, proline rich regions whereas the polar regions have been described as being "amorphous". Extensive studies of model polytripeptides resembling the non-polar regions of collagen have been carried out(2). While the emphasis has been on the non-polar regions of collagen which actually comprise only 40-50% of collagen, model polytripeptides for the polar regions of collagen have received little attention. Our efforts have been directed toward the structural characterization of model polytripeptides which resemble the polar regions of collagen.

We wish to report our results on the synthesis and preliminary characterization of three polytripeptides which are models

for the polar regions of collagen. These three biopolymers are $[ala\text{-}glu(OEt)\text{-}gly]_n$, $[ala\text{-}gly\text{-}gly]_n$ and $[glu(OEt)\text{-}gly\text{-}gly]_n$ (3). The monomeric tripeptides were synthesized in a manner which provided a glycyl residue in the C-terminal position to avoid racemization. Polymerizations were carried out using the para-nitrophenyl tripeptide esters (4).

Materials and Methods

1. Z-glu(OEt)-gly-ONP. A solution of Z-glu(OEt)(15.4 grams, 50 mmole) and triethylamine (7.5 ml, 50 mmole) in anhydrous acetonitrile (200 ml) was cooled to below -10° in an isopropanol ice bath. Ethyl chloroformate (7.5 ml, 50 mmole) was added dropwise with vigorous magnetic stirring. After 45 minutes, gly-ONP,HBr(14.0 grams, 50 mmole) was added, followed by the dropwise addition of triethylamine (7.5 ml, 50 mmole) in 40 ml of acetonitrile. The reaction was allowed to proceed for one hour before the bath was removed.

After an additional hour at room temperature, the reaction mixture was filtered and the product, Z-glu(OEt)-gly-ONP, washed with dilute acid, water and diethyl ether. The yield was 14.6 grams (60%), m.p. 153° (lit. m.p. 154-155°(4)).

2. Glu(OEt)-gly-ONP,HBr. Z-glu(OEt)-gly-ONP (20.0 grams, 41 mmole) was dissolved in 100 mls of 25% HBr in glacial acetic acid. After 45 minutes at room temperature, the reaction solution was diluted with diethyl ether and glu(OEt)-gly-ONP,HBr precipitated as an extremely viscous oil upon cooling. The oil was dried in vacuo over P_2O_5 and a hygroscopic powder (14 grams) was obtained. Amino acid analysis gave gly:glu = 1.00:1.04.

3. Z-ala-glu(OEt)-gly-ONP. This compound was prepared from Z-ala and glu(OEt)-gly-ONP,HBr by the mixed anhydride procedure previously described in 1. The final yield was 7.0 grams (50%).

4. Ala-glu(OEt)-gly-ONP,HBr. This compound was prepared as in 2 from Z-ala-glu(OEt)-gly-ONP. Amino acid analysis gave gly:ala:glu = 1.00:0.93:0.95. Before polymerization, this compound was thoroughly dried in vacuo over P_2O_5.

5. Glu(OEt)-gly-gly-ONP,HBr. The preparation of this compound has been previously described (4).

6. Ala-gly-gly-ONP,HBr. This compound was prepared in the same manner as glu(OEt)-gly-gly-ONP,HBr (4).

7. Polymerization of Tripeptide Active Esters. The three tripeptide esters were polymerized in dimethylformamide solutions greater than 2.5 M. Polymerization was initiated by the addition of 1.5 equivalents of triethylamine with rapid stirring. The reaction mixture solidified in 30 minutes and was kept at room temperature for 3 days. The solid was washed repeatedly with dimethylformamide and ethanol until the wash solution was colorless,

Table 1.

Molecular Weights

Polymer	Molecular Weight	DP[a]	Method[b]
$[ala\text{-}glu(OEt)\text{-}gly]_n$	16,700	55	L.S.
	8,800	29	ONP
$[glu(OEt)\text{-}gly\text{-}gly]_n$	6,800	25	DNP
	4,600	17	ONP
$[ala\text{-}gly\text{-}gly]_n$	14,100	69	DNP
	8,400	41	ONP

a. Degree of polymerization for the tripeptide.

b. L.S. - light scattering.
ONP - para-nitrophenyl ester spectrophotometric method.
DNP - amino end group method.

and then lyophilized twice from water to yield a white, powdery material.

Molecular Weight Determinations. Molecular weights were determined by end group analyses (amine-DNP and ONP) and light scattering methods. Light scattering molecular weights were determined in formic acid; dichloroacetic acid was the solvent for the ONP spectrophotometric method. All molecular weights were determined at room temperature and are found in Table 1.

Spectrophotometric Methods. Infrared absorption measurements were made using a Perkin-Elmer Model 521 grating spectrophotometer, and, unless otherwise stated, the spectra were taken from KBr discs prepared from the solids lyophilized from aqueous solutions or suspensions. The optical rotatory dispersion and circular dichroism spectra for poly[ala-glu(OEt)-gly] films cast from formic acid onto quartz windows were obtained with a Cary 60 spectropolarimeter and circular dichroism attachment.

Results and Discussion

A summary of the major peptide peaks for the polymers is presented in Table 2. The positions of the Amide I, Amide II, and Amide V bands for poly[glu(OEt)-gly-gly] and poly[ala-gly-gly] are consistent with a random conformation (5) when the polymers are lyophilized from aqueous solutions. However, the positions for these same bands in the spectrum of poly[ala-glu(OEt)-gly], lyophilized from an aqueous suspension, suggests an extended conformation (5). The Amide I appears at 1628 cm^{-1} and the Amide V at 697 cm^{-1}; further, the spectrum obtained from a film cast onto a silver chloride plate from formic acid showed an improvement in peak sharpness. A weak peak at 1695 cm^{-1} suggests a β sheet with anti-parallel chains (6).

Apart from the Amide peaks mentioned above there are several

Table 2.

Infrared absorption peaks for polypeptides containing glycine, alanine and ethyl ester of glutamic acid

$[glu(OEt)\text{-}gly\text{-}gly]_n$	$[ala\text{-}gly\text{-}gly]_n$	$[ala\text{-}glu(OEt)\text{-}gly]_n$	Assignments
3295 (vs)	3295 (vs)	3290 (vs)	Amide A
3072 (w)	3074 (w)	3065 (w)	Amide B
1720 (m)	--	1730 (m)	ester CO stretching
		1695* w	Amide I (Antiparallel pleated sheet)
1650 (vs)	1650 (vs)	1628 (vs)	Amide I
1528 (s)	1538 (s)	1518 (s)	Amide II
1020 m	1018 m	--	gly-gly mode
660 m	660 m	697 m	Amide V

*For a film cast from formic acid onto a 0.5 mm silver chloride plate.

other interesting features in the spectra tabulated. The frequency shift of the Amide A and B bands in passing from the random structure to the extended pleated sheet of poly[ala-glu(OEt)-gly] is consistent with the role of C=O···HN bonding in structure integrity. Another difference between the random and ordered form is seen in the carbonyl stretching frequency associated with the glutamyl esters -- that this is probably a reflection of conformation on carbonyl environment rather than the change in polymer sequence is supported by the observation that this band appears at 1722 ± 1 cm^{-1} for both the tripeptide monomers. Finally, it is interesting to note a band at about 1020 cm^{-1} for both the polymers containing diglycine. This band has also been found in the tripeptide precursers with this sequence and Blout, et al (7) have noted the occurrence of this band in peptides and polypeptides with the diglycyl group.

In further support of the assignment of a β structure to poly[ala-glu(OEt)-gly], the optical rotatory dispersion and circular dichroism spectra of films cast from formic acid were obtained. Both the optical rotatory dispersion spectrum (troughs at 231 and 190 mμ, crossovers at 223 and 197 mμ, and a peak at 203 mμ) and the circular dichroism spectrum (trough at 217 mμ, peak at 196 mμ, and a crossover at 206 mμ) agree with those reported for the I-β family of anti-parallel β sheets (8,9).

X-ray diffraction patterns of poly[ala-glu(OEt)-gly] may also be indexed as β sheet, a detailed analysis of the X-ray and electron diffraction, and the rather unusual morphology of the polytripeptides will be presented later.

It appears, therefore, that non-proline containing glycyl polytripeptides do not readily achieve the distorted poly-L-

proline conformation typical of the most prevalent structure in collagen. Such observations are in accord with recent suggestions that the tropocollagen molecule is non-uniform in conformation (10,11).

References

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