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A SEQUENTIAL POLYHEPTAPEPTIDE AS A MODEL FOR THE DOUBLE-STRANDED α-HELICAL COILED-COIL STRUCTURE OF TROPOMYOSIN

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SUMMARY

Preliminary conformational studies of $[Lys\text{-}Leu\text{-}Glu\text{-}Ser\text{-}Leu\text{-}Glu\text{-}Ser]_n$ by circular dichroism and its molecular weight determination in benign and denaturing media strongly suggest that the polypeptide can adopt an α-helical, double-stranded coiled-coil structure in aqueous solution. The hypothesis that the double-stranded coiled-coil is stabilized by repeating hydrophobic residues is verified by this model polyheptapeptide where only non-polar residues occupy positions 2 and 5 of the repeating heptad sequence. It is suggested that the remarkable increase in thermal stability of this peptide relative to tropomyosin is a consequence of having only leucine residues in the hydrophobic positions that stabilize the coiled-coil.

INTRODUCTION

Based on both physical studies and X-ray evidence, the three highly helical fibrous proteins tropomyosin, paramyosin and the rod-portion of the myosin molecule are believed to be arranged in a two-stranded coiled-coil whose structure is stabilized by hydrophobic interactions (1, 2). The analysis of the primary structure of the C-terminal region of tropomyosin led Hodges et al. (3) to propose that the two-stranded coiled-coils were stabilized by hydrophobic residues situated at positions 2 and 5 of the repeating heptad $[X\text{-}N\text{-}X\text{-}X\text{-}N\text{-}X\text{-}X]_n$, where N is a non-polar residue. This hypothesis was further supported when the complete amino acid sequence of tropomyosin was elucidated (4). In the following communication, we present

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Abbreviations: CD, circular dichroism; CM-, carboxymethyl; SDS, sodium dodecyl sulfate; HFIP, hexafluoroisopropanol.

conformational studies made with sequential polypeptides containing respectively seven, four, three and two repeating residues, providing further experimental evidence in favor of the above hypothesis.

MATERIALS AND METHODS

Protected precursors of $[Lys\text{-}Leu\text{-}Glu\text{-}Ser\text{-}Leu\text{-}Glu\text{-}Ser]_n$ (Polypeptide I), $[Leu\text{-}Glu\text{-}Ser\text{-}Lys]_n$ (Polypeptide II), $[Glu\text{-}Ser\text{-}Lys)_n$, (Polypeptide III) and $[Glu\text{-}Ser]_n$ (Polypeptide IV) have been synthesized by usual solution techniques (5), as described in another publication (6). After polymerization and purification, the polypeptides were deprotected in HBr-acetic acid and the polyheptapeptide was fractionated on a pre-calibrated column of Sephadex G-100 in a 8 M urea-0.2 M KCl-0.05 M Tris buffer at pH 7.0. The fractions were dialyzed in Spectrapor 1 dialysis tubes (molecular weight cut-off 6,000-8,000 daltons), consecutively against 1 M KCl and 0.001 N HCl solutions and lyophilized. The first fractions containing the highest molecular weight material were used for the circular dichroism and the ultracentrifuge measurements. The serine-containing polypeptides were deacetylated by dissolution in a 0.37 M barium hydroxide solution followed by dialysis against 0.001 N HCl for 2 days. A 1.1 M KCl-0.05 M phosphate buffer at pH 7.0 was used for CD measurements and column chromatography in benign medium. The molecular weight of the polypeptides in benign medium has been determined on a column of Bio-Gel A (0.5 million). The column had been previously calibrated, using the following rod-shaped molecules: CM-tropomyosin (MW 66,000) and its fragments of molecular weights 22,000 and 32,000. Circular dichroism spectra were recorded on a Cary 60 spectropolarimeter equipped with a Model 6001 CD attachment. Molecular weight determination of selected fractions of polyheptapeptide in the above denaturing medium have been performed independently by SDS-gel electrophoresis, analytical ultracentrifugation and analytical column chromatography. Sedimentation equilibrium experiments were conducted using a Beckman E analytical ultracentrifuge.

RESULTS AND DISCUSSION

CD measurements of non-fractionated samples of polypeptides I, II, III and IV were made in benign medium after the deprotection in HBr-acetic acid and dialysis of each polypeptide in Spectrapor 1. CD results indicated that only the polyheptapeptide had any helical content. The latter was then fractionated on a column of Sephadex G-100 in 8 M urea as shown in Fig. 1. The elution curve was divided into narrow molecular weight fractions of 2-3 mg each. Fig. 2 shows the CD spectra of fractions 5 and 11 in benign medium. It is seen that the helical content of the polypeptide (estimated from the ellipticity value at 220 nm) decreases regularly with decreasing chain length.

Obviously, the best way to determine if we are dealing with a coiled-

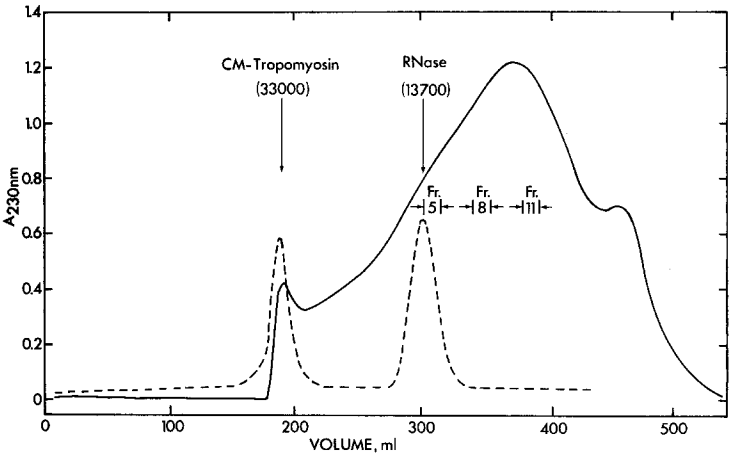

Figure 1: Fractionation curve of $[Lys\text{-}Leu\text{-}Glu\text{-}Ser\text{-}Leu\text{-}Glu\text{-}Ser]_n$ (——) by gel-filtration on a Sephadex G-100 column in 0.2 M KCl-8 M urea 0.05 M Tris buffer, pH 7.0, calibrated with known molecular weight standards (- - -) CM-tropomyosin (33,000 daltons) and ribonuclease A (13,700 daltons). The effluent was monitored for absorbancy at 230 nm.

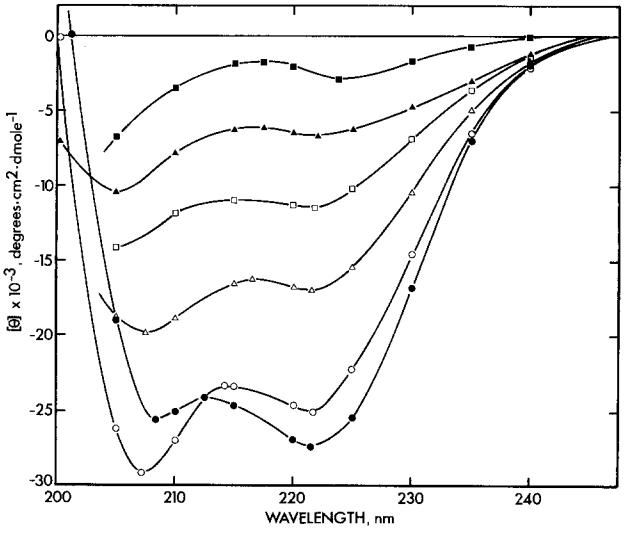

Figure 2: Circular dichroism spectra of CM-tropomyosin and its model polyheptapeptide $[Lys\text{-}Leu\text{-}Glu\text{-}Ser\text{-}Leu\text{-}Glu\text{-}Ser]_n$ in the presence and absence of HFIP in benign buffer. The symbols (▲——▲), (■——■) and (●——●), in that order, represent fraction 5, fraction 11 and CM-tropomyosin in benign buffer, without HFIP. (△——△), (□——□) and (○——○) are symbols for fraction 5, fraction 11 and CM-tropomyosin in solutions containing 80%, 80% and 90% HFIP, respectively.

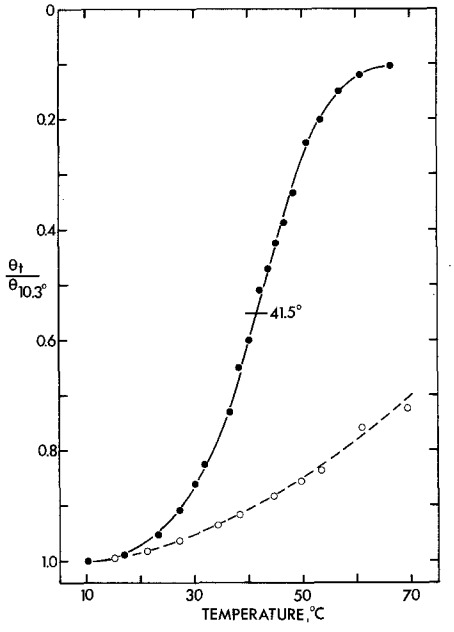

Figure 3: Melting curves of CM-tropomyosin (●——●) and of fraction 4 of the polyheptapeptide in benign buffer (○——○). $\theta_t/\theta_{10.3°}$ represents the ratio of the ellipticity at 220 nm at the indicated temperature to the ellipticity at 10.3° C.

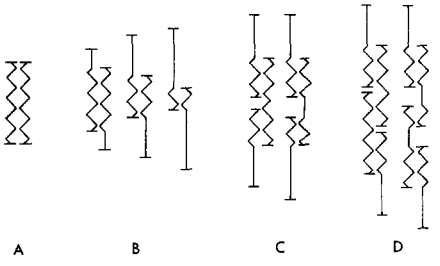

Figure 4: Schematic representation of two-stranded, α-helical coiled-coils. A represents the chains in register as in the case of tropomyosin; B, C and D symbolize the non-specific formation of the coiled-coil structure consisting of the staggering of two, three and four chains of the polyheptapeptide, respectively. (∧∧∧∧) α-helical regions; (————) non-helical regions.

coil structure is to evaluate the molecular weight of the polypeptide in benign and in denaturing media. The formation of a coiled-coil in benign

medium would result in the doubling of the molecular weight obtained in denaturing medium. Unfortunately, our synthetic model has no sequence specificity by which the two chains can be in register, like tropomyosin (Fig. 4A). Our chains could rather stagger randomly, as shown in figures 4C and 4D with the result that the joining of chains with approximately the same size will give rise to a large distribution of molecular weights for the associated chains. The occurrence of this phenomenon became apparent on comparing the narrow molecular weight distribution of fraction 5 in denaturant (10,000 daltons) to the broad distribution in benign medium (20,000-40,000 daltons) (Table 1). Our second clue came from comparative measurements of CM-tropomyosin and the polyheptapeptide in HFIP-containing solution. Since it has been shown that tropomyosin chains are in register (7), no important increase in helicity was expected from the addition of a solvent that can induce helicity in a single-chained, potentially helical polypeptide (8). That is exactly what happens with CM-tropomyosin, as shown in Fig. 2 and Table 1. On the contrary, small addition of HFIP (5%) to an aqueous solution of the polyheptapeptide increases noticeably its helicity. This phenomenon immediately suggested to us the existence of permanent regions of coiled-coil structure in the polyheptapeptide that are interrupted by regions of non-helical structure of varied lengths, as symbolized in Fig. 4. These regions that do not contain the coiled-coil structure would result in lowering the molar ellipticity values of the polyheptapeptide relative to tropomyosin (Table 1). The two serine residues in the heptad sequence could also lower the helicity of the polypeptide since serine is not considered as a good helix-forming residue (9). It is suggested that replacing the serine residues by alanine and removing the non-helical regions by enzymatic digestion, for example, would enhance the helical structure of the polypeptide models and, consequently, increase the ellipticity values to those of tropomyosin. The presence of very stable coiled-coil regions is confirmed

TABLE I. Circular Dichroism and Molecular Weight Determination Data of the Synthetic Polypeptides Compared to those of CM-Tropomyosin

Polypeptide	% HFIP[a]	$[\theta]^{b}_{220}$	% helix[c]	Molecular weight	
	v/v	deg cm^2 $dmole^{-1}$		Benign[b]	Denaturant
I, fraction 5	0	- 6,650	20	20-40,000[f]	9-10,000[d,e,f]
	4.5	-12,000	35	-	-
	80	-16,800	49	-	-
I, fraction 8	0	- 5,500	16	-	8,500[f]
	80	-13,200	39	-	
I, fraction II	0	- 2,650	8	-	6,000[f]
	80	-11,200	33	-	-
II, not fractionated	0	- 600	2	-	>6,000[g]
III, not fractionated	0	0	0	-	>6,000
IV, not fractionated	0	0	0	-	>6,000
CM-tropomyosin	0	-34,000	100	66,000	33,000
	90	-30,900	91	-	-

a) The solutions were prepared from a stock solution of polypeptide in benign buffer and desired quantities of HFIP and water were added, in order to keep the peptide concentration constant. *b)* A 1.1 M KCl-0.05 M phosphate buffer at pH 7.0 was used for all benign measurements. *c)* % helix was based on a value of CM-tropomyosin taken at 100%. *d)* From SDS-gel electrophoresis. *e)* From sedimentation equilibrium. *f)* From column chromatography. *g)* From dialysis bags molecular weight cut-off specifications.

by the melting curves shown in Fig. 3, where we compare the effect of temperature on CM-tropomyosin and the sequential polypeptide. It is seen that 75% of the original helicity of the latter is preserved even at 70° C, at which temperature CM-tropomyosin is already denatured. It is suggested that this increased thermal stability of the sequential polypeptide is due to the presence of only leucine residues in the hydrophobic positions that stabilize the coiled-coil. The sequence $[Leu\text{-}Glu\text{-}Ser\text{-}Lys]_n$ containing three out of four good α-helix forming residues (9) does not show any sign of helicity by CD in benign buffer. In this sequence, the hydrophobic residue repeats at every fourth residue rather than in the case where the position 2 and the position 5 hydrophobes of the heptad sequence each repeat at seven residue intervals.

This model polypeptide sequence verifies the hypothesis of Hodges et al. (3) that these positions are responsible for the hydrophobic interactions stabilizing the two-stranded α-helical coiled-coils. Work is in progress in our laboratory to determine the minimum size requirement for a hydrophobic residue in positions 2 and 5 and the importance of the residues in the other positions of the heptad to stabilize the coiled-coil structure.

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