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# POLYPENTAPEPTIDE OF ELASTIN: TEMPERATURE DEPENDENCE OF ELLIPTICITY AND CORRELATION WITH ELASTOMERIC FORCE

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Circular dichroism (CD) is used to follow the conformational changes that attend temperature dependent aggregation leading to the viscoelastic (coacervate) state of the polypentapeptide of elastin in water. Two concentrations are used, 2.3 mg/ml and 0.023 mg/ml. The former results in aggregates of a size that exhibit much particulate distortion of the CD spectra whereas the latter results in spectra that are relatively free of distortions. Given the CD spectra of the temperature dependent aggregation of the lower concentration, it is possible to show that the same conformational change is occurring at high concentration. The structure of the polypentapeptide is one of limited order below 20°C which undergoes an inverse temperature transition to a conformation characterized by a regularly recurring β-turn at 40°C. The temperature profile for the conformational change is compared to the temperature dependence of elastomeric force of γ-irradiation cross-linked polypentapeptide coacervate. The curves virtually superimpose. When there is little order, there is little elastomeric force and elastomeric force develops to a near maximal value as the repeating Type II β-turn conformation develops. Not only is the elastomeric state non-random, the more nearly random state has very little elasticity. These results are the inverse of expectations based on the classical theory of rubber elasticity.

The polypentapeptide of elastin, $(\text{L}\cdot\text{Val}^1\text{-L}\cdot\text{Pro}^2\text{-Gly}^3\text{-L}\cdot\text{Val}^4\text{-Gly}^5)_n$ where n is as large as eleven in pig (1) and thirteen in chick (2), is the most striking primary structural feature of tropoelastin, the precursor protein of fibrous elastin which is obtained after removal of a fine microfibrillar glycoprotein coat from the biological elastic fiber (1). The polypentapeptide, like tropoelastin and α-elastin (a chemical degradation product of fibrous elastin), is soluble in all proportions in water below 20°C, but on raising the temperature toward the physiological range aggregation occurs. On standing the aggregates settle to form a viscoelastic phase, called a coacervate, which is about 60% water, as is natural fibrous elastin (3,4). The polypentapeptide coacervate,

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when cross-linked either by chemical means (5) or by γ-irradiation (6) is elastomeric and exhibits an elastic modulus that is similar to that of fibrous elastin. Also γ-irradiation cross-linked polypentapeptide and fibrous elastin exhibit similar temperature dependences of elastomeric force in that there is a dramatic rise in elastomeric force between 20°C and 40°C, and then the elastomeric force remains nearly constant, when normalized to °K, as the temperature is raised above 40°C (3). It has previously been shown by means of dielectric relaxation studies on the polypentapeptide at the coacervate concentration that a 25 MHz peptide librational mode also develops as the temperature is increased above 20°C (7) and that the temperature dependence of the real part of the dielectric permittivity resulting from this relaxation correlates with the temperature dependence of elastomeric force (8). Furthermore carbon-13 nuclear magnetic resonance relaxation studies on $(1\text{-}^{13}C)Pro^{2}$-polypentapeptide and $(1\text{-}^{13}C)Gly^{5}$-polypentapeptide at coacervate concentration demonstrate an inverse temperature transition (defined as decreasing mobility with increasing temperature) in the same 20°C to 40°C temperature interval (9).

In the present report circular dichroism of the coacervation process is followed at different concentrations in such a way as to be able to conclude that the change in structure of the polypentapeptide in water is one of changing from a state where the circular dichroism spectrum indicates little order at 20°C to a state at 40°C where the circular dichroism pattern is typical of a polypeptide that contains a regularly repeating β-turn. Importantly, the temperature dependence of this structural transition closely parallels the temperature dependence of elastomeric force of γ-irradiation cross-linked polypentapeptide coacervate. Strikingly, there is little elastomeric force when the polypentapeptide exhibits the least order and elastomeric force develops as the polypentapeptide converts to a structure containing a regularly recurring β-turn.

## Materials and Methods:

Preparation and characterization of the polypentapeptide of elastin: the synthesis of the polypentapeptide of elastin was as previously described

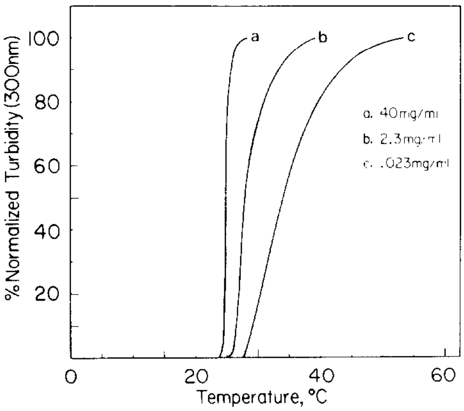


Figure 1: Temperature profiles for coacervation of the polypentapeptide of elastin, determined as turbidity measured at 300 nm.

(10,11). The synthesis procedure utilized mixed anhydride coupling reactions (12-14) in the presence 1-hydroxybenzotriazole (10) for preparation of the particular pentamer permutation (Gly-L•Val-Gly-L•Val-L•Pro) utilized in the polymerization to form the polypentapeptide. Prolyl carboxyl activation of the pentamer for polymerization used bis-(p-nitrophenyl)-carbonate (15) or p-nitrophenyl trifluoroacetate (16). The intermediates and high polymer were verified by thin layer chromatography, elemental analyses, aminoacid analyses, and proton and carbon-13 nuclear magnetic resonance (10,11). For the present studies a high molecular weight fraction was used which was retained by equilibrium dialysis using 50,000 molecular weight dialysis tubing. Based on the mid-point of the temperature profile for coacervation (11), the mean molecular weight of the polypentapeptide is close to 100,000 daltons. The temperature profile for the high concentration limit (40 mg/ml in this case) above which the profile no longer shifts to lower temperatures is given in Figure 1 along with the temperature profiles for 2.3 mg/ml and 0.023 mg/ml, which are the concentrations used in the present circular dichroism studies.

The Cary Model 60 spectropolarimeter, equipped with the Model 6001 circular dichroism (CD) accessory modified for high frequency modulation, was used for following the coacervation process. This CD beam configuration is favorable for these studies as the sample cell is positioned within a few centimers of the photomultiplier with an interceding focusing lens. Before the CD spectra were obtained, the instrument was tested and aligned to eliminate instrumental artifacts due to high absorbance and light scattering samples. The lyophilized polypentapeptide was dissolved in cold water (deionized/glass distilled with quartz immersion heater). The CD spectra collection was begun immediately starting at 15°C, increasing in about 5°C increments up to 40°C and then continuing in 10°C increments up to 80°C. The data were collected at a rate whereby the study was complete within an eight hour period. Two concentrations of samples were used, 2.3 mg/ml and 0.023 mg/ml in 0.1 mm and 10 mm path length cells, respectively. The two different concentrations were used because, as shown in Figure 1, the temperature profiles are concentration dependent and because at lower concentrations the average aggregate size is smaller which results in a more limited distortion of the CD spectra due to the effects of particle size.

## Results and Discussion:

Circular dichroism spectra for the 2.3 mg/ml sample are given in Figure 2 at 15°, 30°, 35° and 40°C. At 15°C the spectrum is characterized by a negative

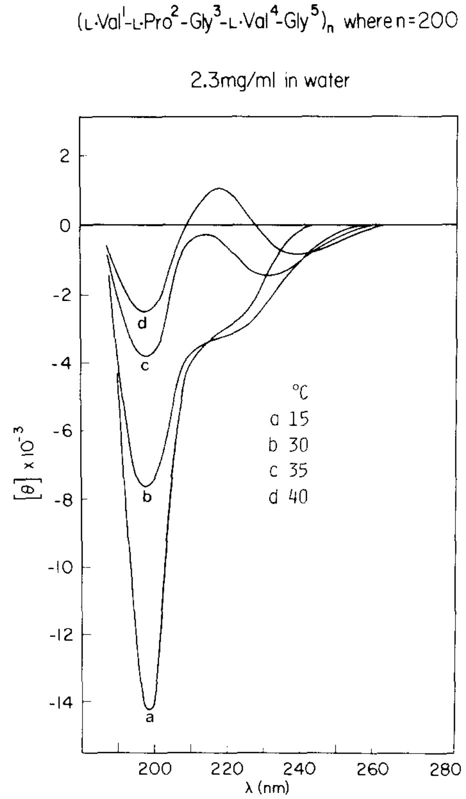


Figure 2: Circular dichroism spectra of 2.3 mg/ml polypentapeptide in water. At this concentration the CD spectrum at 40°C is distorted due to the particulate nature of the sample. The distortions, however, are understood sufficiently to be able to deduce what the general form of the correct CD spectrum should be which is as obtained in Figure 4. See text for further discussion.

shoulder near 220 nm and a negative extremum near 197 nm. The location of the 197 nm extremum is close to that characteristic of random or disordered polypeptides (~ 195 nm) but the intensity, about $-1.4 \times 10^4$, is lower than the generally accepted reference value for random polypeptides, i.e., $[\theta]_{195} \simeq -4 \times 10^4$ (17). Also, the negative shoulder, rather than a near zero or positive value near 220 nm as considered indicative of disorder (17,18), suggests the presence of some order. As the temperature is raised, the negative extremum at 197 nm decreases in intensity and approaches zero at 40°C and at higher temperatures. The ellipticities at 197 nm are plotted as a function of temperature in Figure 3, curve a. Curve a of Figure 3 approximates curve b of Figure 1 with the suggestion that the negative band begins to decrease in magnitude before light scattering is detected at 300 nm, that is, before the distortions due to the

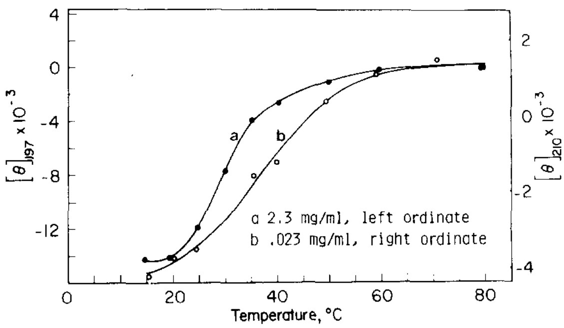
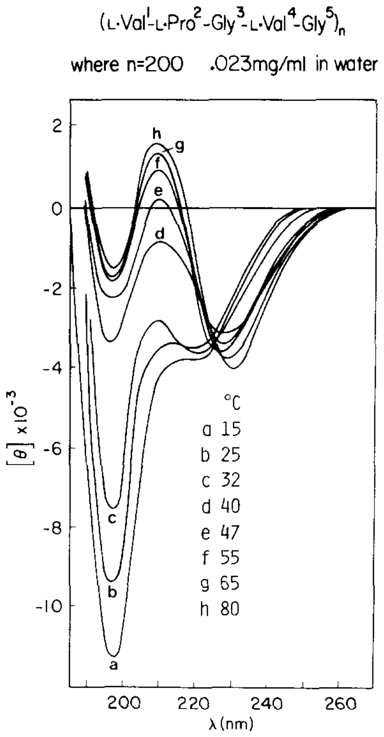


Figure 3: Plots as a function of temperature of the mean residue ellipticity of the 2.3 mg/ml sample at 197 nm (curve a, plotted with respect to left-hand ordinate) and of the 0.023 mg/ml sample at 210 nm (curve b plotted with respect to the right-hand ordinate).

particulate nature of the sample begin to occur (18-20). Once above 30°C, however, it is clear that the light scattering (particulate) distortions are present. The three types of distortions, as first described by this Laboratory (for a detailed review please refer to 19 and references therein), are differential absorption flattening and differential light scattering of the left and right circularly polarized beams as they pass through the sample and concentration obscurring due to light scattering. While corrections have been demonstrated in detail for aggregates that are dominantly α-helical, it is sufficient here to be aware of the nature of the distortions. It is then possible to compare the results at 2.3 mg/ml where the distortions of the CD spectra become severe with the results at 0.023 mg/ml where the distortions are relatively minimal.

For large optically active particles with a diameter of about 0.5 μm, the differential light scattering effects become very apparent and the localized Gaussian shaped CD curve becomes transformed into a flattened optical rotatory dispersion curve. The anomalous optical rotatory dispersion curve (Cotton effect) relates to a circular dichroism band just as the anomalous refractive index (dispersion) curve relates to an absorption band, except that the CD band may be negative or positive. For example, an isolated negative CD band centered at 225 nm and with half-width at $|[\theta]|_{max}/e$ of 15 nm would have a Cotton effect with a negative extremum at 240 nm, with a value of zero at 225 nm and with a positive extremum at 210 nm. If a negative CD band near 225 nm were associated with a positive CD band near 210 nm, then the associated particulate distorted CD spectrum would exhibit a negative extremum near 240 nm, would have a value of zero near 225 nm, would exhibit a larger positive lobe between 225 and 210 nm, would again pass through zero near 210 nm and then would exhibit a negative extremum at wave lengths shorter than 210 nm. Indeed at 40°C and higher temperatures (see Figure 2), the spectra, dominated as they are by differential light scattering, take on the appearance that would arise from a negative CD band near 225 nm and a positive CD band near 210 nm. When the data is collected at 0.023 mg/ml (where the average particle size is much smaller), indeed, this is what is observed at 40°C and above (see Figure 4). This indicates that the spectra obtained at lower concentrations exhibit much more limited distortions. Importantly, it is therefore possible to argue that the confor-


$(\text{L-Val}^1\text{-L-Pro}^2\text{-Gly}^3\text{-L-Val}^4\text{-Gly}^5)_n$

where n=200 .023mg/ml in water

Figure 4: Circular dichroism spectra of 0.023 mg/ml polypentapeptide in water. At this concentration the CD spectra exhibit little particulate distortion and the CD spectrum at 40°C, for example, can be used on introducing the particulate distortions to derive the distorted spectrum at 40°C in Figure 2. This means that the same conformational change is occurring at higher concentration. See text for further discussion.

mation is the same within the aggregates whether the associations are formed from solutions at lower or higher concentration.

On the basis of these studies, it is possible to conclude that coacervation results in a large change in conformation. Furthermore, the CD spectrum in Figure 4 at 40°C and above is characteristic of a Type II β-turn (18,21) indicating that the resulting conformation is one in which each repeating pentamer contains the β-turn. It has previously been shown that films of the coacervate exhibit CD spectra characteristic of a Type II β-turn; also, solution studies in several solvents using proton and carbon-13 nuclear magnetic resonance and x-ray diffraction studies of crystalline $\mathrm{cyclo(L{\cdot}Val^1\text{-}L{\cdot}Pro^2\text{-}Gly^3\text{-}L{\cdot}Val^4\text{-}Gly^5)_3}$ (which in solution has a nearly identical conformation to the linear polypentapeptide) specifically demonstrate the Type II β-turn. These data are reviewed in 3. What is unique in the present studies is that it is possible to determine the temperature profile for the conversion from a less ordered state to a regularly recurring β-turn conformation at different concentrations. At low concentrations the particulate distortions are relatively minimal and at higher concentration, where the particulate distortions of the CD spectra are greater, it is yet possible to verify the same conformational change.

Now it is of interest to compare this higher concentration temperature profile with the temperature dependence of elastomeric force of γ-irradiation (20 Mrad) cross-linked polypentapeptide coacervate. These data are plotted for comparison in Figure 5. The correspondence is dramatic. Thus, one is brought to

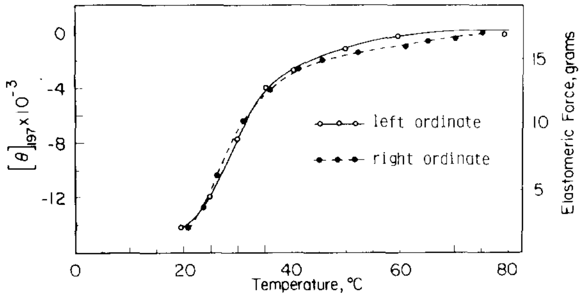


Figure 5: Elastomeric force of γ-irradiation cross-linked polypentapeptide coacervate plotted as a function of temperature on the right-hand ordinate and a replot of curve a of Figure 3 on the left-hand ordinate. The curves are almost superimposed over the entire temperature range. Thus elastomeric force develops as the repeating Type II β-turn conformation develops.

the striking perspective that, below 20°C where there is limited order in the polypeptide, there is little elastomeric force and that the elastomeric force develops in proportion to the development of a regular structure described as one containing a regularly recurring β-turn. Not only is the elastomeric state non-random; the more nearly random state has very little elasticity. This represents the exact inverse of what would be expected on the basis of the classical theory of rubber elasticity.

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