Vol. 90, No. 1, 1979 **BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS**
September 12, 1979 Pages 194-198

PROLYL HYDROXYLATION OF THE POLYPENTAPEPTIDE MODEL OF ELASTIN IMPAIRS FIBER FORMATION

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Received June 28,1979

SUMMARY:

With elastogenesis described as a process dominated by intermolecular hydrophobic interactions and the polypentapeptide, (Val-Pro-Gly-Val-Gly)$_n$, presented as a model for the dominant dynamic elements of the elastic fiber, it is demonstrated that hydroxylation of proline residues of the polypentapeptide unfavorably affects the inverse temperature transition leading to fiber formation such that even with only 10% of the proline residues hydroxylated very little fiber formation occurs at 37°C. Significantly higher temperatures are required. As prolyl hydroxylase, elaborated during a fibrogenic response, hydroxylates elastin, this result raises the question as to whether hydroxylation of the precursor protein of the elastic fiber may similarly impair in vivo fiber formation.

INTRODUCTION:

On raising the temperature of aqueous solutions of tropoelastin (the precursor protein of the elastic fiber), of α-elastin (a chemical fragmentation product of the elastic fiber), of the polypentapeptide (Val-Pro-Gly-Val-Gly)$_n$, and of the polytetrapeptide (Val-Pro-Gly-Gly)$_n$, a phase separation, called coacervation, occurs in which the molecules order themselves into filamentous structures (1-4) with periodicities verified by optical diffraction of the electron micrographs (3,4) to be closely similar to those of native elastin (5). Coacervation of these peptides, therefore, is a process of fiber formation.

0006-291X/79/170194-05$01.00/0

This has been demonstrated conclusively by simultaneous coacervation and covalent cross-linking of the synthetic polypentapeptide where, without flow orientation, there is a self assembly into fibers which are visible in the light microscope and in the scanning electron microscope where the fibers are seen to splay out into many fine fibrils and to recoalesce back into a single fiber (6). These synthetic polypentapeptide fibers, when cross-linked with flow orientation to form a matrix, are elastomeric and can, with the appropriate water content, have the same elastic modulus as aortic fibrous elastin (6). Because of this, because in the carbon-13 magnetic resonance spectra the five carbonyl carbon resonances of the polypentapeptide coincide exactly with the five dominant carbonyl carbon resonances of α-elastin (7), because large peptide fragments have been isolated from elastin with the pentamer composition (8-11), and because the polypentapeptide coacervate has mobility (12) similar to that found in the native elastic fiber (13), the polypentapeptide is a compelling model for the more dynamic elastomeric elements of the elastic fiber.

It is also interesting that the temperature profile of coacervation of the polypentapeptide (see Figure 1, curve a), which gives the temperature profile for fiber formation, is delicately balanced such that just below physiological temperatures the polypentapeptide is in the solution state but on raising to physiological temperatures it has converted to the coacervate, i.e. to the fibrous state. This filamentous ordering on raising the temperature is an inverse temperature transition indicative of dominant intermolecular hydrophobic association (14). Since the polypentapeptide fiber formation is due to intermolecular hydrophobic interactions, since the proline side chain is a significant hydrophobic side chain, since many proline residues of tropoelastin become partially hydroxylated during a fibrosis when the enzyme activity of prolyl hydroxylase is raised, and since the polypeptide is a compelling model for the elastic fiber, it becomes of particular interest to determine the effect of hydroxyproline (HyP) incorporation on the temperature profile of coacervation of the polypentapeptide.

MATERIALS AND METHODS:

When pentamer polymerization is carried out with (Val-HyP-Gly-Val-Gly) to (Val-Pro-Gly-Val-Gly) ratios of 1:99, 1:9 and 1:0, the 1%, 10% and 100% derivatives are formed. The monomeric pentapeptide units, TFA·H-Val-Pro-Gly-Val-Gly-ONp (I) and TFA·H-Val-HyP(Bzl)-Gly-Val-Gly-ONp (II), were synthesized by the classical solution methods, the complete details of which will be published elsewhere. Briefly, polymerization of the hydroxyproline containing peptide (II) was carried out in DMSO for 14 days after which time HCl-H-Val-OCH_3 was added to terminate the reaction. The diluted reaction mixture was dialyzed against water and lyophilized to obtain H-(Val-HyP(Bzl)-Gly-Val-$Gly)_n$-Val-OCH_3. The protecting benzyl groups were removed by HBr/TFA treatment and after the usual work up, the (100% HyP)-polypentapeptide was obtained after lyophilizing from water. Similarly prepared were the (1% HyP)- and (10% HyP)-polypentapeptides by copolymerizing I and II in the corresponding ratios. The factor n (extent of polymerization) was calculated to be greater than 40 by nuclear magnetic resonance end group analysis of the three polymeric peptides. Amino acid analysis confirmed incorporation to be as expected from the ratios of the added pentamers.

The temperature dependence of coacervation was followed spectroscopically with a Cary 14 spectrophotometer by monitoring the 300 nm optical density due to light scattering from the coacervating suspension. The temperature of the sample was increased at the rate of 30°C per hour with a Haake temperature controller and constant sample agitation was achieved with a 300 kHz vibrator. The 30°C per hour rate was chosen because, at this rate, equilibrium is maintained throughout the temperature scan such that a similar curve is obtained on going from high to low temperatures. The total optical density changes were 0.6, 1.4, 1.0 and 0.2 for the non-hydroxylated polypentapeptide, the 1%, the 10% and the 100% hydroxylated polypentapeptides respectively. These values were nomalized individually so that each curve's maximum absorbance was defined as 100% for that curve. The absolute optical densities differed, in part, due to different rates of settling. The concentrations of polymers in the equilibrium solution after settling were 0.42 mg/ml, 0.26 mg/ml and 0.17 mg/ml for the 1%, 10% and 100% hydroxylated polypentapeptides.

RESULTS AND DISCUSSION:

As seen in Figure 1, the 1% HyP polymers exhibits a temperature profile which is essentially the same as that of the parent polymer but 10% HyP causes a significant shift to higher temperatures such that there is very little fiber formation at 37°C. Complete conversion to the HyP polypentapeptide shifts the process of fiber formation to 60°C or above. Similarly when the polypentapeptide is enzymatically hydroxylated by prolyl hydroxylase (15), the process of fiber formation is shifted above physiological temperatures (see curve e of Figure 1). While enzymatic hydroxylation of the polypentapeptide is found to be 1% (15), the effect is greater than with the synthetic HyP incorporation. Presumably this is due to differences in the distribution of HyP in the polypentapeptide. Consistent with fiber formation being a dominantly intermolecular

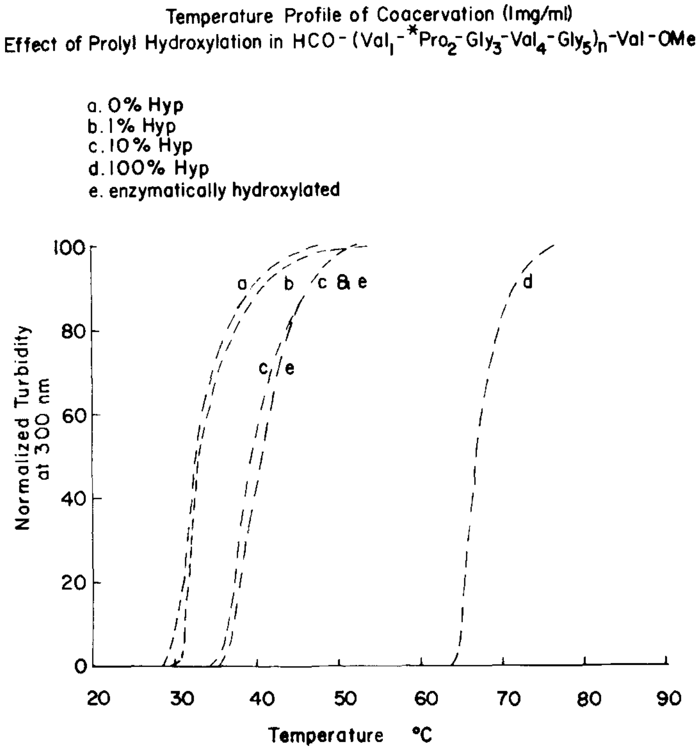

FIGURE 1: Temperature profile of coacervation in water of the polypentapeptide as a function of extent of hydroxylation of the prolyl residue (i.e. HyP). The biological HyP isomer was used. Curves a through d are at 1 mg/ml and curve e is at 5 mg/ml. The effect of increased concentration as well as increased ionic strength is to shift the temperature profile of coacervation to lower temperatures (14). While fiber formation is the balance of many factors the effect of hydroxylation is one of impeding association leading to fiber formation. The observation that 0.42 mg/ml, 0.26 mg/ml and 0.1 mg/ml remained in the equilibrium solutions for the 1%, 10% and 100% hydroxylated polypentapeptides, respectively, indicates that the observed translation of the curve to higher temperatures in these studies is a conservative result and that the effect of hydroxylation per se can even be greater than demonstrated here and would be consistent with enzymatic hydroxylation having an even greater effect.

hydrophobic process, hydroxylation of proline is seen to impair fiber formation at physiological temperatures.

While hydroxylation of collagen by prolyl hydroxylase is important for release of collagen from the cell (16), for increased strength and thermal stability of collagen (17-20) and for resistance of collagen to non-specific proteolytic degradation (17,18), the question must now be asked as to whether hydroxylation of proline residues in elastin by the same prolyl hydroxylase enzyme (16,21,22) may impair elastic fiber formation.

ACKNOWLEDGMENT

This work was supported in part by the National Institutes of Health, Grant No. HL-11310. M. M. Long is an Established Investigator with the American Heart Association.

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