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Calorimetric and Dynamic Mechanical Analysis of Thermal Transitions in Collagen

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Abstract

DSC and DMA measurements were carried out on a collagen sample. Effect of humidity, Tensil modulus and Thermal denaturation between 30 °C and 130 °C were studied.

Introduction

Collagen, a fibrous protein, is the most abundant of all proteins in higher animals, making up one-third of the total body protein (1).

Thermal and mechanical properties of collagen play an important role in a number of applications. In food industry, for instance, collagen is used as a hydrocolloid to modify food texture or stability. Collagen is also used in a wide variety of products including cosmetic skin creams, industrial filters, fining agents for beverages or medical wound dressing.

The molecular structure of collagen has been extensively studied in relationship to a number of effects including aging (2), prosthetic rejection and genetic disorders but, the complete molecular structure is not fully understood as of yet (3, 4).

Depending upon the tissue type and the state of development, the structure of collagen can vary. Figure 1 shows that a fiber bundle or filament contains fibers and fibrils and is surrounded by an outer sheath. A bundle is bound together by heat-labile reducible disulfide cross-links among fibers and sheath. The fiber bundles range in

diameter from 100,000 to 200,000 Angstrom and up to 20 millimeters in length.

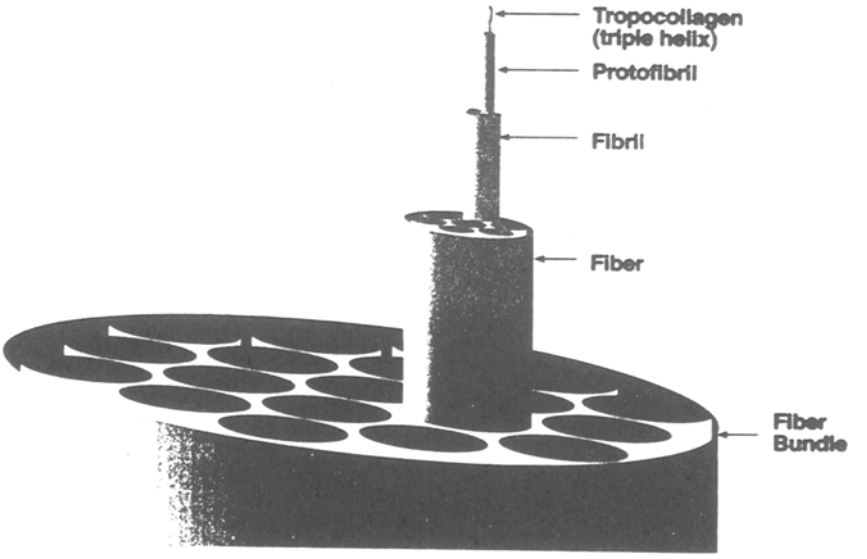

Figure 1 Structural Components of Collagen Fiber

Collagen fibrils contain proto-fibrils, that are aggregates of tropocollagen molecules. They are covalently cross-linked through an enzymatic reaction between two lysine residues of adjacent tropocollagen subunits. These are the heat stable cross links associated with aging. Tropocollagen is built up of protein chains forming a triple helix. Recent work suggests that there are several sites and states of water fixation within the proto-fibrils and microfibrils, each with different energies of dissociation (5).

Object of that study is to investigate the mechanical and thermal properties of a collagen sample and to assign those to processes on a molecular level.

Experimental

Collagen samples were analyzed in a Dynamical Mechanical Analyzer (Perkin Elmer DMA 7e) and a power compensated Differential Scanning Calorimeter (Perkin Elmer DSC 7). Results obtained by a DMA are typically storage modules as a measurement for the energy stored in a sample and loss modules related to the energy dissipated by activated processes. A power compensated DSC allows the direct measurement of enthalpy effects caused by thermal transitions. These quantities are sensitive towards any changes of molecular structure.

Type I native bovine collagen fibers and fiber bundles were extracted, in the form of a fiber mat, from the Corium layer of bovine hide. The samples were washed, purified and placed in a 9 % solids dispersion with normal saline solution. The samples were frozen until testing.

For DSC measurement the dispersion was thawed at room temperature and a sample of single fibers and fiber bundles was cut from the dispersion. A sample mass of 61.7 milligrams was placed in a 75 microliters hermetically sealed capsule and analyzed at 1,2 and 5 °C per minute. Heat flow was plotted, following the thermodynamic convention of endotherm up versus temperature.

For DMA measurement the diameter of the specimen was measured using an infrared microscope. The extension measuring system was mounted. A static force of 4mN, dynamic force of 3mN and a frequency of 1 Hz was selected. For Temperature Mode measurements the furnace was raised and the temperature programmed to 30 °C. Temp 1 was set to 30 °C, Rate was set to 1 °C/min and Temp 2 was set to 130 °C.

A stainless steel cylinder (6) closed on one end was placed inside the standard furnace and filled with about 10 milliliters of normal saline solution. The cylinder and the solution were temperature controlled using the standard furnace while providing a saturated saline solution environment for the sample.

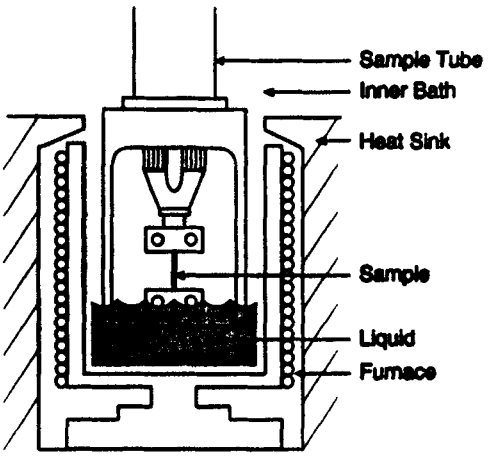

Figure 2 DMA 7e Inner Bath Stetup

Results and Discussion

The Tensile modulus and the DMA modulus were characterized using a stress/strain analysis. The Tensile modulus is obtained by increasing the static strain (i.e. an increasing static force is applied). The slope of the stress/strain curve before the yield, gives a Tensile modulus of 2.77 x 10^9 Pa. The Tensile modulus is very similar to Youngs modulus of elasticity.

The DMA modulus is obtained by increasing the dynamic stress (i.e. an oscillating force is applied). As shown in Fig. 2 a DMA modulus of 6.6 x 10^9 Pa is determined.

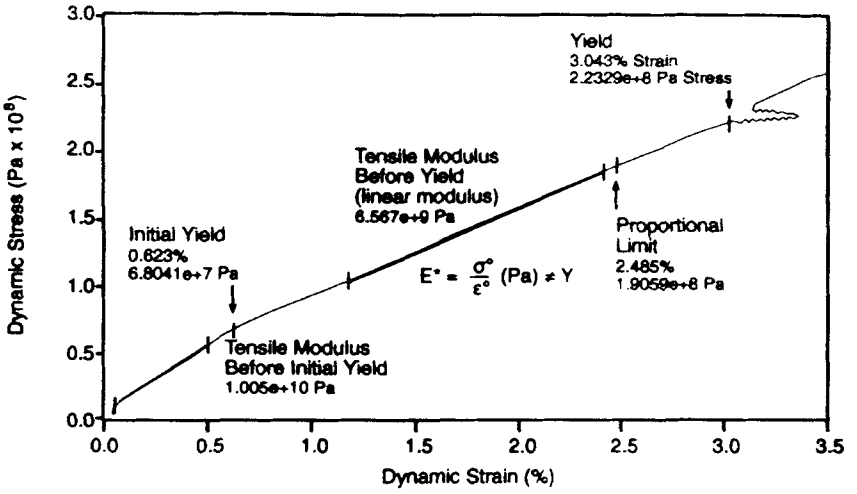

Figure 3 DMA Modulus Tensile behaviour of Collagen at 100 % RH

The effect of humidity on the fiber length and on the modulus has been studied. The fiber was first exposed to ambient laboratory conditions (40 % relative humidity) for approximately 10 minutes and then emerged into a normal saline solution. The fiber became lenghtened and the modulus increased (Fig. 4, 5) Lengthening of collagen samples is confirmed by HIT (Hydrothermal Isometric Tension) measurements (7) but the effect of humidity on collagen modulus does not appear in literature.

Storage modulus, Loss modulus, Tengent Delta, Heat Flow and Heat Capacity were recorded as a function of temperature as shown in figure 6 and 7.

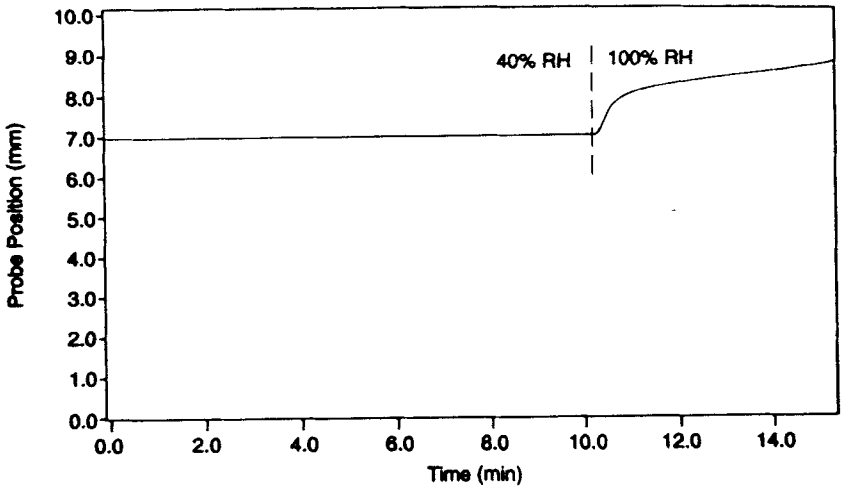

Figure 4 Effect of Humidity on Sample Length

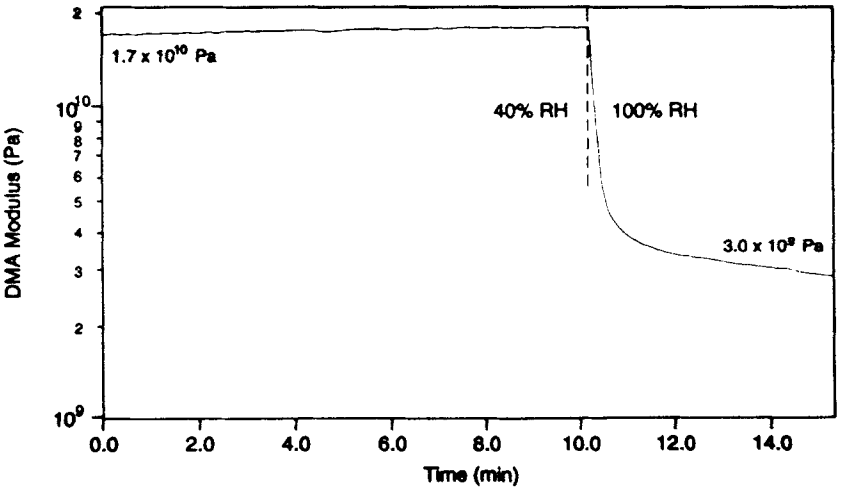

Figure 5 Effect of Humidity on Modulus

There is an increase in the DMA modulus from 38 to 45 °C. Initial evidence suggested that this modulus increase was due to the rubber-elastic properties which denaturation confers on the collagen network (8, 9). However, this has been more recently attributed to collagen fiber sheath denaturation (10, 11).

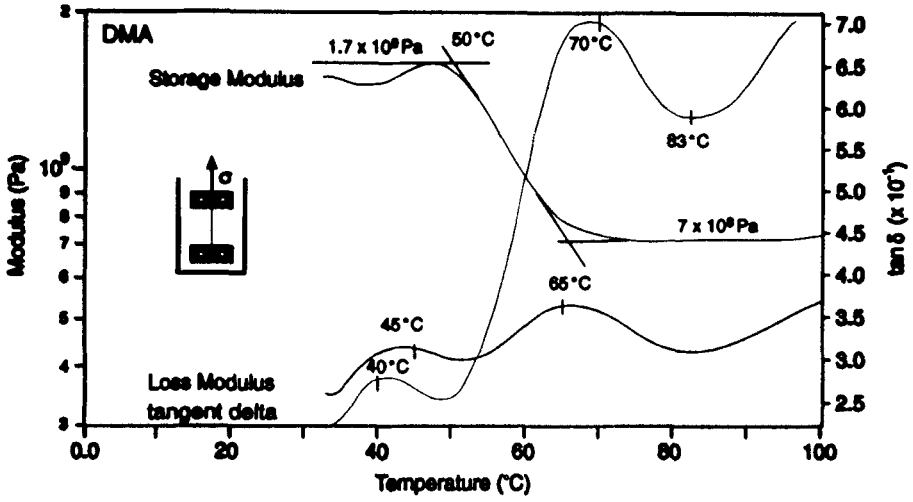

Figure 6 Thermal Denaturation of Collagen by DMA

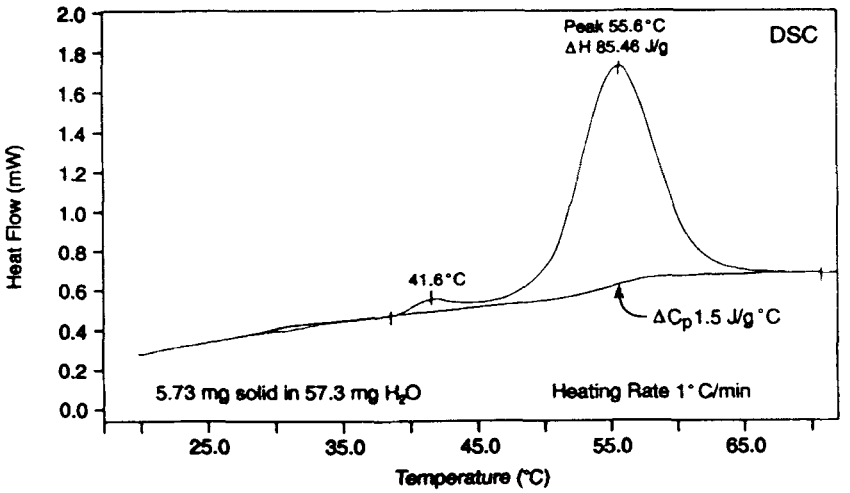

Figure 7 Thermal Denaturation of Collagen by DSC

This new evidence suggests that destruction of heat-labile (reducible) disulfide cross-links formed by dehydro HHMD (histidino-hydroxy-merodesmosine) and dehydro HLNL (hydroxy-lysino-norleucine) of the outer sheath is the major contributor of this modulus increase. Each cllagen filament contains thousands of fibers and fibrils, all bound together by di-sulfide cross-links and

a sheath. The fibers range in diameter from 10,000 to 20,000 Angstroms. When the cross-links of this sheath are destroyed, the filament contracts, as shown here by the probe position in figure 8.

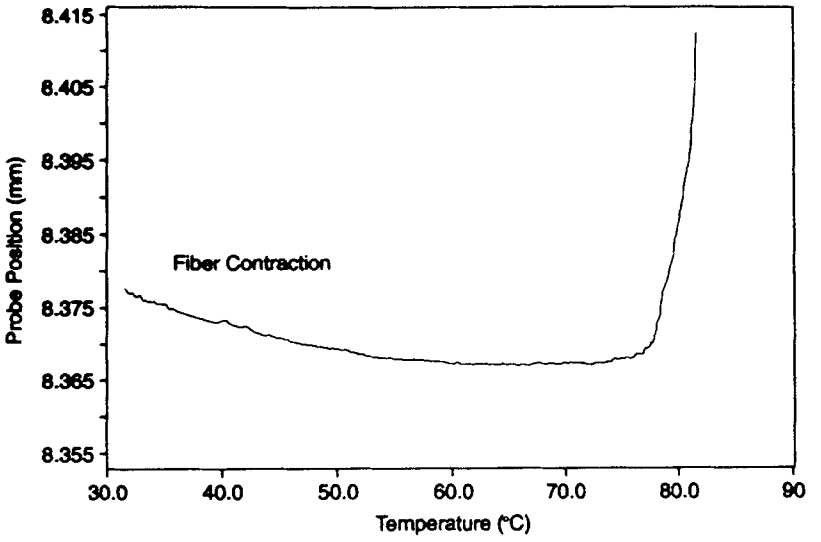

Figure 8 Thermal Denaturation of Collagen by TMA

Beside heat labile cross-links mentioned above also heat stable cross-links exist, that are the covalent bonds between two lysine residues of adjacent tropocollagen subunits. Recent work on the collagen of human skin, which contains only heat-stable cross-links confirms that when heat-stable cross-links are present, the rate of denaturation of the triple-helix decreases and results in a overall decrease in the rate of denaturation of the collagen network (12).

The peak in the DMA modulus observed at 50 °C is due to the loss of fixed water and collapse of the triple-helix. This transition can also be observed under refraction as a colorful product. Recent work suggests that there are several sites and states of water fixation within the protofibrils and microfibrils, each with different energies of disassociation (5). It was suggested that water fixation in collagen is similiar to water fixation in Nylon 6 (13).

The DSC peak at 55 °C is due to denaturation of collagen and collapse of triple-helix of tropocollagen. The temperature at which collagen denaturates in equilibrium conditions is known to vary with fiber diameter and organization, mainly due to intra-water content.

Figure 7 shows an increase in heat capacity. Such a step is often associated with changes in hydrogen bonding. The helical arrangement of collagen allows every peptide bond to participate in hydrogen bonding and hydrophobic interactions. They are oriented to give nearly maximal strength.

By 65 °C there is a complete loss of order of the collagen network. This transition can also be observed under refraction as a loss of color.

The protein denaturation, or "thermal melting" observed at 55°C is the unfolding of the protein chain from the native, biologically active form, without breaking of covalent bonds. Recent calorimetric data (14), indicating protein unfolding is a kinetic, thermodynamic process involving several intermediate states. Statistical mechanics for the prediction of protein unfolding also predicts multiple conformational states (15, 16). Also, the formation of domains and subdomains within the protein suggests that there are multiple pathways for folding. In fact a kinetic pathway for triple helix formation and directionality for folding has been recently established (17, 18). Computer simulations indicate a residual hydrophobic core, a "dry" interior, exists inside the triple-helix (19).

Summary

Denaturation or thermal melting of collagen has been studied using the techniques of DSC and DMA. Both methods are suitable for the investigation of a wide variety of ordered structures that was demonstrated on a collagen sample.

A table summarizing the events observed is given below.

Thermal Melting of Collagen by DSC and DMA

DSC °C	DMA °C	Event	Structural Unit	Other Observations
42	40	Heat-labile cross-links, Hydrogen bonds	Collagen and fiber bundle sheath	Contraction
	50	Reducible cross-links	Tropocollagen	
		Heat-labile cross-links and random domain denaturation	Polypeptide	
55		Triple helix collapse hydrophobic and heat-labile hydrolyzed	Tropocollagen	Colorful under refraction
	65	Loss of order	Tropocollagen	Loss of color
	70	Heat-stable cross-links	Protofibrils	
	83	Hydrolysis	Polypeptide	

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