

A Research Note

Changes in the Denaturation Characteristics of Collagen Induced by Bacterial Collagenase Preparations

MICHAEL H. TUNICK

ABSTRACT

A differential scanning calorimetric procedure adapted from the literature was used to study the effects of collagenase on the thermal behavior of collagenous material. Two different preparations of the enzyme and two sources of collagen were used. The samples were treated in the same fashion as in the published procedure, and were found to have similar decreases in their denaturation enthalpies. However, the previously reported decrease in denaturation temperature of collagenase-treated material was not duplicated in any sample. Alternate interpretations of the previous results are presented.

INTRODUCTION

COLLAGENASES are enzymes that cause hydrolytic cleavage of peptide bonds in the helical regions of the native collagen molecule. General proteases such as trypsin are not considered collagenases since they attack the nonhelical regions of collagen (Keil, 1979; Seifter and Harper, 1971). The degradation of collagen by *Clostridium histolyticum* was first demonstrated over 70 years ago (Keil, 1979). The enzyme isolated from this bacteria is clostridiopeptidase A, one of the two purified collagenases generally available. Commercial preparations are contaminated with proteases and peptidases, which are other components of the proteolytic system of *C. histolyticum* (Hagihara, 1960). Clostridial collagenase extensively degrades the collagen molecule, which then becomes susceptible to the action of the other proteases (Nordwig, 1971; Seifter and Harper, 1971).

An objective of the meat industry is the restructuring of less desirable cuts into steak-like products. When high levels of connective tissue are present, the resulting products are unacceptably tough due to the presence of fibrillar collagen. Enzymatic breakdown of this protein into fragments has been suggested as a method of tenderization (Kang and Rice, 1970; Foegeding and Larick, 1986). The purpose of this study was to investigate enzymatic breakdown of collagen as a way of reducing connective tissue in restructured beef. Bernal and Stanley (1986) reported that treatment of bovine tendon collagen with *C. histolyticum* collagenase resulted in reductions in the enthalpy and temperature of denaturation. This means that the amount of heat required to denature the collagen as well as the temperature at which the denaturation occurs would be lowered. The purpose of this study was to analyze the effects of two collagenase preparations on the enthalpy and temperature of denaturation of connective tissue isolated from meat.

MATERIALS & METHODS

INSOLUBLE ENDOMYSIAL and perimysial connective tissue was isolated from a sample of beef chuck obtained from a 1-yr-old animal approximately 36 hr post-mortem (Cronlund and Woychik, 1986). The dry-blending method of McClain (1969) was used, after which strands of white connective tissue

were removed from the pulverized muscle with forceps, washed for 3 hr at 5°C with 0.15M NaCl, rinsed with and dialyzed against deionized water, and lyophilized. An amino acid analysis of the isolated material demonstrated it to be essentially collagen. Bovine Achilles tendon collagen (Type V, No. C-4387) was obtained from Sigma Chemical Co., (St. Louis, MO), as was *C. histolyticum* collagenase (Type VII, No. C-0773), described as "a chromatographically purified lyophilized powder substantially free of nonspecific protease, clostripain, and tryptic activities." *C. histolyticum* collagenase (Type IV, No. C-5138), described as being "characteristically low in tryptic activity relative to crude collagenase," was also purchased from Sigma. The collagen types mentioned here are designations used by Sigma and are not related to the genetic types of collagen.

As in the previous publication (Bernal and Stanley, 1986), lyophilized samples of collagenous material were rehydrated in 50 mM CaCl₂-1.0M Tris (hydroxymethyl) aminomethane buffer (pH 7.4) for 24 hr at 4°C. After the addition of 1.0 mg collagenase per 25 mg sample and 7-hr incubation at 25°C, the samples were washed with 0.2M phosphate buffer (pH 7.4) and stored at 4°C.

Differential scanning calorimetry (DSC) experiments were performed in a Perkin-Elmer DSC-2 (Perkin-Elmer Corp., Norwalk, CT). The procedure of Bernal and Stanley (1986) was generally followed, using 8-16mg rehydrated sample in phosphate buffer, sealed in aluminum volatile sample pans. Samples were heated from 20 to 100°C at a rate of 5°C/min with nitrogen as the purge gas. The peak temperature was taken to be the temperature of denaturation (T_D). Peak areas, in mcal, were determined by the cut-and-weigh method. The dry matter content of the samples was found by puncturing the sample pan lids following DSC analysis, allowing the water to evaporate, and weighing. The enthalpy of denaturation (ΔH_D) for each sample was then calculated by dividing mcal by mg.

RESULTS & DISCUSSION

TYPICAL THERMAL CURVES of collagenous material from bovine Achilles tendon are shown in Fig. 1a, 1b, and 1c. The curve of the control sample, which was incubated in buffer but not enzymatically treated, is shown with the curves of samples that were treated with the two preparations of *C. histolyticum* collagenase. DSC peak areas are proportional to both weight and enthalpy; however, when ΔH_D alone is examined (Table 1), trends can be observed. The ΔH_D decreased by 18% when the collagen was treated with purified (Type VII) collagenase, and by 52% when the less pure (Type IV) collagenase was used. The latter result is comparable to that observed by Bernal and Stanley (1986). Thermal curves of collagenous material from beef chuck, both untreated and treated with the enzymes, are shown in Fig. 1d, 1e, and 1f. The ΔH_D values were 1 cal/g higher than those of the tendon collagen in the present work, and the decreases in ΔH_D with the Type VII and Type IV collagenases were 16% and 48%, respectively. All of these enthalpy decreases are indicative of collagen breakdown and would be desirable in the tenderizing of meat.

DENATURATION CHARACTERISTICS OF COLLAGEN.

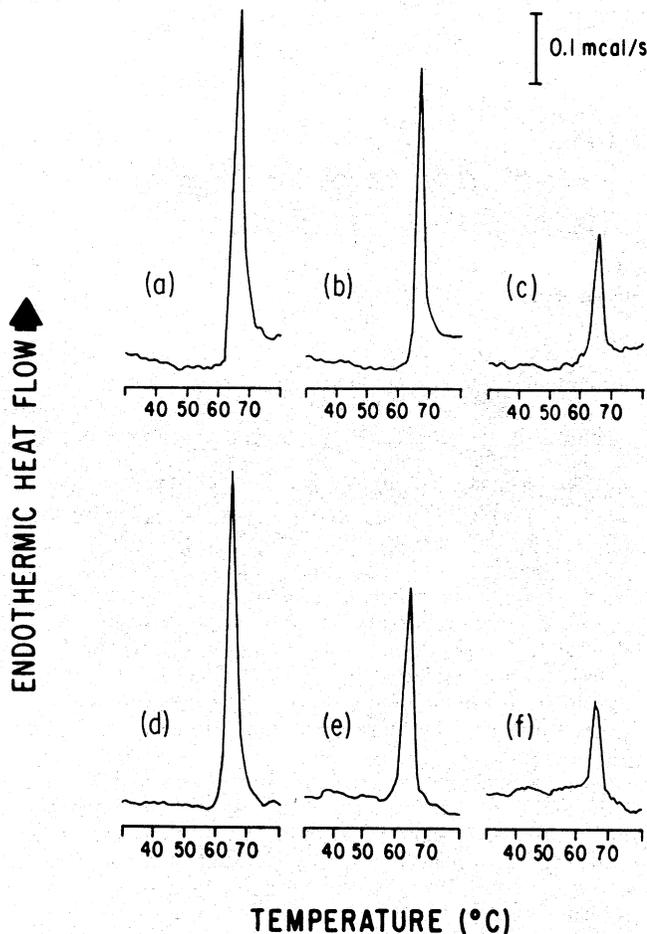


Fig. 1—Thermal denaturation curves of untreated and collagenase-treated collagenous material from bovine tendon (a-c) or beef chuck (d-f) in pH 7.4 phosphate buffer (dry weights in parentheses): (a) No enzyme added (2.40 mg); (b) Treated with purified collagenase (1.75 mg); (c) Treated with less pure collagenase (1.32 mg); (d) No enzyme added (2.41 mg); (e) Treated with purified collagenase (1.74 mg); (f) Treated with less pure collagenase (1.95 mg).

Table 1—Temperature and enthalpy of denaturation of intact and collagenase-treated fibrillar collagens determined by differential scanning calorimetry

Collagenase used	Denaturation temperature (°C)	Denaturation enthalpy (cal/g dry sample)
Bovine tendon collagen		
none	67.3 ± 0.9 ^b	8.82 ± 0.16
Type VII	65.9 ± 0.4	7.23 ± 0.24
Type IV	66.6 ± 0.9	4.20 ± 0.32
none ^a	61.6 ± 0.9	10.60 ± 0.06
Type IV ^a	43.6 ± 0.9	6.24 ± 0.15
Beef chuck collagen		
none	68.5 ± 0.8	9.90 ± 0.70
Type VII	67.3 ± 0.6	8.27 ± 0.30
Type IV	66.0 ± 0.2	5.19 ± 0.67

^a Data from Bernal and Stanley (1986).

^b Standard deviation of 4 samples.

The differences in the ΔH_D decreases between the two collagenases may be explained by the presence of other enzymes. Commercial collagenases are isolated from media containing several different proteases, and can be contaminated by variable amounts of them (Seifter and Harper, 1971). The Type

IV collagenase employed in the previous work was not highly purified, while the Type VII enzyme used in the present work is described in the manufacturer's literature as being of a high purity and substantially free of contaminating proteolytic activities. A recent comparison of commercial collagenase preparations showed that the specific activities of contaminating proteases present in Sigma Type IV collagenase are much greater than those in Type VII (Bond and Van Wart, 1984). Proteases present in the Type IV preparation probably attacked the non-helical sections of the collagen molecules, producing a greater decrease in ΔH_D than would be expected if only collagenase were present.

The T_D for the intact collagen was somewhat higher than the previously reported results (Table 1). However, the T_D of the enzyme-treated collagen showed little decrease, with no evidence of the denaturation around 44°C that was observed in the previous work (Fig. 1). Although the baselines of the thermal curves of the enzyme-treated samples did become rougher, no measurable peaks were present. Conversion of fibrillar collagen into molecular collagen resulted in a T_D decrease of 25°C (Aberle and Mills, 1983), while the ΔH_D did not change (McClain and Wiley, 1972). Although Bernal and Stanley (1986) concluded that their enzyme-treated samples were degraded into molecular collagen, there is no indication that this took place when their procedure was followed exactly. Even when other sets of samples were incubated at 37°C, no decrease in T_D was found. Instead, it appears that the collagen was merely broken up into smaller pieces, thus lowering the ΔH_D while not affecting the T_D . Previous conclusions about disruption of noncovalent intermolecular bonds resulting in thermodynamic changes are therefore not valid in light of these results.

Collagenase reduces the enthalpy of denaturation of fibrillar collagen, although the temperature of the denaturation does not change. The observed breakdown of collagenous material should be enough to improve the eating quality of restructured meat. An optimum level of tenderness could then be obtained by variation of experimental parameters.

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