THE RELATIONSHIP BETWEEN PYRIDINOLINE AND THERMAL STABILITY OF MUSCLE COLLAGENS

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SUMMARY

The thermal transition temperature and pyridinoline concentration were determined for collagen prepared from goat longissimus dorsi muscles. Pyridinoline concentration increased with animal age (0.25 to 12.4 years) from 0.19 to 0.33 mole pyridinoline/mole collagen for intramuscular connective tissue (IMCT). The initial relatively high level of pyridinoline in IMCT casts some doubt on the traditional maturation model of collagen crosslinking. Thermal transition temperature also increased with animal age from 60.37 to 64.61°C for IMCT, and from 56.27 to 62.97°C for tendon. A linear relationship can be proposed between IMCT pyridinoline concentration and thermal transition temperature with a correlation of 0.63 which is significant at the 99% confidence level. The results show that pyridinoline is a mature crosslink of IMCT collagen and that it contributes to the thermal stability of collagen. Ample scope exists for the presence of other mature crosslinks in tendon and IMCT.

INTRODUCTION

Essential steps in collagen crosslink formation (reviewed by Eyre et al. 1984) are: (i) enzymic oxidation of the sidechain of a lysine or hydroxylysine residue to an aldehyde, (ii) reaction of this aldehyde with an e-NH_2 group of lysine or hydroxylysine to form an aldimine linking two polypeptide chains, (iii) in the case of an hydroxylysine aldehyde precursor, rearrangement of the aldimine to a ketoimine which is thermally more stable, (iv) further reaction of the aldimine or ketoimine with a functional group in another collagen molecule to form a mature crosslink.

In the traditional model of collagen maturation, step (iv) is thought to occur much more slowly than the earlier ones (eg. Eyre and Oguchi 1980). Mature crosslinks formed via the lysine aldehyde pathway differ from those of the hydroxylysine aldehyde/ketoimine pathway. One crosslink formed via the latter pathway is pyridinoline (Fujimoto et al. 1978; Eyre and Oguchi 1980) which links three polypeptide chains. The importance of pyridinoline as a heat stable crosslink in cooked meat has been dismissed by Light (1986), due to the reported instability of isolated pyridinoline to heat (Fujimoto et al. 1978). However Sakura and Fujimoto (1981), and Gunja-Smith and Woessner (1985) have reported that pyridinoline, when present as a crosslink in collagen, is more stable than free pyridinoline.

Previous workers have found that the number and nature of crosslinks is likely to be the major factor determining the thermal stability of collagen (Flandin et al. 1984) and that thermal transition temperature increases with increasing animal age (King 1987).

The aim of this paper is to determine if there is a relationship between the thermal transition temperature of muscle collagen and its pyridinoline concentration.

METHODS

Goats of known birthdate were slaughtered and the dressed carcasses pelvic hung in a chiller at 2°C for 48 h. Muscles were then removed and frozen at -20°C until used. Intramuscular connective tissue (IMCT) was prepared by homogenizing (3 times) diced (<5 mm) muscle trimmed of tendon, fat and epimysium in 50 mM sodium citrate, 10 mM EDTA buffer pH 6.0. The IMCT accumulating on the homogenizer blades was retained. A portion of this material was used for thermal transition determination by differential scanning calorimetry (King 1987). The remainder of the IMCT was freeze dried and then approximately 100 mg was acid hydrolysed (Meltzer et al. 1987) and taken up in 5 ml 0.1 M HCl. 100 µl was withdrawn for hydroxyproline analysis and the remainder fractionated on a phosphocellulose column (1.6 x 7 cm) by washing with 0.1 M HCl (1 h) and eluting the pyridinoline with 1 M HCl. The pyridinoline containing fraction was dried by rotary evaporation and taken up in 400 µl of 0.2 M sodium citrate pH 4.25. 200 µl was applied to an Interaction AA911 amino acid analyser column. Pyridinoline concentration was determined by comparison to the ninhydrin developed peak area of a known amount of pyridinoline prepared by the method of Fujimoto et al. (1977).

Tendon samples were treated similarly except that they were not homogenized but soaked overnight in buffer prior to measurement of thermal transition temperature.

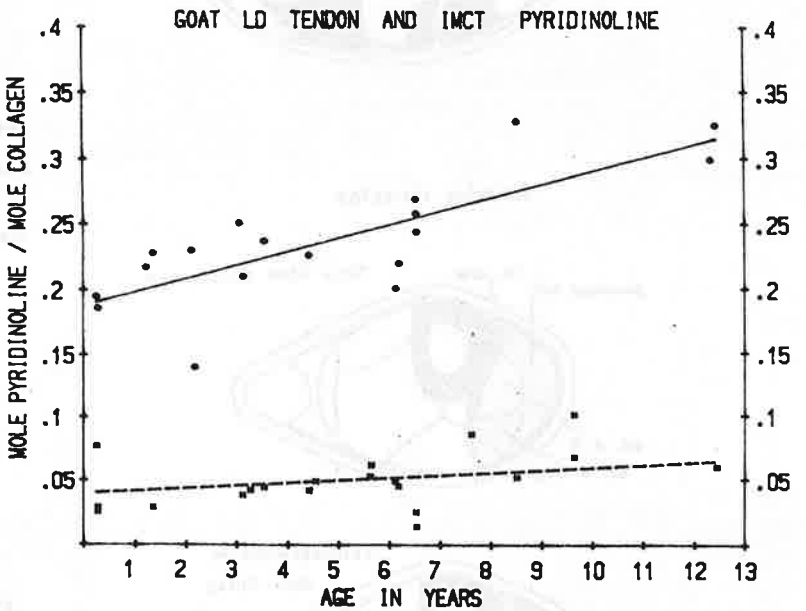

Figure 1 Pyridinoline concentration as a function of animal age. Pyridinoline concentration is expressed as moles pyridinoline per mole (MWt = 300,000) collagen. Continuous line = Intramuscular connective tissue. Dashed line = Tendon.

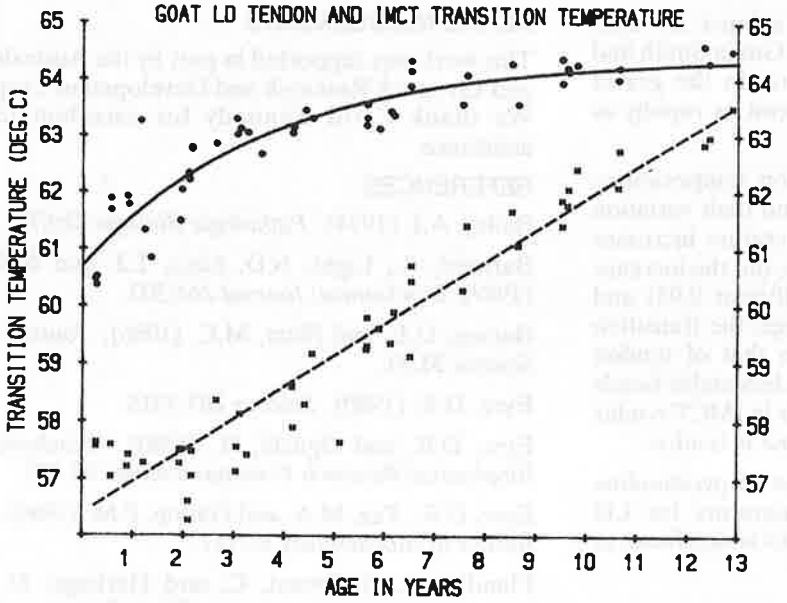

Figure 2 Thermal transition temperature as a function of animal age. Continuous line = Intramuscular connective tissue. The curve was obtained by fitting an equation of the type y = a + b exp(kt) where t is animal age, and a, b and k are constants. Dashed line = Tendon.

RESULTS AND DISCUSSION

The collagen content of the IMCT preparation was approximately 40% on a dry weight basis representing a 20-40 fold enrichment in collagen concentration by the preparative procedure. On average, 65% on a dry weight basis of the tendon was collagen.

The thermal stability of pyridinoline was confirmed by analysis of IMCT that had been heated at 80°C during the determination of its thermal transition temperature. However, it was more convenient to analyse larger samples of IMCT for pyridinoline as detailed in the methods.

Figure 1 shows the pyridinoline concentrations determined in longissimus dorsi (LD) IMCT and tendon and their variation with animal age. Pyridinoline concentration does not significantly increase (at 95% confidence level) with age in tendon, however in IMCT there is an age related increase which is highly significant (at)99% confidence level).It is clear that pyridinoline occurs in different concentration ranges in these two types of collagenous tissue. The 4-5 fold higher concentration of pyridinoline in IMCT compared to tendon at any given age accords with the higher proportion of dihydroxylysinonorleucine to hydroxylysinonorleucine in reduced IMCT compared to tendon (Shimokomaki et al. 1972). Whilst the IMCT contains a mixture of predominantly types I and III collagen in the ratio of approximately 7:3 (Light et al. 1985, Burson and Hunt 1986), compared to predominantly type I collagen in tendon, this is unlikely to produce the variation in crosslink concentration observed since both types of collagen have conserved the same homologous sites of crosslinking (Eyre et al. 1984).

The IMCT data shown in Fig. 1 agree with results we obtained for bovine muscle, where a similar concentration change was observed in samples from animals aged 2 weeks and 11 years old. The relatively high levels of pyridinoline found in young animals are consistent with a biphasic production of this crosslink rather than a slow and steady maturation type reaction. Thus there could be an initial burst of pyridinoline production arising from rapid condensation of ketoamines in 4D staggered fibrils, followed by a slower steady production of pyridinoline by condensation of ketoamines in 0D staggered fibrils growing laterally. Eyre (1980) has demonstrated that pyridinoline could form in both of these packing arrangements. However, biphasic production of pyridinoline is not the only possible interpretation of the results in Fig. 1. An alternative explanation is that pyridinoline may be formed only within a limited time period after collagen molecules are synthesised, and, during subsequent collagen turnover, there may be selective catabolism of less crosslinked molecules leading to a gradual increase in pyridinoline-rich collagen during the advancing years of an animal's life. This explanation is supported by the findings of Uchiyama et al. (1981) who reported that (i) during in vitro incubation of decalcified chick bone collagen, the pyridinoline content reached a plateau after six weeks and (ii) susceptibility of collagen fibres to pepsin digestion decreased as pyridinoline

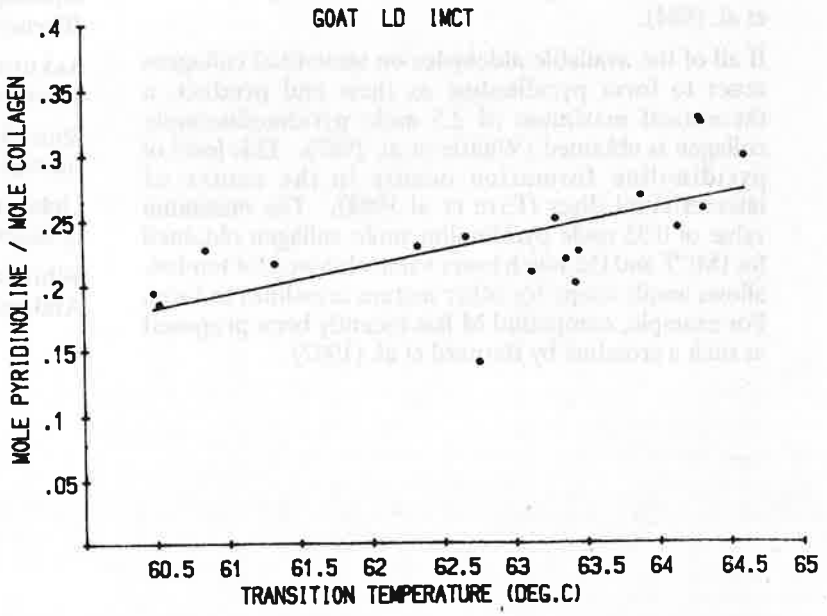

Figure 3 Pyridinoline concentration as a function of thermal transition temperature for longissimus dorsi intramuscular connective tissue.

content increased. The concept of a burst of rapid pyridinoline formation is supported by Gunja-Smith and Woessner (1985) who have found that in the gravid human uterus, pyridinoline forms at least as rapidly as new collagen is deposited.

Figure 2 shows the thermal transition temperatures recorded for LD IMCT and tendon and their variation with animal age. The transition temperature increases with animal age for both types of tissue, but the increase is linear for tendon (correlation coefficient 0.93) and curvilinear for IMCT. At any given age, the transition temperature of IMCT is higher than that of tendon indicating that the IMCT contains more heat stable bonds than tendon, or alternatively, the bonds in IMCT confer greater heat stability per bond than those in tendon.

Figure 3 shows a linear regression plot of pyridinoline concentration against transition temperature for LD IMCT. The correlation coefficient of 0.63 is significant at the 99% confidence level.

CONCLUSIONS

The age related increase and the four fold higher pyridinoline concentration in IMCT over that in tendon highlight the tissue specificity of collagen crosslink formation (Bailey 1974; Eyre et al. 1984).

The initial relatively high level of pyridinoline in IMCT casts some doubt on the traditional maturation model of collagen crosslinking (Robins et al. 1973; Eyre and Oguchi 1980). Pyridinoline qualifies as a mature crosslink in IMCT because it is formed from reducible crosslinks and increases in concentration throughout the life of the animal; yet it is present in relatively high concentration in very young animals.

The results in Fig. 3 show that there is a relationship between the pyridinoline concentration and thermal transition temperature. This supports the view that the thermal stability of collagen is largely determined by the number and stability of the crosslinks in collagen (Flandin et al. 1984).

If all of the available aldehydes on interstitial collagens react to form pyridinoline as their end product, a theoretical maximum of 2.5 mole pyridinoline/mole collagen is obtained (Whittle et al. 1987). This level of pyridinoline formation occurs in the centre of intervertebral discs (Eyre et al 1984). The maximum value of 0.33 mole pyridinoline/mole collagen obtained for IMCT and the much lower value obtained for tendon, allows ample scope for other mature crosslinks to form. For example, compound M has recently been proposed as such a crosslink by Barnard et al. (1987).

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