AMSA International Lectureship Award

The Chemistry of Collagen Cross-Links and Their Role in Meat Texture

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Introduction

It has long been recognized that the high tensile strength of collagen is due to formation of intermolecular cross-links within the fiber. Following identification of the first of these intermolecular divalent cross-links in 1968 (Bailey and Peach, 1968), several other divalent and multivalent cross-links have been proposed. Some of these proposed cross-links have not stood the test of time, subsequently being shown to be artefacts or incorrectly characterized. However, although the overall mechanism is as yet incomplete, the enzyme initiation, the chemistry of formation, the location of the divalent cross-links and the basis of the age-related changes to multivalent cross-links is now well established (for reviews, see Bailey et al., 1974; 1980; Eyre et al., 1984).

The specificity of the cross-links, in terms of nature and location, depends very much on the structure of the collagen molecule and the organization of the molecules in the fiber. The precision of the molecular alignment in the fiber allows a minimal number of cross-links, that is, about one residue per 6,000 amino acid residues, to be highly effective in converting a fiber of negligible tensile strength to one comparable to that of some metals. The ultimate tensile strength of tendon is about 100 Nnm^{-2} following an extension of only 10% to 15%, and can be compared with aluminum at 220 Nnm^{-2}.

Collagen is the basic supporting structure of all animal tissues and plays a particularly important role in the action of muscle providing animal locomotion. It has also been shown to be important in the tenderness of meat (for reviews, see Bailey, 1984; Bailey and Light, 1989) and in the context of this conference, I shall be concentrating on its latter role.

Some two decades ago, I embarked on a program to determine the role of collagen in the texture of meat by attempting to identify the stabilizing intermolecular cross-links. Since then, we have in fact proposed that collagen is actually the determining factor in the textural differences between various meat muscles. The proposal is based primarily on the nature of the stabilizing intermolecular cross-links but obviously must also include the total amount of collagen. To understand the role of these cross-links, it is necessary to appreciate the structure of the collagen fibers in muscle and the various types of collagen, at least those of interest to the meat scientist.

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The Nature of Collagen

The collagen molecules are, in fact, a family of closely related proteins, possessing a basic structure of three polypeptide chains with a -Gly-X-Y- repeat forming tightly bound triple helices which subsequently aggregate to form various types of supporting structures (Piez and Reddi, 1985; Mayne and Burgeson, 1987; Bailey, 1987).

The collagen aggregates exist as thick striated fibers, as non-fibrous networks in basement membranes, or as cell-associated non-striated filaments (Fig. 1). These morphologically different collagen structures are aggregates of one of about a dozen different collagen molecules, but in all cases the tensile properties are totally dependent on the formation of intermolecular cross-links between the molecules in the aggregate, rather than a property of the aggregate itself.

The Fibrous Collagens

The collagen molecules in this group self-assemble to form fibers possessing a characteristic band pattern with a periodicity of 67 nm, identifiable in the electron microscope. The collagen types in the group are Types I, II III and the minor collagens V and XI.

The Non-Fibrous Collagens

The only member of this group is Type IV collagen, and these molecules self-assemble to form a "chicken wire" network structure which acts as the basic framework for all basement membranes.

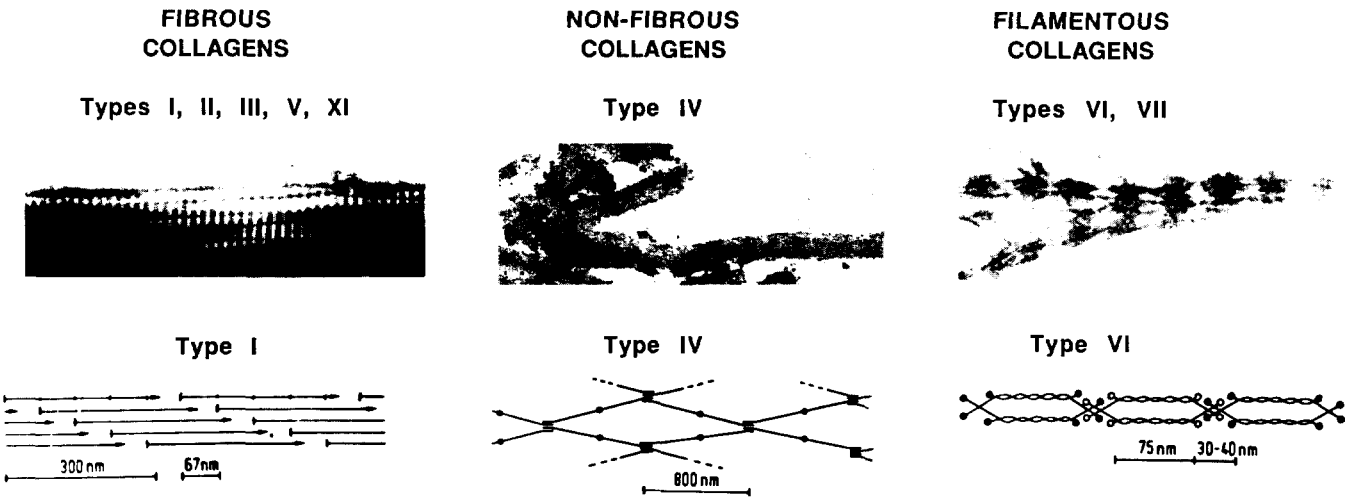
Filamentous Collagens

A number of recently-identified minor collagens possessing variable molecular lengths form a variety of filamentous structures. Type VI is observed as a loosely packed filamentous structure with an axial repeat of 100 nm and is formed by anti-parallel alignment of the individual molecules. Type VII microfibers underlie some basement membranes acting as anchoring fibrils between the membrane and the underlying matrix.

Formation of Intermolecular Cross-links

Immediately following secretion of molecules from the cell and the formation of fibrils, cross-linking precursors are formed through the oxidative-deamination of specific lysine residues in the terminal non-helical region of the collagen molecule. The enzyme involved, lysyl oxidase, is a copper metallo-enzyme that requires pyridoxal phosphate as a cofactor (Siegel, 1979). Lysyl oxidase acts only on the fibrillar form of collagen which is precisely aligned in a quarter-

Figure 1



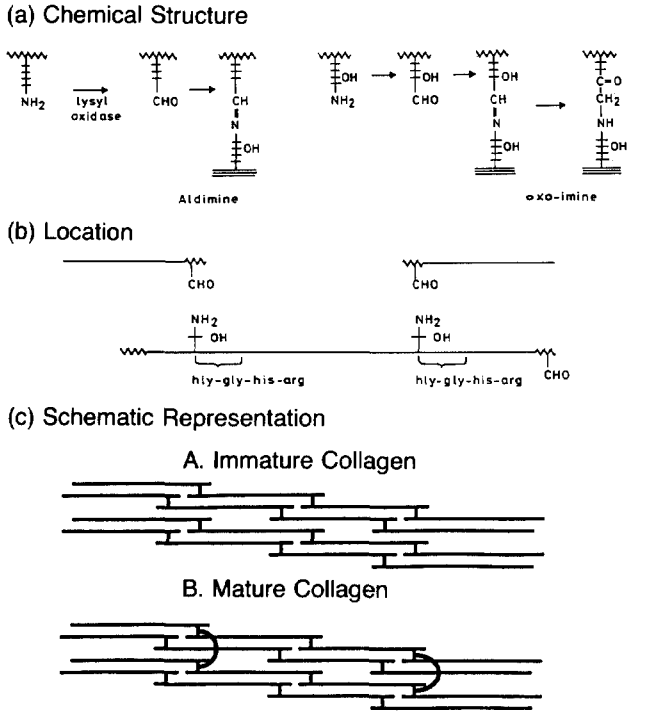
Grouping of collagen types into fibrous, non-fibrous and filamentous, depicting the ultrastructures of the various groups in the electron microscope and a schematic diagram of the organization of the collagen molecules.

stagger array in the fibril such that the non-helical region is in register with a domain in the helix with the amino acid sequence Hyl-Gly-His-Arg. We have previously suggested that this sequence acts as the binding domain for the enzyme from which it can catalyze the oxidative-deamination of the lysyl residue in the non-helical region. The lysyl oxidase converts the lysine or hydroxylysine in the telopeptide to a lysine-derived aldehyde.

The lysyl-aldehyde thus formed spontaneously undergoes condensation with the ε-NH_2 group of the hydroxylysine residue in the above sequence to form a divalent intermolecular cross-link of the aldimine type (Fig. 2a). However, if the residue in the non-helical region is hydroxylysine-aldehyde, the aldimine bond initially formed undergoes a spontaneous Amadori rearrangement to form a chemically stable oxo-imine bond (Fig. 2a). Both of these cross-links are termed reducible because they are readily reduced by reagents such as sodium borohydride. Detailed analysis of the CNBr peptides from cross-linked collagen has shown that these bonds are confined to the end-overlap regions, thus providing a head-to-tail cross-linking of the molecules within a fiber (Fig. 2b).

The relative proportion of the oxo-imine cross-link and the aldimine cross-link in any collagen varies with the age and nature of the collagenous tissue, and is determined by the extent of hydroxylation of the lysines in the non-helical region. Variation of the cross-links between tissues is clearly demonstrated by the predominance of the aldimine in dermal collagen, an approximately equal proportion of the two in Achilles tendon, and exclusively the oxo-inime in cartilage collagen. Variation with age is illustrated by the predominance of the oxo-imine in embryonic dermal collagen and a complete changeover to the aldimine form in the dermis of the young animal. The development of the dermis also involves a changeover in the collagen type from Type III to Type I. However, it has been shown that both Type I and III collagens in embryonic collagen possess the oxo-imine cross-link, and that post-natally both contain the aldimine cross-link (Bailey and Sims, 1976). The extent of hydroxylation of the lysyl residues in collagen probably depends on the level of activity of the enzyme lysyl hydroxylase rather than on inherent differences in the collagens. Whether the hydroxylation of the lysyl residues in the non-helical

Figure 2



(a) Chemical structure of the divalent aldimine and oxo-imine cross-links. (b) Location of the divalent cross-links polymerizing the collagen molecules in head-to-tail linkage, at conserved Hyl-Gly-His-Arg sequences. (c) Schematic representation of the location of the cross-links within the collagen fiber showing (A) stage one involving head-to-tail longitudinal cross-linking of the molecules in quarter-stagger array, and (B) transverse cross-linking between microfibrils in register in mature collagen.

region involves a different enzyme to those in the triple helix is unknown.

The physiological significance of the two different types of divalent reducible cross-link in immature collagen has not been established. Although chemically different in vitro, under physiological conditions both constitute a stable intermolecular cross-link. It is unlikely that the chemical differences affect physical properties, or susceptibility to attack by degradative enzymes, since there does not appear to be any relationship between the function, mechanical properties of a tissue or rate of turnover and the presence of the chemically more stable oxo-imine cross-link. However, this contention is by no means certain since we have recently demonstrated a higher proportion of the stable oxo cross-link in the extensor tendon than the flexor tendon, which may relate to the greater activity of the extensor tendon.

Age-Related Changes

The proportion of the aldimine and oxo-imine cross-links slowly reduces to low levels during maturation (Bailey et al., 1974), the actual final level depending on the ultimate rate of turnover of that particular tissue. To account for this decrease and the concomitant increase in stability of mature collagen, we proposed that the reducible aldimines and oxo-imine cross-links present in immature tissues are intermediate cross-links that subsequently act as precursors of more stable, multivalent cross-links. This proposal is supported by the identification of a cross-linked polymer isolated from mature Type I collagen following cyanogen bromide digestion (Light and Bailey, 1980). This polymer (denoted by poly-α1CB6) comprised the lysine-aldehyde bearing C-terminal CNBr peptide (α1CB6) and the N-terminal helical peptide containing the recipient hydroxylysine residue (α1CB5). The polymer 'poly-α1CB6' in mature collagen did not contain any reducible cross-links, yet contained the peptides α1CB6 and α1CB5 known to link the molecules in a head-to-tail fashion in young tissue by aldimine and oxo-imine cross-links. This clearly indicates that further reaction of these reducible cross-links is occurring to form the stable, non-reducible cross-link(s) of mature collagen. For this type of interaction to occur, the molecules must be in register rather than quarter-staggered. We suggested, therefore, that the polymerization of collagen occurs in two stages:

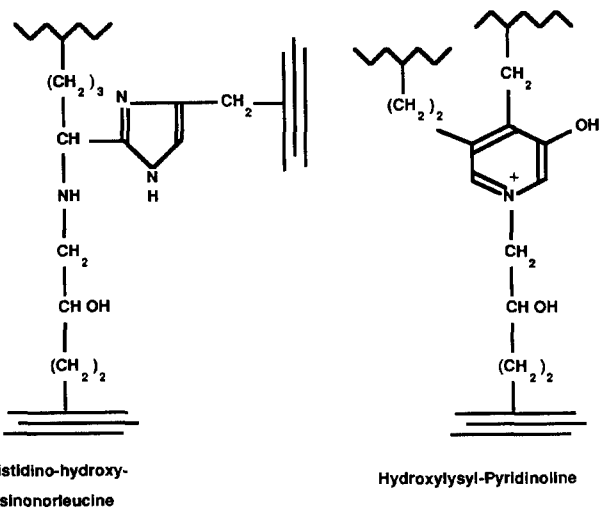
(i) initially, by end-overlap head-to-tail cross-linking to form a longitudinal polymeric fibril in which the adjacent molecules are quarter-staggered, and

(ii) by transverse cross-linking through interaction of the reducible cross-links of specific molecules in register (Light and Bailey, 1980; Bailey et al., 1980).

In this way the fibrils build up a three-dimensional network of cross-links which can readily account for the increased stability of mature collagen (Fig. 2c).

The nature of the mature collagen cross-link has been extensively investigated and several proposals have been made (Housley et al., 1975; Fujimoto et al., 1977; Scott et al., 1981), although to date only one of them has been confirmed. The compound identified by Housley et al. (1975) as hydroxyaldol-histidine has recently been re-evaluated as histidino-hydroxylysinonorleucine (Yamauchi et al., 1987) (Fig. 3a). This structure is compatible with the presence of lysyl aldehides in the telopeptides, and may, therefore, be the

Figure 3



Chemical structure of histidinohydroxy-lysinonorleucine (HHL) and hydroxylysyl-pyridinoline. HHL can form a trivalent cross-link between three differnt molecules, as suggested in Fig. 2(c). Pyridinoline is a trivalent cross-link but probably only links two molecules, both hydroxylysine-aldehydes being derived from the same molecule.

major cross-link in tissues such as skin. A fluorescent cyclic compound, 3-hydroxy-pyridinoline, has received considerable attention since its identification by Fujimoto et al. (1977) (Fig. 3b). Two mechanisms have been proposed (Eyre and Oguchi, 1980; Robins and Duncan, 1983), both mechanisms involving two hydroxylysine-aldehydes and a hydroxylysine. One can, therefore, predict that pyridinoline would be found in tissues with a high hydroxylation level (e.g. cartilage collagen), and should be absent from dermal collagen which contains little, if any, hydroxylysine-aldehyde. Indeed, Eyre has reported values of 1-2.6 residues/mole of collagen in cartilage, 0.4 residues/mole of collagen in tendon, and 0.1-0.2 residues/mole of collagen in bone collagen, and its absence from skin collagen.

The pyrrole derivatives (Ehrlich chromogens) identified by Scott et al. (1981) have not to date been fully characterized or confirmed as cross-links. Recent analysis of intramuscular collagen has shown high EC chromagen levels, hence, it would appear that these compounds warrant further attention.

In contrast to this direct approach of using an acid hydrolysate of whole collagen, we have isolated pure poly-α1CB6 from mature tissue (which by definition must contain the 'mature' cross-links), yet failed to detect the presence of pyridinoline (Light and Bailey, 1980). On the other hand, examination of the lower molecular weight non-cross-linked peptides revealed that the pyridinoline was associated only with α2CB3,5. We have suggested (Light and Bailey, 1985), therefore, that since the C-terminal non-helical sequence of the α2 chain lacks lysine, pyridinoline must be formed by condensation of the hydroxylysine-aldehyde in the N-terminal non-helical region with a pre-existing oxo-imine cross-link between the N-terminal non-helical sequence of the same molecule and the α2CB3,5 region of an adjacent molecule. Pyridinoline would, therefore, only cross-link two collagen molecules, and would be unable to build up the transverse

cross-link network required to bring about the age-related changes in mechanical properties observed in mature collagen. (This may not be necessary in certain tissues containing small diameter fibers, e.g. cartilage). We have previously pointed out that the absence of pyridinoline from, for example, mature dermal collagen would suggest that an alternative mechanism would be required for tissues stabilized by the aldimine cross-link. Recently, Eyre et al. (1984) have proposed that such a mechanism exists, the oxo-imine maturing to pyridinoline and the aldimine maturing to hydroxyaldol histidine (Housley et al., 1975). This latter structure would involve the unlikely production of a hydroxy-allysine at hydroxylysine residue 87 in the helix. However, the recent re-evaluation of this component as histidino-hydroxylysinonorleucine (Yamauchi et al., 1987) provides an acceptable structure.

The nature of the multivalent cross-link in poly-α1CB6 has not yet been completely characterized but our recent studies have revealed the presence of a novel amino acid which increases in content as the tissue matures. The amino acid has been isolated as two isomers with a molecular weight of 446, consistent with a trivalent cross-link. This compound M appears at about the same concentration in all the mature tissues so far examined (including the non-fibrous basement membrane — see below). If this proposal is confirmed, it would provide a more acceptable common mechanism for the maturation of all collagens in all tissues.

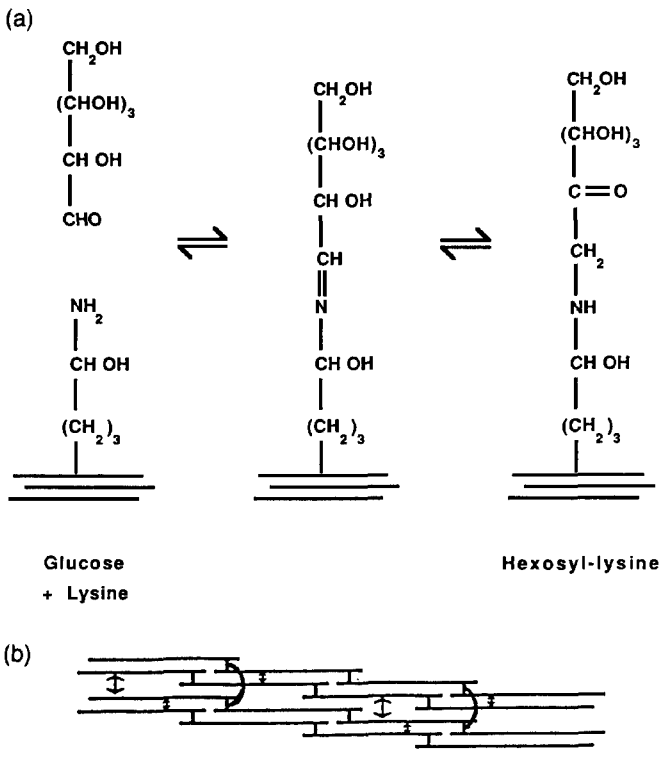
Non-Enzymatic Glycosylation in Mature Collagen

Early studies on the age-related changes in collagen revealed that glucose slowly reacted with the ε-NH2 groups of a few lysine residues in the long-lived collagen molecules to form hexosyl-lysines (Robins and Bailey, 1972; Le Pape et al., 1981) (Fig. 4a). The stability of these complexes and detailed chemical analysis demonstrated that the addition of the glucose occurred non-enzymatically through the formation of a Schiff-base which was stabilized by undergoing the Amadori rearrangement. More importantly, the addition of the glucose was concomitant with an increase in the stability of the fibril.

It has been reported that collagen from animals with experimentally-induced diabetes appear to undergo accelerated aging as evidenced by increased stability and is analogous to the effects observed by human diabetes. This has increased interest in the glycosylation process and various theories have been proposed to account for this increased stability, including activation of the normal cross-linking mechanism.

We have clearly demonstrated, using a simple in-vitro system, that new covalent cross-links are formed in collagen incubated with glucose (Kent et al., 1984). These cross-links are heat stable and are believed to result from further reaction of the hexosyl-lysine, presumably involving a similar mechanism to the Browning reaction. This is supported by the presence of fluorescent compounds and the isolation of putative cross-links by several workers. An imidazole-derived compound has been isolated from a model system of polylysine and glucose by Cerami and his colleagues (Ponger et al., 1984). Recently, the formation of this compound has been shown to be artefactual (Njoroge et al. 1988). Monnier and his colleagues have identified and synthesized an alternative structure (Pentosidine) capable of acting as a cross-link (personal communication). In our own studies, we have identified a different compound of molecular weight 596 but have not yet determined its structure. Although the putative glucose-mediated cross-links present in collagen have not yet been fully proven, an important aspect is the effect of this type of cross-linking in vivo. Certainly inter-helical cross-links would stabilize the fibril (Fig. 5b), particularly against degradative enzymes, and the modification of the lysine residues could alter the fibril's interaction with other connective tissue components. Similar changes in the properties of collagen occur in aging and diabetes. However, before any conclusions can be drawn, the nature and extent of glucose-mediated cross-linking in vivo needs to be established before one can identify a precise role for the increased cross-linking and modification in charge.

Figure 4

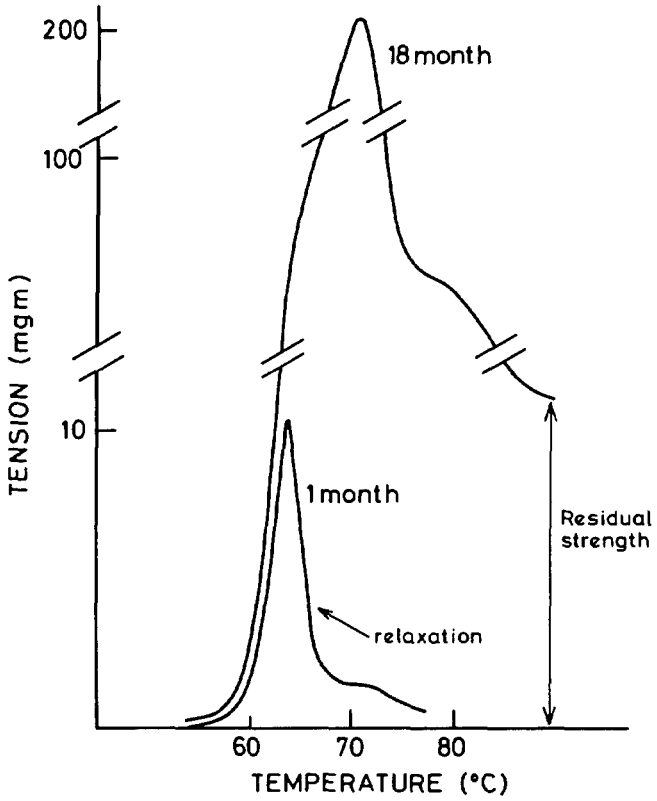


(a) Reaction of glucose with the ε-NH_2 group of hydroxylysine residues in the helical part of the molecule, and the stabilization of the aldimine to an oxo-imine through the Amadori rearrangement. (b) Proposed location of the hexosyl-lysine derived cross-links (↕) as inter-helical cross-links

Cross-Linking of Non-Fibrous Collagen

Basement membrane collagen (Type IV) appears microscopically to be non-fibrous in nature and a network structure compatible with this has been proposed by Timpl and Kühn (Timple et al., 1981). Their proposed structure consisted of 'chicken wire net' made up of units of four collagen molecules in which the N-terminal of each are bound together in an anti-parallel fashion. Despite this novel organization of the mole-

Figure 5



Variation in isometric tension generated with increasing age of tendon collagen. Note relaxation of young tendon and residual strength of mature tendon on further heating.

cules compared to fibrous collagen, analysis of the cross-links revealed the presence of the oxo-imine cross-links in immature tissues, e.g. placental membrane. In an analysis of cross-link biosynthesis in cultured lens capsules, Heathcote et al. (1980) confirmed the predominance of the oxo-imine cross-link. In recent, more detailed investigations of the location of the cross-link within the Type IV tetramer, we showed that both the N-terminal domain (7S fragment) and the C-terminal helical regions contain the oxo-imine cross-link in addition to disulphide bonds (Bailey et al., 1984). The location of the hydroxylysyl-aldehyde is almost certainly in the globular end-regions, as in the fibrous collagens, and the recipient hydroxylysine in the helical region. A possible modification of the model of Timpl and Kühn incorporating these data would involve some lateral alignment and end-overlapping of the Type IV collagen molecules, thus forming cross-links in the same manner as the fibrous collagens (Bailey et al., 1984). In this way, the tetramer could build up to form a stable open network.

Recent amino acid sequence data in the N-terminal 7S region of the α1(IV) chain have revealed the sequence Hyl-Gly-Glu-Arg which is strikingly similar to the lysyl oxidase binding sequence in fibrous collagen, Glu replacing histidine. There are several of these sequences along the molecule, suggesting there may be other cross-linking sites consistent with some lateral packing and overlapping of the molecules. Support for lateral packing has been obtained by X-ray diffraction studies (Barnard et al., 1987) and electron microscopy. However, further confirmation is required.

During maturation of basement membrane, there is an analogous decrease in the proportion of the oxo-imine cross-links to that of the fibrous collagen although the rate of maturation appears to be faster. We, therefore, further proposed that a secondary reaction of the oxo-imine cross-link analogous to that observed in fibrous collagen occurs (Bailey et al., 1984). To achieve this with the molecular structure proposed would require the sheets in the 'chicken wire' conformation to be in register, such that the chains cross-linked by the oxo-imine overlap similar regions of chains in the next sheet, thus permitting further reaction (polymerization) of the cross-link to form the 'mature' cross-link. Despite a careful analysis for pyridinoline in mature basement membrane, we failed to detect this cross-link. As in the case of the fibrous collagens, the nature of the mature cross-links forming a polymeric network has not been elucidated but clearly a similar mechanism occurs since we were able to demonstrate in basement membrane the presence of the putative cross-link (compound M) previously identified in poly-α1CB6.

Intramuscular Collagen

Muscle collagen can be distinguished morphologically as three separate hierarchies (Fawcett, 1968), the epimysium or outer muscle sheath, the perimysium or the intramuscular connective tissue binding the bundles of muscle fibers and the endomysium or individual muscle fiber sheath.

Meat can, therefore, be considered as a 'two component' system being composed of a complex intracellular contractile apparatus and the compositionally minor extracellular collagen. The contractile muscle proteins contribute the major element of meat texture whilst the connective tissues which comprise less than 2% of most skeletal muscles have long been associated with background textural effects. Indeed, a role for collagen in the texture of meat was made as long ago as the beginning of the century (Lehman, 1907), but correlations with a single parameter, for example, the total amount or the solubility of collagen (Ramsbottom et al., 1945), only gave partial or conflicting relationships. This lack of understanding of the fundamental properties of collagen presented considerable difficulties in developing a rationale for the role of collagen in determining the texture of cooked meat. However, from our work during the 1970s it became clear, based primarily on a basic study of the cross-links and their crucial role in determining the changes in properties with age and with heat-denaturation of the fiber, that it was the 'quality' of the collagen, not the quantity, that was critical.

When heated, the collagen is denatured and because of its partially crystalline nature it shrinks sharply at about 65°C to form insoluble gelatin. The fiber is converted from an inextensible highly organized fiber to a randomly organized elastic fiber. It is the nature of the cross-linking that will determine its solubility, the extent of shrinkage and the tension generated on shrinkage. We have shown that the tension generated under isometric conditions in tissues cross-linked by dehydro-HLNL does not achieve its potential due to the thermal instability of this cross-link, and that as the temperature is increased following maximum tension, the cross-links are increasingly ruptured leading to a dramatic

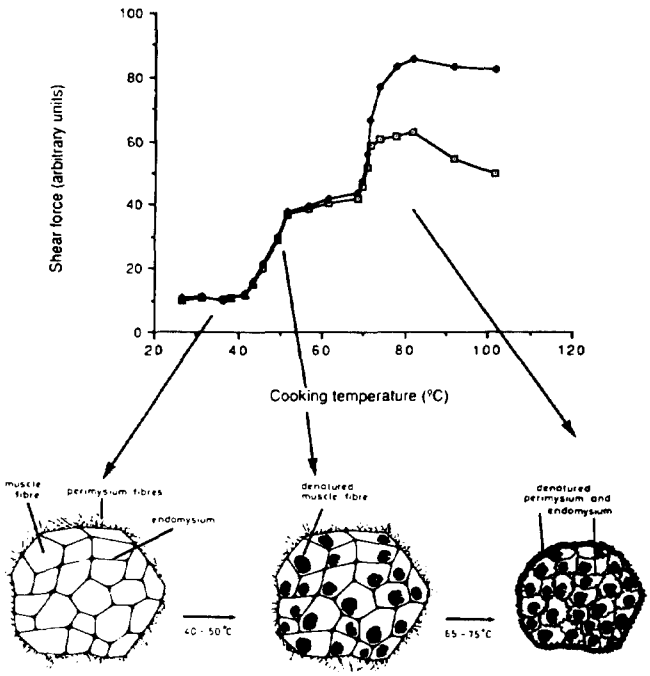
loss of tension (Bailey and Lister, 1968; Allain et al., 1978). In tissues cross-linked by the thermally stable dehydro-DHLNL, maximum tension is achieved with little loss of the cross-link; and on further heating, there is only a small relaxation of the tension. With increasing age, the formation of the mature multivalent transverse cross-links results in a dramatic increase in the tension generated and a reduction in the relaxation of that maximum tension at higher temperature (Fig. 5). The residual tension maintained means that the fiber still has a significant mechanical strength despite being denatured. This can readily be explained by the heat stability of the increasing number of mature cross-links maintaining some strength in the denatured fiber.

Attempts to define a relationship between the cross-links and texture were initially based on the thesis that the cross-links determine the tension generated during thermal shrinkage (Bailey and Sims, 1977; 1981). Early studies demonstrated the perimysium generally possessed a higher content of the relatively heat-stable oxo-imine cross-link than the epimysium (Shimokomaki et al., 1972). As anticipated, the best correlation was found with the content of the heat stable cross-link oxo-imine of the perimysium; but a few muscles, for example *psoas major*, possessed a high content of DHLNL yet produced tender meat (Shimokomaki et al., 1972; Light et al., 1984). This is almost certainly due to the low collagen content and the high sarcomere length of *psoas major* due to stretching during rigor hanging. Clearly the relationship is complex. It is certainly possible to have a collagenous tissue of high DHLNL content, which is consequently very insoluble in acid but is incapable of generating a significant tension on shrinkage. An extreme case is the low tensile strength of cuverian tubules despite a high proportion of DHLNL making the fibers insoluble (Bailey et al., 1982a). The obvious answer is that both the intermediate and the mature cross-links need to be determined.

These analyses of different cross-links, and their partial correlation with texture, together with the fundamental studies on the connective tissues have allowed a rational description of the role of collagen in meat texture to be developed over the past few years. The hypothesis has developed such that it can be shown that collagen is the major determinant of the texture of cooked meat, and that it is the quality as well as the quantity of the collagen that accounts for the variability in texture.

To illustrate this primary role of collagen, consider the nature of the changes taking place in meat as it is cooked. The myofibrillar proteins, primarily actomyosin, denature at 40° to 50° resulting in an increase in loss of fluid and shear value. At these temperatures, the epi- and endomysium are unaffected, but at 60° to 65° both these connective tissue tracts begin to shrink, resulting in an increased fluid loss and a second increase in shear value (Fig. 6). The extent of this shrinkage of collagen varies with the heat stability of the perimysium, which in turn is determined by the nature and extent of cross-linking. Further, the older the animal, the higher the proportion of heat-stable cross-links and the greater the tension generated, with a consequent increase in toughness of the meat. The contribution of the endomysium to the shrinkage of the meat may be significant but is likely to be small compared with that of the much greater mass of perimysial fibers.

Figure 6



Change in shear force with increasing temperature during the cooking of muscle (top). Diagrammatic representation of the change in cross-section of the muscle illustrating the initial denaturation of the acto-myosin and, at a higher temperature, the endo- and perimysium. The denaturation of the collagen results in a shrinkage in cross-sectional area.

In addition to their effect on the overall shrinkage of the meat, the collagen has a second role. Following shrinkage, the muscle fibers are held together by denatured perimysium. The strength of this adhesion is clearly important in the texture of meat, and is again dependent on the proportion of heat-stable cross-links remaining and holding the collagen chains together. The fibers are weaker in young animals and pull apart more easily.

At higher temperatures consistent with prolonged cooking, the shear values decrease again. This can be interpreted to be due to the slow cleavage of peptide bonds and mature cross-links. The former is likely to be the primary effect since we have evidence that peptide bond cleavage does occur under these conditions whereas the cross-links appear to be stable. The myofibrillar proteins may degrade, but the role of denatured collagen in maintaining the adherence of the muscle fibers would suggest that their degradation results in a more dramatic drop in shear value than random cleavage of the myofibrillar proteins.

The complete reversal of the properties of meat proteins on heating, the myofibrillar proteins denaturing to a more rigid aggregate and the collagen denaturing to a weaker elastic polymer, leaves the question of which is now the weaker component. The tensile properties of cooked meat are readily observed to be much stronger longitudinally than the force required to separate the fibers transversely. This difference can be quantified using fracture mechanics (Purslow, 1985) and shown to be ten times greater along the longitudinal axis. The lateral binding of the muscle fibers is,

therefore, clearly much weaker than the muscle fibers themselves. The relative strength of the perimysium and muscle fiber will vary from muscle to muscle and with age as the quality of the collagen varies, in contrast to the muscle proteins which remain fairly constant. Collagen can therefore be considered as a major determinant in the texture of cooked meat.

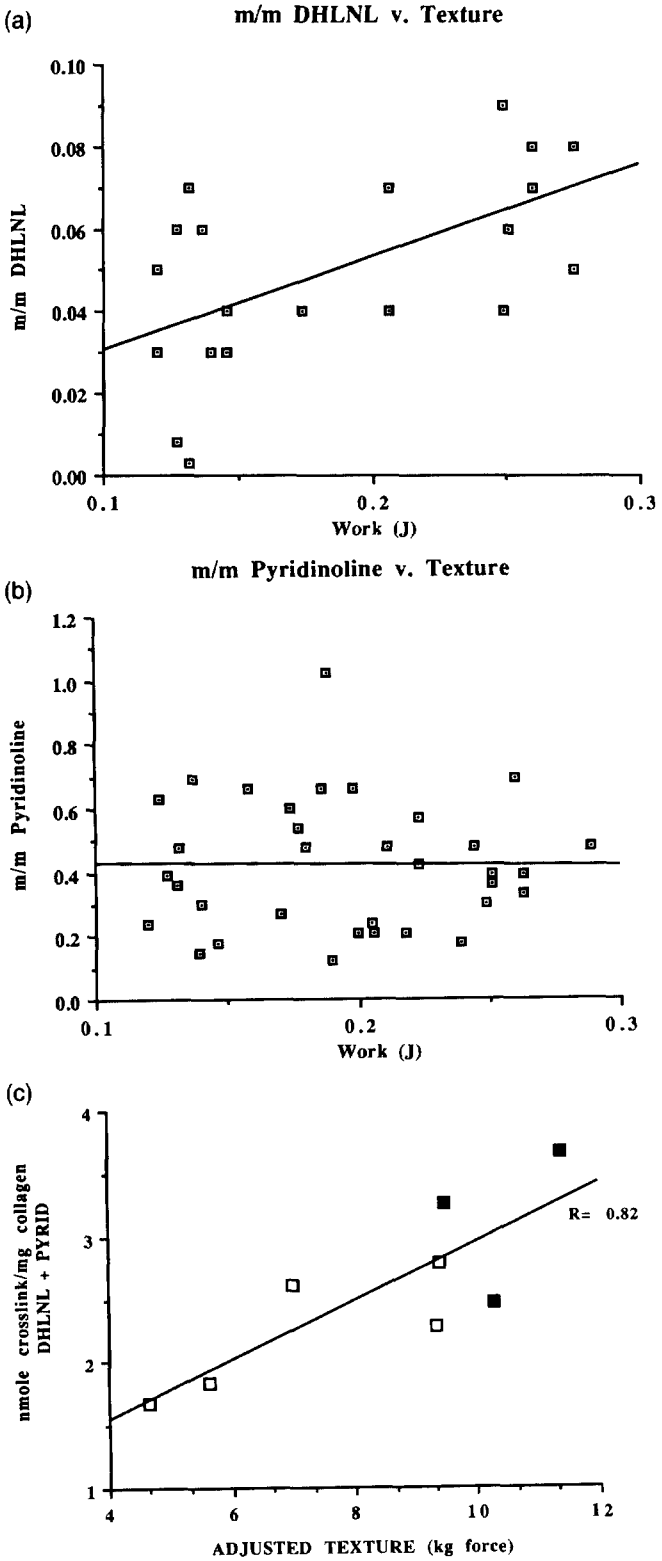
In the final analysis, to confirm this hypothesis we must obtain a direct correlation of the texture measurement with the total heat-stable cross-links (oxo-imine, pyridinoline and compound M) per mg of intramuscular collagen. Such correlation would allow prediction of texture by determination of the cross-links. The latter should also correlate with the isometric tension generated in the muscle, although this is notoriously difficult to determine reproducibly.

In an attempt to correlate cross-links with texture, we have carried out an extensive analysis of a large number of different muscles, comparing texture determined by shear values and sensory analysis with cross-link analysis for animals of the same age. Previous studies (Shimokomaki et al., 1972) had indicated a correlation with the stable oxo-imine cross-link, and using more accurate techniques a significant correlation was once again obtained (Fig. 7a). It would not be expected to obtain a perfect correlation because the rate of conversion of the oxo-imine to the mature cross-links may be different in the various muscles. The sum of the oxo-imine and mature cross-links should provide a better correlation. An attempt was therefore made to correlate with the one identifiable mature cross-link, pyridinoline. In this case, there was no correlation with the pyridinoline content over a wide range of textures (Fig. 7b). Although pyridinoline is derived from the oxo-imine, it may not be a major pathway, and there is some evidence that it is not a truly 'mature' cross-link (Fig. 8). We are currently attempting to correlate texture with compound M, our as yet uncharacterized mature cross-link , which if a trivalent cross-link, as believed, could provide a better correlation with texture.

The nature and extent of cross-linking depends on the age, growth rate of the animal and the rate of turnover of the particular tissue analyzed. For example, bulls, steers and double-muscled animals would be expected to have different cross-link profiles. Certainly differences in texture have been reported but few correlations with cross-linking have been carried out, e.g. Bailey et al. (1982); Bailey (1985).

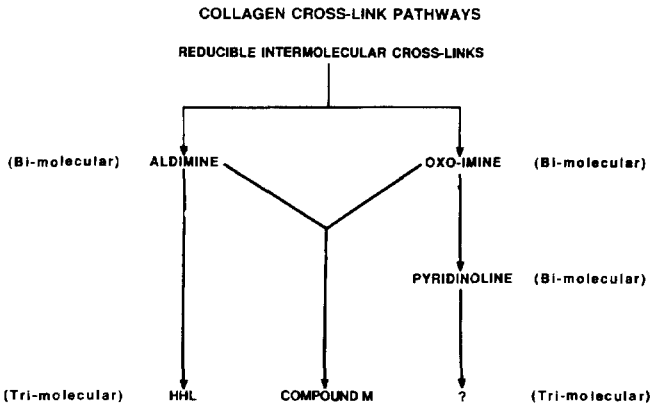
Growth-promoting agents also provide the opportunity to check the hypothesis. We have carried out a preliminary analysis of animals fed β-agonists, compared with controls and animals four and eight weeks after withdrawal of the drug. The texture increased dramatically following drug adminstration, but fell slowly back to the control values after eight weeks. The total intramuscular collagen did not change but there was a significant difference in the cross-linking (Fig. 7c). The values of both the oxo-imine and pyridinoline were higher in the β-agonist-treated animals. This reflects an increase in synthesis to elevate to oxo-imine cross-links and the inhibition of catabolism allowing the collagen to mature and, hence, increasing the amount of pyridinoline. The overall increase in these heat-stable cross-links accounts in part for the increased toughness of the meat when cooked. Further studies on the other mature cross-links are clearly warranted.

Figure 7



(a) Relationship between the heat-stable divalent cross-link oxo-imine (determined in the reduced form as DHLNL) and the texture in terms of work. (b) Relationship between pyridinoline and texture in terms of work. (c) Relationship between the sum of DHLNL and pyridinoline and the texture (adjusted for pH). □ normal animals. ■ animals treated with β-agonist.

Figure 8



Possible pathways for the collagen cross-links during maturation.

In summary, the substance of meat is provided by the denatured myofibrillar proteins which constitute over 80% of the protein, but the expression of the texture is determined by the squeezing together of the muscle bundles and consequent loss of fluid due to the pressure exerted during the thermal contraction of collagen, and the residual strength of the denatured collagen binding the muscle bundles together. These properties of collagen are determined by the nature and extent of the intermolecular cross-links.

Cross-linking chemistry has, therefore, provided the meat scientist with the basic rationale for the role of collagen as the determining factor in the texture of meat. The relative importance of the different mature cross-links in both the perimysium and endomysium, and the relationship between the various muscles with age and collagen types remains to be determined. However, the essentials of the hypothesis have been laid.

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