Amino Acids (1991) 1: 293–306

Amino
Acids

The chemistry of natural enzyme-induced cross-links of proteins

Review Article

A. J. Bailey

Muscle & Collagen Research Group, University of Bristol, Langford, Bristol, England

Summary. The cross-linking of protein molecules to form stable supramolecular aggregates capable of acting as protective and supporting structures is a common feature of organisms coping with the stresses of life. These new polymeric forms range from thick rigid structures to thin flexible membranes. The formation of such cross-links must be carefully controlled since more or less than optimal cross-linking could lead to malfunction or even death of the organism. The chemistry of the amino acids converted or directly involved in the formation of these cross-links is complex and a range of new amino acids has been identified. Di- and tri-tyrosines are formed by the action of peroxidases, quinones by catechol oxidases, γ glutamyl lysine iso-peptide bonds by glutamyl transferase and a complex series of lysine- aldehyde derived cross-links induced by lysyl oxidase. These cross-linking mechanisms provide an insight into the complex changes in tissue function during growth of the organism and their effects on the properties of foods.

Keywords: Amino acids – Cross-links – Peroxidases – Catechol oxidase – Glutamyl transferase – Lysyl oxidase – Resilin – Fibrin – Collagen

Introduction

Natural cross-linking, ie. the formation of covalent inter and intra molecular bonds between proteins induced by highly specific enzymes, is utilized by nature to create new entities with properties completely different from the original monomeric form. These new polymeric forms range from rigid structures through elastic fibres to thin flexible membranes. The chemistry of the amino acid residues converted or directly involved in the formation of these cross-links has proved both complex and fascinating in the range of new amino acids identified. The results of cross-linking are not only important to physiologists and biochemists studying the normal structure-function relationships of tissues, but to medical researchers studying the effects on function of tissues, and to food scientists studying the role of these cross-links on the properties of foodstuffs and their effects on nutrition.

Cross-linking of proteins can readily be effected by exogenous agents, but this paper will be confined to natural enzymatic cross-linking.

Peroxidase induced tyrosine cross-links

Tyrosine derived cross-links occur as stabilising cross-links in many proteins throughout the animal kingdom.

The di-tyrosine cross-link was first isolated from resilin by Andersen (1963). Resilin occurs in all insects investigated, also in crustacean but is not present in arachnids. The locust has been the most extensively investigated where resilin is present in many structures but the wing ligaments are the tissue generally used in analysis. These ligaments are 80% resilin and the remaining part is chitin. The main characteristics of resilin are its elasticity and insolubility. The ligaments can be stretched up to three times their original length without breaking, and return to their original length on release, in effect acting as an ideal rubber. The molecular weight between cross-links has been calculated to be about 3,000 as determined by elastic modulus. The stable tyrosine cross-links (Fig. 1) connect the resilin molecules in a three dimensional network, the peptide chains between the cross-links being hydrophobic in nature with a random distribution of Gly, Ala, Pro, thus ensuring a random polypeptide structure.

The protein is devoid of secondary structure rather like elastin, and this random form is crucial to its functioning as an elastic element.

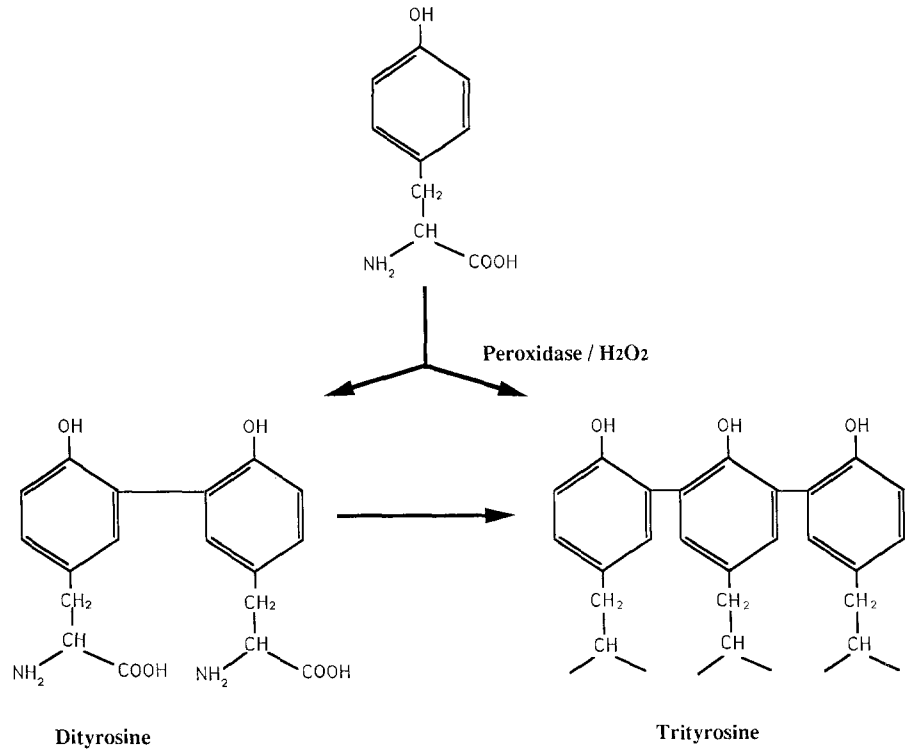


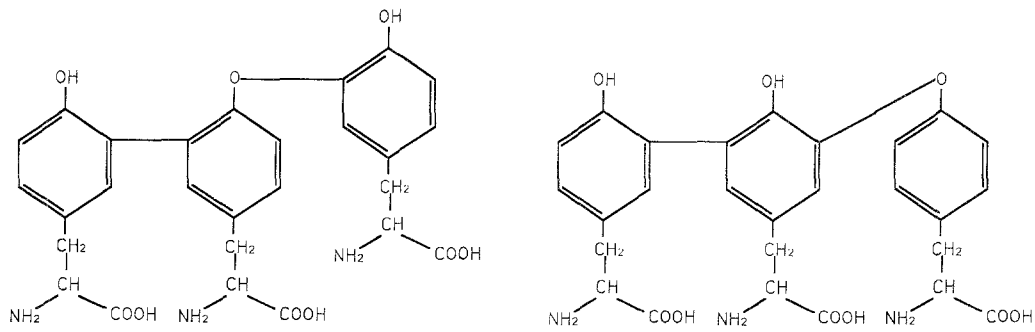
Fig. 1

About one third of the amino acid residues in resilin are glycine and this allows easier contact between the bulky tyrosine residues to form cross-links. The amino acid sequence around the tyrosine must determine which tyrosines are oxidised by the enzyme and subsequently participate in the cross-link formation. It would be interesting to know whether there are a greater number of glycine residues in the proximity of the di and tri tyrosine cross-links.

The formation of these cross-links is believed to be through the action of a peroxidase, based mainly on the ability of this enzyme to convert tyrosine to di-tyrosine in vitro. Only in the case of the membrane of the sea-urchin has the formation of these cross-links been definitely associated with the presence of ovoperoxidase activity (Foerder et al., 1977). The fertilization envelope is a huge complex of proteins, and the electrophoretic band pattern varies considerably with the type of sea urchin, but the presence of the enzyme ovoperoxidase, Mr 70KD appears to be conservative amongst the different species.

Their formation in Mytilus Byssus, tussah silk or crab carapace may be related to the presence of phenol oxidase, but the evidence is not conclusive. The reaction mechanism for the formation of these cross-links is generally agreed to involve the phenoxy radical (Gross and Sizer, 1959) and the formation of a tyrosine cation radical may be present, but the presence of super oxides may also be involved. (Takahashi et al., 1989)

An unusual tyrosine derived cross-link has been reported by Fujimoto et al., 1981 in the cuticle of Ascaris Lumbricoides. The cross-link is a tri-tyrosine in which the third tyrosine is linked through an ether link to di-tyrosine (Fig. 2). The compound has been given the trivial name iso-trityrosine. The cuticle collagen contains iso-trityrosine but only traces of tri and di-tyrosine, whereas cuticlin, another component of the cuticle, contains appreciable amounts of both di- and tri-tyrosine, but does not contain iso-trityrosine. Ascaris cuticle collagen has an unusual structure of a single polypeptide chain that forms a triple helix by self-folding rather than three separate α-chains (McBride & Harrington, 1967). Whether this unusual cross-link plays a role in the organization of this unique collagen remains to be elucidated.



Iso-trityrosine Pulcherosine

Fig. 2. Structure of ether linked tyrosine crosslinks

A similar ether-linked tyrosine cross-link has been isolated from the fertilization envelope of the sea urchin embryo (H. pulcherrimus) in addition to the di- and tri-tyrosines (Nomura et al., 1990). The compound is another tri tyrosine with an ether linkage and is therefore an isomer of the iso-trityrosine found in Ascaris cuticle collagen. The compound termed Pulcherosine is present in the ratio 4-1.5-1 for di- tri- and pulcherosine. It is interesting to note that of the 30 tyrosine residues per 1000 resídues in the H pulcherrimus fertilization envelope only 17% are converted to the three types of tyrosine cross-link, whilst in yeast spore wall almost 90% of the even higher level of tyrosines are converted to di-tyrosine. However, they are not converted to the higher types of tyrosine cross-link. The mechanism of formation of these ether-linked tri-tyrosines has not yet been elucidated.

Di-tyrosine has also been reported in the cateractous human lens capsule proteins, and the formation of these compounds may be important in ageing and in diabetes, hence research on the mechanism should be of value in determining methods of inhibiting their formation. However, the mechanism may be different in that the formation may occur through external forces eg UV in which the formation of tyrosine cross-links may be due to the action of free radicals formed by the UV irradiation (Garcia et al., 1978). Similarly, its identification in collagen or elastin from old human skin may also be due to UV irradiation (Waykole & Heidemann, 1976). However, we will confine ourselves to instances where the formation of the cross-links are enzymatically mediated.

Catechol oxidase induced quinone cross-links

The formation of quinone cross-links (Fig. 3a) by the action of catechol oxidases have been reported in many proteins, particularly invertebrate exo-skeletons, insect cuticles, molluscs and annelides, helminth egg cases etc. Although quinones have been identified no specific quinone cross-link has been characterised to date possibly due to the complexity of the chemistry involved. Andersen (1976) suggested that two different enzymes are involved and that the cross-links are formed through quinones based on dopamine.

In the sea there is a diversity of organisms that specialise in sticking to all types of wet surfaces eg barnacles, oysters, and mussels. Considerable progress has been made with the sea mussel (Mytilus edulis) and the external byssus threads involved in its attachments. These threads secreted from the foot of the common mussel consist of a collagenous structure (Gathercole and Keller, 1974) enclosed in a sclerotized cuticular sheath that is quinone tanned . The threads attach to the rocks and must be resistant to the mechanical action of the waves and the chemical action of the components of the sea. Brown (1952) detected catechol oxidase activity in the threads and proposed that the enzyme oxidizes the catechols to o-quinones, from which quinone cross-linking occurs. Waite (1985) demonstrated that the catechol oxidase can be liberated from the threads by 1M Na Cl, and appears to be associated with a large macromolecule but may have sub-units of about 120KD. He further showed that it is derived from a latent enzyme in the foot. This higher molecular weight form of catechol oxidase

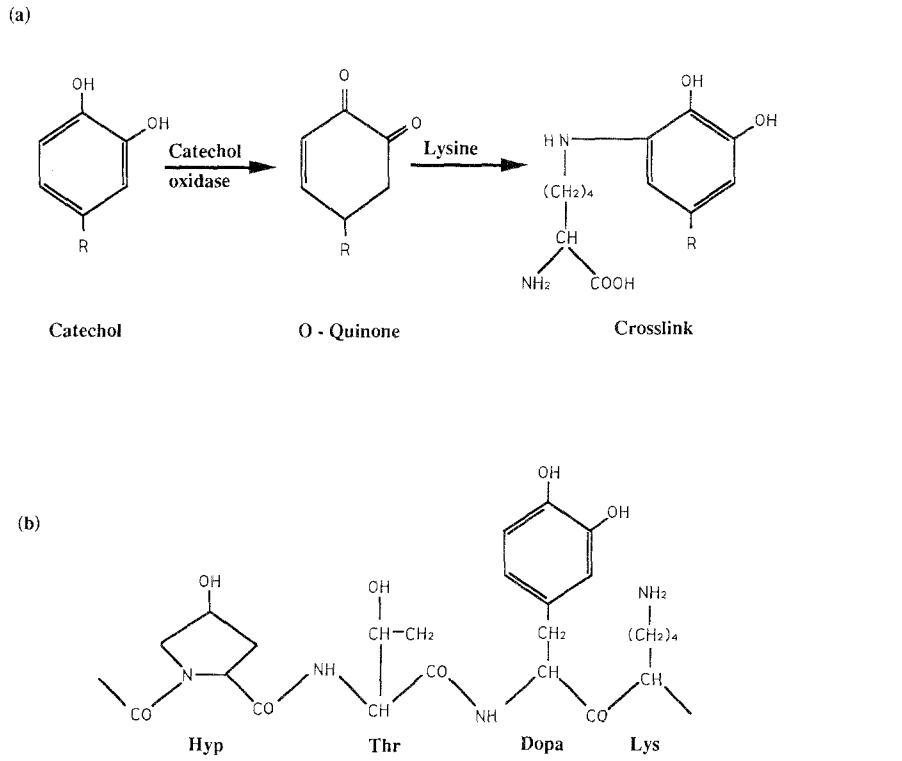


Fig. 3. Dopa containing sequence of repeat decapeptide in the polyphenolic adhesive protein

may be activated by proteases such as α-chymotrypsin. The oxidase then dehydrogenates catechols to o-quinones in the presence of oxygen.

These findings by Waite confirmed the proposal by Brown (1952) that mussels, unlike insects employ catecholic tanning agents that behave like proteins. The tanning agents utilized by insects are not proteins but low molecular catechols such as N-acetyl dopamine which following oxidation can form cross-links between proteins.

The adhesive precursor is encapsulated in micelles. The precursor has a mol wt of 130KD and an open conformation, the encapsulation preventing premature oxidation. The random or open structure of the adhesive polyphenolic protein may be due to the presence of proline, which acts as an α-helix and β-pleated sheet breaker. The requirement for good surface attachment is for energetic interfacial contacts between the adhesive and the substrate surface, hence several possibilities are likely (i) H-bonds with hydrophilic polymers (The use of catechol groups is well established in the tanning or cross-linking of leather with vegetable tannins, the groups compete with water displacing it from the collagen backbone and forming strong H-bonds). (ii) Catechol groups are also strong metal chelating agents – the stability constants being extremely high. (iii) a quinhydrone charge-transfer complex. (iv) The fourth possibility is the formation of covalent bonds. A Michael type nucleophilic condensation product by

reaction of the o-quinone with a primary amine takes place (Fig. 3a). This latter option of oxidation to quinones and the formation of covalent bonds is presumably the major mode of stabilisation. It is possible that the other modes of attachment are employed on a temporary basis depending on the surface, but the final stabilization is through the formation of covalent cross-links. Unfortunately the characterisation of these cross-links has not yet been achieved to confirm this proposal.

The chemical nature of the polyphenolic proteins as the basis for a synthetic underwater adhesive has been the subject of considerable research activity. The attachment of the byssus threads is by adhesive plaques (Brown, 1952) These plaques contain a glue called the polyphenolic protein (Waite et al., 1985) secreted from the foot as a foam. The protein is highly basic, 90% of the amino acids are accounted for by eight residues Lys, Trp, Ala, Thr, Pro, Tyr and 3,4 di-hydroxy phenyl dopamine (DOPA). Repetitive sequences of Ala-Lys-Pro-Ser-Tyr-Hyp-Thr-Dopa occur in the protein (Fig 3b). The adhesiveness of the protein may be related to the repetition of the Dopa residues – which have a strong metal chelating capability. In addition, and probably more importantly, oxidation to o-quinones would enhance its adhesive properties by the subsequent formation of covalent bonds with lysine and cysteine.

Attempts to utilise the properties of this protein are being tackled by chemical synthesis of the peptides and by cloning of the gene of the adhesive protein followed by expression in bacteria or yeasts.

Glutamyl transferase induced iso-peptide cross-links

The glutamyl-lysine iso-peptide cross-links have been detected in many different types of protein, although it is perhaps best known for the stabilisation of fibrin clots. The clot is produced by spontaneous assembly of fibrin molecules on losing their highly charged terminal peptides, which includes tyrosine O-sulphate, by the action of thrombin. It is initially quite fragile but is subsequently stabilised by enzymatic cross-linking through iso-peptide bonds. The donor groups were identified as the ε-amino groups of lysine residues (Fuller & Doolittle, 1966) and the acceptor residues as the glutamic groups (Matacic & Loewy, 1968) and the crosslink isolated and characterised as ε-(γ-glutamyl) lysine by Lorand et al. (1968) (Fig. 4).

The formation of this cross-link is catalysed by the pro- enzyme glutamyl transaminase which is activated by thrombin and is a calcium requiring enzyme. The action of the enzyme is to transfer the ε-amino group of particular lysyl residues to specific glutamyl residues on another fibrin molecule, resulting in an iso-peptide polymer network of fibrin molecules. The $\gamma - \gamma$ dimer is first formed and the subsequent α-chain polymerisation is much slower and is believed to result in lateral association of the molecules.

The iso-peptide bond by its very nature is destroyed by both acid and alkaline hydrolysis, and can therefore only be demonstrated directly as the ε-(γ-glutamyl) lysine detected after extensive proteolytic degradation of the protein. Its presence

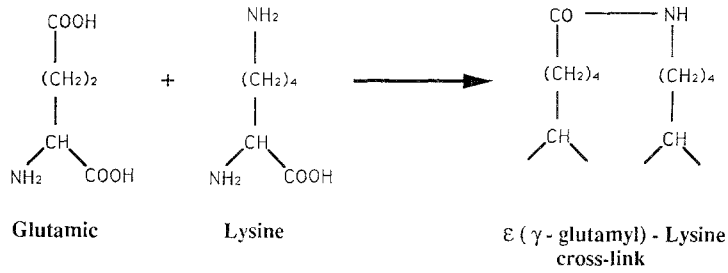


Fig. 4

may be detected indirectly by amino acid analysis before and after cyanoethylation or FDNB treatment of the free ε-amino groups of the free lysines.

Defects in the crosslink formation have been identified in humans as due to a deficiency of the transamidase enzyme, which leads to a pronounced tendency to bleed.

Combined isopeptide and dityrosine cross-linking

The sea-urchin fertilization envelope provides an interesting model to study the stabilization of the extra-cellular matrix. Assembly of the envelope involves a number of discrete components that are synthesised by a single cell. Covalent cross-linking by transglutaminase is initially utilized to modify the envelope. (Battaglia and Shapiro, 1988). Additional proteins attach to this matrix and one protein, protoliaisin, attaches ovoperoxidase to the envelope. The ovoperoxidase then hardens the envelope by producing hydrogen peroxide and forming dityrosine cross-links between the various proteins. These tyrosine derived cross-links play an essential role in rendering the fertilization envelope resistant to physical forces once fertilization has occured (Foerder & Shapiro, 1977). The fertilization envelope hardens within 10 minutes of fertilization. The mature envelope is refractory to chemical, enzymatic and mechanical disruption, thus allowing developmental events in the embryo to occur in a protected environment.

The activity of the ovoperoxidase has to be closely regulated. Prior to fertilization it is present in a low pH environment where the enzyme is inactive, but when exposed to the higher pH of sea water it becomes active producing a burst of H_2O_2. This only occurs after the assembly of the fertilization envelope. The enzyme is sequestered in the envelope restricting its activity to the nearby substrates. It does not act as a diffusible enzyme, but is active only within the matrix and thus catalyses only 5–10 cross-links. The major substrate for ovoperoxidase is protoliaisin which in fact contains very few tyrosines (about 50), being composed primarily of glu, asp and cyst. Ovoperoxidase binds to proteoliaisin which in turn binds to receptors in the matrix, thus the action of ovoperoxidase is both spatially controlled, and temporally regulated by pH (Shapiro, 1991).

Lysyl oxidase derived cross-links

The cross-linking of the connective tissue proteins collagen and elastin is mediated through the enzyme lysyl oxidase which catalyses the oxidative deamination of the ε-amino groups of specific lysines to aldehydes (Siegel 1979). Cross-linking is crucial for the polymerisation and subsequent functioning of both collagen and elastin. Inhibition of the enzyme by lathyritic agents such as β-amino propionitrile which binds to and inhibits the action of the enzyme results in fragile tissues which rupture under load. Lysyl oxidase is a copper containing enzyme and consequently copper deficiency reduces its activity producing a similar effect on the tissues.

The lysyl oxidase catalyses the conversion of almost all the ε-NH_2 lysines in elastin to lysine aldehydes, but in the case of collagen confines itself to the specific lysines in the non-helical terminal regions which are of variable amino acid sequence. The specificity of the enzyme is therefore not yet clear.

Elastin

Newly synthesised tropoelastin spontaneously coacervates to an insoluble aggregate following secretion from the cell. Lysyl oxidase acts on this aggregate to stabilise it by the formation of cross-links. The lysyl residues oxidatively deaminated by the enzyme are present within the more structured region of these peptides, which are rich in ala and possess an α-helical configuration About 85% of the lysines in elastin are involved in cross-linking. It has been suggested that

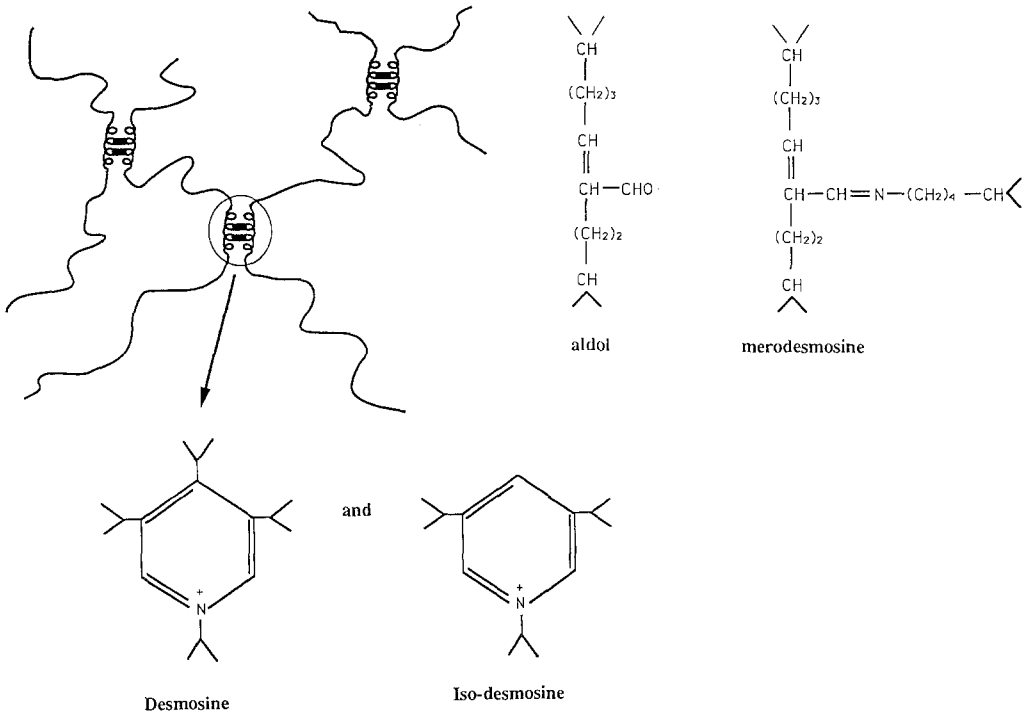


Fig. 5

the lysines next to the tyrosines are not oxidised but this remains to be confirmed in all cases. A series of divalent aldols and Schiff bases and the trivalent merodesmosine are initially formed and these are rapidly converted to the tetravalent desmosines (Partridge et al., 1963). The latter are present as isomers desmosines and isodesmosine (Fig. 5). In an age-related study (Francis et al., 1973) the aldol and merodesmosine decrease rapidly and are clearly precursors of the desmosines. The formation of these tetravalent cross-links confirms the proposition that the elastin possesses sufficient structure for the lysines to be in the correct location for the formation of the desmosines. The cross-linking of elastin therefore occurs within specific regions along the peptide chains, the remainder of the chain possesses mainly hydrophobic groups and randomly distributed proline and glycine resulting in the random organization of this part of the polypeptide. The random order allows stretching of this part of the chains, over-extension being prevented by the cross-links, and results in almost complete reversibility of the extension (Bailey & Etherington, 1980). Elastin is therefore present in tissues where elastic recoil is an essential part of its function eg the major arteries, lung and ligaments.

Collagen

The consequences of the cross-linking of collagen contrast markedly with those of elastin. This is primarily due to the precise packing of the collagen polypeptide chains into a rigid triple helix and the self-assembly of these molecules into fibrils, which consequently limits the number of residues accessible to lysyl oxidase, and secondly, the presence of hydroxylysine in collagen modifies the subsequent reactions of the initial cross-links. The lysyl oxidase binds to the newly formed fibril and oxidises the lysine or hydroxylysine residue present in the short non-helical N and C terminal regions of the molecule. The enzyme does not act on the individual molecules, only on the fibril, attaching to the helical regions of one molecule which because of the end-overlap packing is opposite the non-helical terminal region of an adjacent molecule (Fig. 6). In fact these helical regions possess the sequence Hyl-Gly-His-Arg and this sequence is conserved throughout the fibril forming collagens. The histidine residue may be involved in stabilization of the complex through proton donation. The Hyl residue of the sequence acts as the receptor for the lysyl-aldehyde thus forming a Schiff base or aldimine bond. This cross-link was first isolated in its reduced form as Hydroxylysinonorleucine (Bailey & Peach, 1968). If the aldehyde is in fact hydroxylysyl aldehyde then a spontaneous Amadori rearrangement of the Schiff's base takes place to form the stable keto-imine divalent cross-link. These two divalent cross-links polymerise the fibrillar molecules in a head to tail fashion thus providing the fibril with mechanical strength.

We have proposed that these aldimine and keto-imine bonds are intermediates and are converted to tri-valent cross-links to form a 3-dimensional network by cross-linking the linear polymers, or microfibrils, in a transverse fashion (Bailey et al., 1980). Such increases in the cross-linking would account for the increase in mechanical strength of collagen fibres with age.

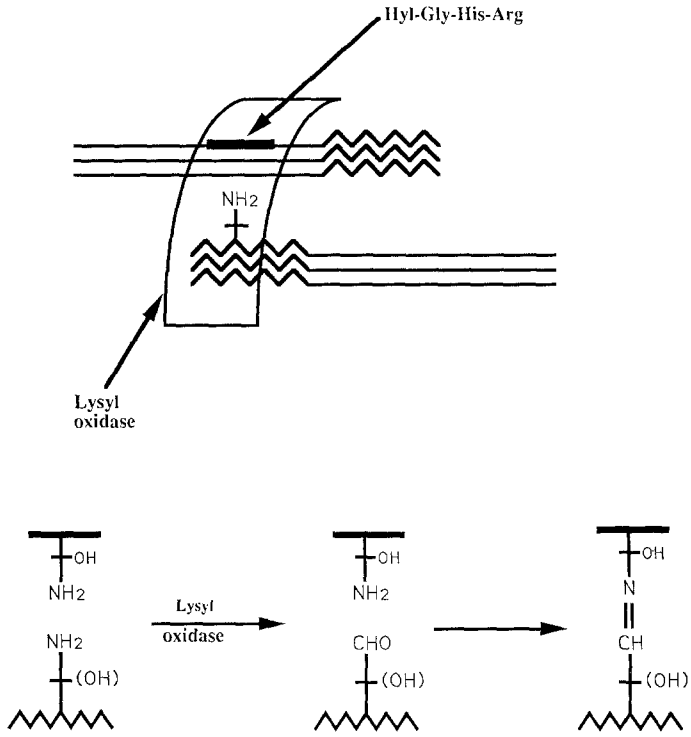
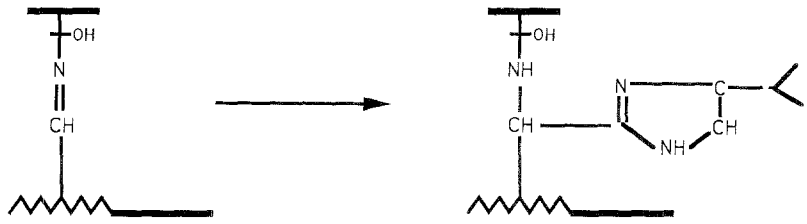


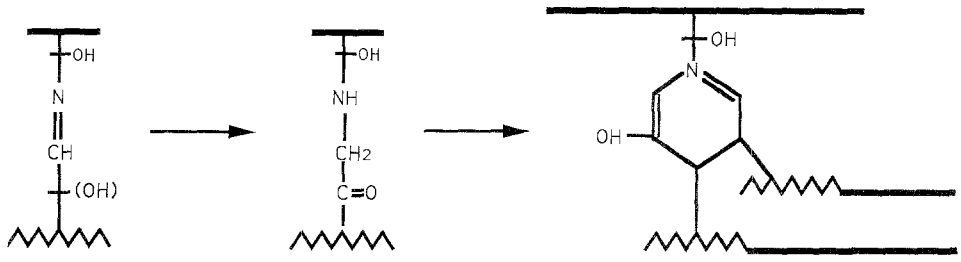
Fig. 6. Action of lysyl oxidase on collagen fibril and formation of aldimine intermediate crosslink

As the animal matures the rate of synthesis decreases, thus at maturity the turnover of collagen is only a fraction of that of the rapidly growing infant, and consequently there is little newly synthesised collagen present. Indeed analysis of the cross-links reveals a virtual absence of the aldimine and keto-imine forms but a preponderance of the trivalent forms. Two such compounds have been characterised. Pyridinoline (Fig. 7) was first reported in very small quantities in tendon (Fujimoto et al., 1977), but later studies identified it as a major cross-link in cartilage (Eyre & Oguchi, 1980). Pyridinoline is derived from the keto-imine either by the interaction of two of these cross-links (Eyre & Oguchi, 1980) or by the addition of a further hydroxylysyl-aldehyde to the keto-imine cross-link. (Robins and Duncan, 1983). It should therefore be referred to as hydroxylysyl-pyridinoline. In bone or dentine collagen there are significant amounts of lysyl-pyridinoline due to the under-hydroxylation of the lysyl residue in the helix that acts as a receptor for hydroxylysyl-aldehyde.

In some tissues eg skin collagen, the hydroxylation of the telopeptide lysine does not occur and clearly these collagens cannot form pyridinoline as the trivalent cross-link. A second stable cross-link was therefore searched for and recently characterised by Yamauchi et al. (1987) as histidinohydroxylysinonor-leucine (Fig. 7). This cross-link is formed by the reaction of histidine with the aldimine bond of the divalent cross-link. The histidine is probably derived from the sequence Hly-Gly-His-Arg in an adjacent molecule which means the cross-linking occurs with the molecules in register ie inter fibrillar link. Alterna-



Histidino-hydroxylysinonorleucine



Pyridinoline

Fig. 7. Formation of mature crosslinks from intermediate divalent crosslinks

tively the histidine could be the derived from the conserved sequence but from the same molecule, in which case the trivalent cross-link only links two molecules.

These studies on the location of the divalent cross-links by identification of the linked peptides confirmed the end-overlap theory of molecular alignment, and the later studies indicated that the linearly polymerised microfibrils cross-link through the trivalent mature cross-links with the molecules in register.

A number of other compounds have been proposed as cross-links but have not been confirmed. An Ehrlich's type compound has been reported by Scott et al. (1981), and a derivative of pyridinoline, deoxypyridinoline, by Barber et al. (1982). In our own studies we have identified a compound that increases with age but owing to its instability following isolation we have been unable to characterise the compound (Barnard et al., 1987). Recently we have demonstrated an increase in two other components of skin with age, and are currently attempting to isolate and characterise them.

Although certainly incomplete these investigations have basically accounted for the mechanical properties of the collagen fibre, a property on which its bodily functions rely.

Over the past few years several different types of collagens have been identified, demonstrating the existence of a family of collagens. They may be roughly classified as fibrous, non-fibrous, filamentous and fibril associated.

Fibrous collagens

The major part of the work described above has been carried out on typically striated fibrous collagens ie Types I, II, III, V and XI. In all cases examined to date these fibres are stabilised by the same cross-linking mechanism.

Non-fibrous Collagens

The network structure of the non-fibrous type IV collagen of basement membrane is also stabilised by the same cross-linking mechanism (Bailey et al., 1984). The known cross-linking sites in the 7s region contain the sequence Hyl-Gly-Glu-Arg which is similar to the lysyl oxidase binding site in the fibrous collagens. This sequence also occurs in the main body of the helix of the type IV molecule (Fig. 8) and cross-links have been identified along these helical domains. The latter suggests that there is some lateral alignment of the type IV molecules in the net (Bailey et al., 1984) rather than a simple single molecule network as proposed. Type VII collagen exists as a dimer attached to type IV and can also be included in the non-fibrous class. To date no studies have been carried out as to whether it is stabilised by lysyl-aldehyde cross-links.

The in vivo structure of type X and its mode of cross-linking has not been determined but it has been suggested that it may have a net-work structure.

Filamentous collagens

The filamentous collagens include type VI. Surprisingly type VI does not appear to possess lysyl-aldehyde cross-links, but is stabilised by di-sulphide bonds.

Fibril associated collagens

Types IX and XII are a new class of collagens in which individual molecules are associated with a fibre of genetically different collagen. For example, type IX exists on the surface of type II fibrils in cartilage and type XII exists on the surface of type I fibres. The type IX has been shown to be cross-linked to the type II by pyridinoline cross-links (Wu and Eyre, 1984) but the mode of attachment of XII to I has not been reported.

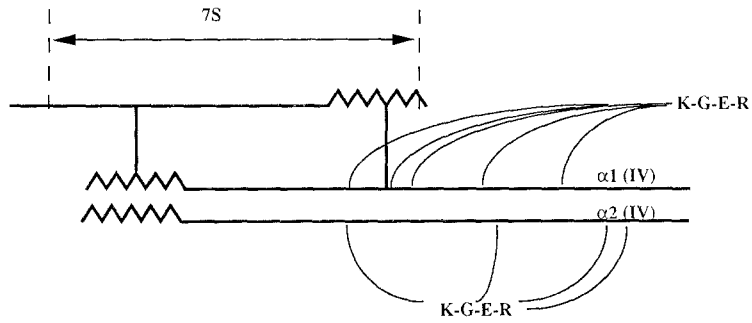


Fig. 8. Location of Hyl-Gly-Glu-Arg (KGER) sequences in type IV collagen molecule

Concluding remarks

The controlled and specific localisation of cross-links by enzymatic action on biological macromolecules has evolved and been widely exploited by organisms throughout the animal kingdom. It is used as a means of supporting and protecting themselves against the stresses of the environment and in providing tissues of specific characteristics such as rigidity or elasticity. The ability to achieve optimal functioning through cross-linking is clearly of paramount importance, particularly since malfunctioning can at worst be lethal. An understanding of the nature of these cross-linking mechanisms should help us provide an insight into their effect on the physical properties of collagenous tissues during growth and maturation, and provide data on the alignment of the molecules in the supermacromolecular structure, and ultimately to understand the cause of tissue function and malfunction.

References

Andersen SO (1964) The cross-links in resilin identified as dityrosine and trityrosine. Biochim Biophys Acta 93: 213–215

Andersen SO (1976) Evidence for two mechanisms of sclerotization in the insect cuticle. Nature 251: 507–508

Bailey AJ, Peach CM (1968) Isolation and structural identification of a labile intermolecular cross-link in collagen. Biochem Biophys Res Commun 33: 812–819

Bailey AJ, Etherington DJ (1980) Metabolism of collagen and elastin. Comp Biochem 19B: 299–460

Bailey AJ, Light ND, Atkins EDT (1980) Chemical cross-linking restrictions on models for the molecular organization of the collagen fibre. Nature 288: 408–410

Bailey AJ, Sims TJ, Light ND (1984) Cross-linking of type IV collagen. Biochem J 218: 713–7

Barber M, Bordoli RS, Elliot GJ, Fujimoto D, Scott JE (1982) The structures of pyridinolines. Biochem Biophys Res Commun 109: 1041–1046

Barnard K, Light ND, Sims TJ, Bailey AJ (1987) Chemistry of the collagen cross-links. Biochem J 244: 303–309

Battaglia DE, Shapiro BM (1988) Hierarchies of protein cross-linking in the extracellular matrix. Involvement of an egg surface transglutaminase in the early stages of fertilization envelope assembly. J Cell Biol 107: 2447

Brown CH (1952) Some structural proteins of Mytilus Edulus. Quat J Micros Sci 93: 487–502

Eyre DR, Oguchi H (1980) The hydroxypyridinium cross-link of skeletal collagen. Biochem Biophys Res Commun 92: 403–410

Foerder CA, Shapiro BM (1977) Release of ovoperoxidase from sea urchin eggs hardens the fertilization membrane with tyrosine cross-links. Proc Natl Acad Sci (USA) 74: 4214–4218

Fujimoto D, Akiba KY, Nakamura N (1977) Isolation and characterization of a fluorescent material in bovine Achilles tendon. Biochem Biophys Res Commun 76: 1124–1129

Fujimoto D, Horiuchi K, Hirama M (1981) Iso trityrosine, a new cross-linking amino acid isolated from Ascaris cuticle collagen. Biochem Biophys Res Commun 99: 637–700

Fuller GM, Doolittle RF (1966) The formation of cross-linked fibrins: evidence for the involvement of Lysine E-amino groups. Biochem Biophys Res Commun 25: 694–700

Garcia-Castinaras S, Dillon J, Spector A (1978) Detection of bityrosine in cataractous human lens protein. Science 199: 897–899

Gathercole LJ, Keller A (1974) Light microscope waveforms in the collagenous tissues and their structural implications. In: Atkins EDT, Keller A (eds) Structure of fibrous proteins. Butterworth, London, pp 153–187

Gross AJ, Sizer IW (1959) The oxidation of tyramine, tyrosine and related compounds by peroxidase. J Biol Chem 234: 1611–1614

Lorand I, Downey J, Gotoh T, Jacobsen A, Tokura S (1968) The transpeptide system which cross-links fibrin by γ-glutamyl E-lysine bonds. Biochem Biophys Res Commun 31: 222–230

Matacic SS, Loewy AG (1968) The identification of isopeptide cross-links in insoluble fibrin. Biochem Biophys Res Commun 30: 356

McBride OW, Harrington WF (1967) Ascaris cuticle collagen: On the disulphide cross-linkages and the molecular properties of the subunits. Biochemistry 6: 1484–1498

Nomura K, Suzuki N, Matsumoto S (1990) Pulcherosine, a novel tyrosine derived trivalent cross-linking amino acid from the fertilization envelope of the sea urchin embryo. Biochemistry 29: 4525–4534

Partridge SM, Elsden DF, Thomas J (1963) Constitution of cross-linkages in elastin. Nature 197: 1297–1298

Robins SP, Duncan A (1983) Cross-linking of collagen, location of pyridinoline in bovine articular cartilage at two sites of the molecules. Biochem J 215: 175182

Scott JE, Hughes EW, Shuttleworth A (1981) Biosci Rep 1: 611–615

Shapiro BM (1991) The control of oxidant stress in fertilization. Science 252: 533–536

Siegel RC (1979) Lysyl oxidase. Int Rev Connect Tissue Res 8: 73–114

Takahashi A, Totshuna-Nakano H, Nakano M, Mashiko S, Suzuki N, Ohma C, Inaba H (1989) Febs Lett 264: 117–119

Waite JH, Housley TJ, Tanzer ML (1985) Peptide repeats in a Mussel glue protein. Biochemistry 24: 5010–5014

Waykole P, Heidemann E (1976) Dityrosine in collagen. Conn Tissue Res 4: 219–222

Wu JJ, Eyre DR (1984) Cartilage type IX is cross-linked by hydroxypyridinium residues. Biochem Biophys Res Commun 123: 1033–1039

Yamauchi M, London RE, Guenat C, Hashimoto F, Mechanic GL (1987) Structure and formation of a stable histidine-based trifunctional cross-link in skin collagen. J Biol Chem 262: 11428–11434

Author's address: Prof. Dr. A. J. Bailey, Muscle & Collagen Research Group, University of Bristol, Langford, Bristol BS 187 DY, England.