Extra cellular materials

Collagens

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The formation of fibrous tissue is the usual process of repair after most types of injury. The major component of the repair tissue is collagen, although other components such as the proteoglycans and glycoproteins are important for the structure and function of the tissue. The structure and biosynthesis of collagen has been well reviewed recently by Grant and Jackson (1976) and Prockop *et al.* (1976) who give detailed references.

Fibrous tissue formation is the end product of the inflammatory response to injury, a response which leads to the invasion into the damaged area of proliferating fibroblasts which lay down the structural components of fibrous tissue.

Thus the first two to three days sets the scene for the appearance and proliferation of the fibroblast. The precursor of the fibroblasts, at least in wounds and granulomas, is not the monocyte or adjacent fibrocytes. Instead an undifferentiated mesenchymal cell originating in the vascular tissues moves into the damaged area. As it does so it divides and develops the apparatus of a secretory cell—multiple nucleoli, extensive rough endoplasmic reticulum, and a large randomly distributed Golgi apparatus. Cell mitosis continues until the cells come into contact, when mitosis and migration cease and the synthesis of the components of fibrous tissue begins, including, of course, the collagens.

Collagen structure

GENETIC POLYMORPHISM

All extracellular collagens so far investigated consist of three polypeptide chains (α -chains) which are wound around each other to form a special type of helix. Unusual features are the presence of hydroxyproline and hydroxylysine, both of which have important roles in defining the final fibre structure. A number of the hydroxylysine residues have sugars attached to them; these glycosyl residues are linked through the hydroxyl group. The overall picture of the collagen molecule (tropocollagen) is of a long, thin, fairly rigid rod about 290×1.4 nm which can

spontaneously aggregate into fibrils of the kind seen by electron microscopy in most connective tissues (Fig. 1). These aggregates are stabilised by covalent cross-links and these give the fibrils their tensile strength (see A. J. Bailey, (1978) at page 49).

Fig. 1 *Electronmicrograph of collagen fibrils* ($\times 44\,000$).

The cells of higher organisms contain several different structural genes directing collagen synthesis. Five different types of α -chains have been identified in vertebrate tissues. They give rise to four different collagen types. There is some degree of specificity in the type of collagen found in various tissues (Table

Table 1 Collagen polymorphism in various tissues

Type	Molecular form	Tissue
I	$[\alpha 1(I)]_2 \alpha 2$	Bone, dermis, tendon, cornea, dentin
II	$[\alpha 1(II)]_3$	Cartilages
III	$[\alpha 1(III)]_3$	Fetal and infant dermis, early scar tissue, granulomas, cardiovascular system
IV	$[\alpha 1(IV)]_3$	Basement membranes

Note. Tissues mentioned contain predominantly the collagen type indicated by many tissues have been found to contain more than one collagen type.

1), indicating the selectivity of gene expression for collagen biosynthesis in certain cell types.

In tissues such as bone, tendon, mature dermis, cornea, and sclera the chain composition is $[\alpha 1(I)]_2 \alpha 2$ —that is, having two identical chains— $\alpha 1(I)$, the third member of the triple-helix, having a different primary structure, $\alpha 2$, and the complete molecule being referred to as type I collagen. The major collagen type in cartilage is type II, which has three identical α -chains of different primary structure to $\alpha 1(I)$, designated $\alpha 1(II)$, with the chain composition $[\alpha 1(II)]_3$. Yet another type of interstitial collagen has been found and named type III. It, too, has three identical α -chains of unique primary structure, the molecule being designated $[\alpha 1(III)]_3$. This collagen is also unusual in that it contains cysteine and is disulphide-bonded. This type of collagen was first found in embryonic dermis. Postnatally there is a switch from type III to type I synthesis, with the result that the bulk of the collagen in adult dermis is type I although 10-15% remains type III.

type III also occurs in the cardiovascular tissues, constituting, for example, some 50% of the collagen of the human aorta.

A class of connective tissues distinct from others is found in the basement membrane. From these a further type of collagen (type IV) has been isolated and characterised. This has three identical α -chains $\alpha 1(IV)$ of unique primary structure and is designated $[\alpha 1(IV)]_3$. Others in addition to these well-defined collagen types have been reported recently but will not be discussed here.

The collagen in fibrosis seems to contain both type I and type III collagen. Initially type III may be formed, and type I added subsequently. This is similar to the process seen, for example, in the development of the dermis. In dermal wounds type III does not exceed 10% of the total even in the early stages of collagen formation.

SECONDARY MODIFICATIONS

Other variations are found in the proportion of lysine residues that are hydroxylated to hydroxylysine and in the degree of glycosylation of these hydroxylysines. Variations may occur within a given collagen type as well as between the different types (Table 2). Thus tendon collagen, type I, has two to six residues of hydroxylysine and only two carbohydrate molecules compared with corneal collagen, also type I, which has 10 residues of each. There are also differences to be found in the relative proportion of Hyl-Gal and Hyl-Gal-Glc. Scar and granuloma collagen at first has a higher hydroxylysine content and a greater degree of glycosylation than the usual type I and type III.

Table 2 Variations in contents of hydroxyproline, hydroxylysine, and hydroxylysine glycosides in several mammalian collagens

Collagen type and source	Residues/1000			Distribution of carbohydrate units (%)	
	Hypro (4-isomer, 3-isomer)	Hylys	Substituted hylys	Hyl-Gal	Hyl-Gal-Glc
Type I					
Human sclera	88.6	6.4	1.7	30	70
.. dermis	92.1	3.6	1.4	30	70
.. tendon	85.9	8.7	1.4	40	60
.. cornea	82.9	8.7	6.4	30	70
Guinea-pig scar	92.0	8.0	2.2	20	80
Type II					
Chick cartilage	103 2	23	9.2	40	60
Type III					
Human aorta	125	5			
Type IV					
Human glomerular Basement membrane	130 11	45	36	5	95
Bovine anterior Lens capsule	132 10	57	42	3	97

Biosynthesis of procollagen

SYNTHESIS OF PRIMARY STRUCTURE

As initially synthesised the polypeptide chains are significantly larger than the extracellular tropocollagen α -chains. The increase in size is due to the presence of non-helical amino- and carboxy-terminal peptides with molecular weights of 15-20 000 and 33-37 000 respectively. These precursor chains,

named pro- α -chains, also differ from tropocollagen in containing cysteine.

HYDROXYLATION

The amino-acids hydroxyproline and hydroxylysine characteristic of all collagens are absent from the pro- α -chains initially synthesised. Specific proline and lysine residues are hydroxylated by prolyl and lysyl hydroxylases, both of which require Fe^{++} ,

Fig. 2 Biosynthesis of procollagen. Diagrammatic representation of assembly of pro- α -chains.

α -ketoglutarate, O_2 , and ascorbic acid as cofactors. These enzymes are bound to the rough endoplasmic reticulum, and the hydroxylation begins on nascent polypeptide chains attached to membrane-bound ribosomes.

GLYCOSYLATION

All vertebrate collagens contain glucose and galactose linked through the hydroxyl group of hydroxylysine residues. Hence glycosylation is subsequent to hydroxylation. The two hexoses are found as galactosyl hydroxylysine or glucosyl-galactosyl hydroxylysine. Galactosyl and glucosyl transferases take part. Galactose is first attached to hydroxylysine and glucose is then attached to the galactosyl hydroxylysine to form a disaccharide. Both enzymes are membrane-bound, largely to the rough endoplasmic reticulum but with a significant proportion bound to the smooth endoplasmic reticulum.

FORMATION OF INTERCHAIN DISULPHIDE BONDS AND THE TRIPLE HELIX

Unlike extracellular type I and type II tropocollagen, the corresponding procollagens contain cysteine residues located in the N-terminal region. Procollagen is secreted by cultured cells in a triple helical conformation which contains interchain disulphide bonds. Recent evidence indicates that soon after the pro- α -chains are released from the ribosomes into the cisternae of the endoplasmic reticulum interchain disulphide bonds are formed and the triple-helical conformation rapidly assumed by the pro- α -chains. This assembly of pro- α -chains apparently begins in the cisternae of rough endoplasmic reticulum and is completed in the cisternae of smooth endoplasmic reticulum. The whole process is summarised in Fig. 2.

The different types of collagen differ in the time required for synthesis and secretion, type I taking the shortest time and type IV the longest. The time of formation of the triple-helix follows the same pattern. Since hydroxylation and glycosylation can occur only while the pro- α -chains are in a random-coil configuration these time differences may be important in deciding the degree of hydroxylation and of glycosylation which take place.

Secretion and fibril formation

Once the assembly of procollagen molecules is complete they are secreted via the smooth endoplasmic reticulum, including the Golgi apparatus, and thence into the extracellular space. There is some evidence that the procollagen is packaged into membrane-bound vesicles in which it is transported to the cell surface and released into the extracellular

space. At this point the terminal peptides are cleaved by specific peptidases to produce extracellular tropocollagen (Fig. 3). Before the loss of the terminal peptides the procollagen molecules are soluble under physiological conditions of temperature and pH, but after this loss the tropocollagen molecules are insoluble under these conditions and can interact spontaneously and precipitate (Fig. 3). This process is analogous to the formation of insoluble fibrin from soluble fibrinogen. In the case of type IV collagen the cleavage of the terminal peptides may not occur. Extracellular type III is also disulphide-bonded, and again possibly the procollagen is secreted unchanged into the extracellular space.

Fig. 3 Diagrammatic representation of collagen fibril formation from procollagen.

One further modification also takes place in the extracellular space—that is, the formation of the precursors of the stabilising crosslinks. An oxidative deamination of specific lysyl or hydroxylysyl residues in the terminal regions occurs involving an aminoxidase which requires Cu^{++} as a cofactor. The subsequent reactions lead to the stabilisation of the collagen by covalent cross-link (Fig. 3). This final phase of collagen biosynthesis is dealt with by A. J. Bailey in the next paper (p. 49).

Conclusions

We are not sure whether the different forms of collagen have specific functions. The pathological consequences of two heritable diseases in which collagen is affected suggest that this may be so. In Ehlers-Danlos type IV there is a failure of synthesis of type III collagen in several tissues including skin, gut, and aorta. In one type of osteogenesis imperfecta there is excess production of type III collagen. It would be of considerable interest to see whether dermal scars on such patients have the same defects

and if there is any effect on the functional status of such scar tissue.

References

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