Hypothesis

A role for glycosaminoglycans in the development of collagen fibrils

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Extensive data on the glycosaminoglycan (GAG) composition and the collagen fibril diameter distribution have been collected for a diverse range of connective tissues. It is shown that tissues with the smallest diameter collagen fibrils (mass-average diameter < 60 nm) have high concentrations of hyaluronic acid and that tissues with the largest diameter collagen fibrils (mass-average diameter ~200 nm) have high concentrations of dermatan sulphate. It is suggested that the lateral growth of fibrils beyond a diameter of about 60 nm is inhibited by the presence of an excess of hyaluronic acid but that this inhibitory effect may be removed by an increasing concentration of chondroitin sulphate and/or dermatan sulphate. It is also postulated that high concentrations of chondroitin sulphate will inhibit fibril growth beyond a mass-average diameter of ~150 nm. Such an inhibition may in turn be removed by an increasing concentration of dermatan sulphate such that it becomes the dominant GAG present in the tissue.

Glycosaminoglycan *Collagen fibril* *Fibrillogenesis* *Connective tissue development*

1. INTRODUCTION

Although there have been extensive studies on the in vitro assembly of collagen molecules into a fibrillar form in the presence of various glycosaminoglycans (GAGs) alone or in combination [1–12], the disparate results of these studies have made it difficult to assign a definitive role for the individual GAGs during either collagen fibrillogenesis or fibril growth. It has been suggested that the duration of the so-called 'lag (or nucleation) phase' of fibril growth, which is altered by the presence of different GAGs, would directly affect the number-density of collagen fibrils so formed, in that collagen fibrils of smaller diameters would be produced when the nucleation phase was long and those of larger diameters when the nucleation phase was short [7–9,13]. However, other experiments have shown that collagen molecules are capable of aggregating into fibrils in the absence of any of the GAGs [4,7,11] and that fibril diameter may be dependent upon glycoprotein content [14], parameters such as pH, ionic strength and temperature [3,15–18], the distribution and number of glycosylated residues along the length of the collagen molecule [19], the degree of copolymerisation in some tissues of types I and III collagen [20–21], collagen–fibronectin interactions [22] and the amount of collagen extension aminopropeptides remaining in the fibril [23].

In spite of these extensive data, there is as yet little understanding of the precise nature of collagen fibril assembly and growth in vivo. For this reason we have studied the relationship between collagen fibril diameter and GAG content of a variety of mammalian tissues at various stages of develop-

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Table 1

Glycosaminoglycan content and mass-average collagen fibril diameter as a function of age in a variety of connective tissues

Tissue	Age	Mass-average fibril diam. (nm)	Total GAG content (% of DW)	% of total glycosaminoglycans		
				Dermatan sulphate	Chondroitin sulphate	Hyaluronic acid
Rat						
Dorsal skin	1 d.		0.297	19.5	20	60.5
	1–3 d.	31.1	0.330	17.5	17	65.5
	5 d.		0.681	9	31.5	59.5
	8 d.		0.408	18.5	36	45.5
	15 d.		0.474	16.5	33	50.5
	1 mo.	61.8	0.493	26.5	36.5	37
	10 w.		0.353	46	26	28
	3 mo.	122.6	0.426	43	26.5	30.5
	3–4 mo.		0.353	48	20	32
	4 mo.		0.400	39	30	31
	5 mo.	113.9				
	11–12–13 mo.		0.233	46	32	22
Ventral skin	5 d.	42.9	0.687	17	34.5	48.5
	8 d.		0.534	16	35	49
	15 d.		0.577	23	33.5	43.5
	1 mo.	102.6	0.447	21	38	41
	10 w.		0.437	24	31.5	44.5
	3 mo.	112.6	0.397	27	33	40
	3–4 mo.		0.359	33	17	50
	4 mo.		0.427	29	24	47
Tail skin	2–5 d.	35.2	0.633	14	42	44
	8 d.		0.514	17	43	40
Human skin						
weight bearing	17 y.		0.815	50	1	49
hypertrophic scar			1.11	61	24	15
Rat						
Tail tendon	1 d.	49.0	2.38	25	41	34
	5 d.		1.44	29.5	37	33.5
	8 d.		1.25	32	37	31
	15 d.	115.0	0.94	34	37	29
	1 mo.		0.50	45	25.5	29.5
	5 w.	210.0				
	2 mo.	320.0	0.31	70	7	23
	3 mo.	340.0		75	3	22
	11–12–13 mo.	333.0	0.26	82	3	15
Rabbit						
Tendo-achilles	Adult	203.5	0.153	74.5	4	21.5
S-region FDP	Adult	150.0	2.3–3.5	20	60	20
Wallaby						
Tendo-achilles	Mature		0.166	71.5	0	28.5

	15 d.		0.490	36.5	38	25.5
	1 mo.	99.3	0.621	33	32	35
	2 mo.		0.427	42	36	22
	10 w.		0.427	43.5	34	22.5
	3–4 mo.	112.3	0.389	63.5	14	22.5
	5 mo.	154.0				
	1 y.	155.8		63		
Guinea pig						
Dorsal skin	4 d.	61.1	0.294	37	32	31
	1 w.		0.286	28	45	27
	2 w.		0.358	32	33	35
	3 w.		0.250	38	39	23
	3 mo.	120.0		36.5	26	37.5
	6 mo.		0.270	41	26	33
	18 mo.		0.220	48	26	26
Ventral skin	0–4 d.	83.6	0.481	16	43	41
	1 w.	87.9	0.274	35	29	36
	2–3 w.	76.6				
	2 mo.	89.6	0.350	31	28	41
	18 mo./mature	71.6	0.252	31.5	27	41.5
Footpad	0–4 d.	70.4	0.465	27	34	39
	1 w.		0.364	28	36	36
	3 w.		0.265	35	30	35
	2 mo.	60.9				
	6 mo.		0.349	38	19	43
	18 mo./mature	61.1	0.340	40.5	19	40.5
Human skin non-weight bearing	14 w. foetal	25.4	0.843	21	14	65
	24 w. foetal	62.0				
	0–6 mo.	70.0	0.698	56	10	34
	5 y.			43	8	49
	17–20 y.	92.6	0.350	48	3	49
	70 y.	77.3		46	8	46
Bovine Tendo-achilles	Mature	197.9	0.413	82	10	8
Dog Tendo-achilles	Adult	213.0	0.426	68	18	14
Flexor digitorum sublimis	Adult	151.8	0.350	61	26	13
(sesamoid)	Adult	48.4	1.994	18	75	7
Chicken flexor tendon	8–10 d. foetal	47		7.5	25	67.5
	12–14 d. foetal	47		12	31	57
	18 d. foetal	55.0	0.696	37.5	49	13.5
	6 w.		0.747	45	50	5
	12 mo.	239.5	0.558	60	33	7
Human Tibialis posterior tendon	5 y.		0.570	77	6	17
Dupuytrens control (palmar fascia)		142.1	0.318	54	11	35
Dupuytrens contracture		40–50	1.396	58	33	9
Dupuytrens nodule		40–50	1.172	58	30	12
Rabbit para-tenon	All ages	40–50	0.600	28	14.5	57.5

[a]The chondroitin sulphate content listed includes some contribution from heparan sulphate

ment and, as a result, have attempted to formulate a new hypothesis of collagen fibrillogenesis and growth. However, at the outset we would emphasise that we are aware of an inherent limitation in our analysis in that technological considerations have limited our studies to the glycosaminoglycans rather than to the true functional unit, the proteoglycan.

2. HYPOTHESIS

In table 1 we report the mass-average diameters of the collagen fibrils and the GAG content from a diversity of connective tissues at different ages. These data have been examined to determine whether there are any correlations between the GAG compositions and the mass-average diameters of the collagen fibrils. No simple (linear) relationships have been found, but when the individual GAGs were expressed as a fraction of the total glycosaminoglycan pool it was noted that tissues containing the smallest fibrils frequently had the highest hyaluronic acid levels, those containing fibrils of intermediate size (mass-average diameter of 60–150 nm) had elevated choindroitin sulphate levels, and those with the largest diameter collagen fibrils contained the highest levels of dermatan sulphate (fig. 1).

It has long been established that hyaluronic acid is the predominant GAG synthesized by foetal connective tissue cells both in vivo and in vitro [19,24] and in connective tissue regeneration and remodelling following wounding [19,25–26]. It has been suggested that hyaluronic acid allows, or at least facilitates the migration of cells to sites of connective tissue development or repair [27–29]. As these are also areas of potential collagen synthesis it may be that hyaluronic acid, by virtue of its water-inclusion properties, could also facilitate the movement and distribution of newly synthesised collagen molecules and fibrils. In these situations, in which the primary stage of fibril growth is occurring, we postulate that the transverse or circumferential growth of collagen fibrils is limited by the hyaluronic acid rich matrix so that only small fibrils < 60 nm in diameter are formed. However, whilst hyaluronic acid, which does not bind to collagen under physiological conditions [10,30–32] may inhibit lateral growth of the fibrils beyond 60 nm diameter, there is no comparable inhibition

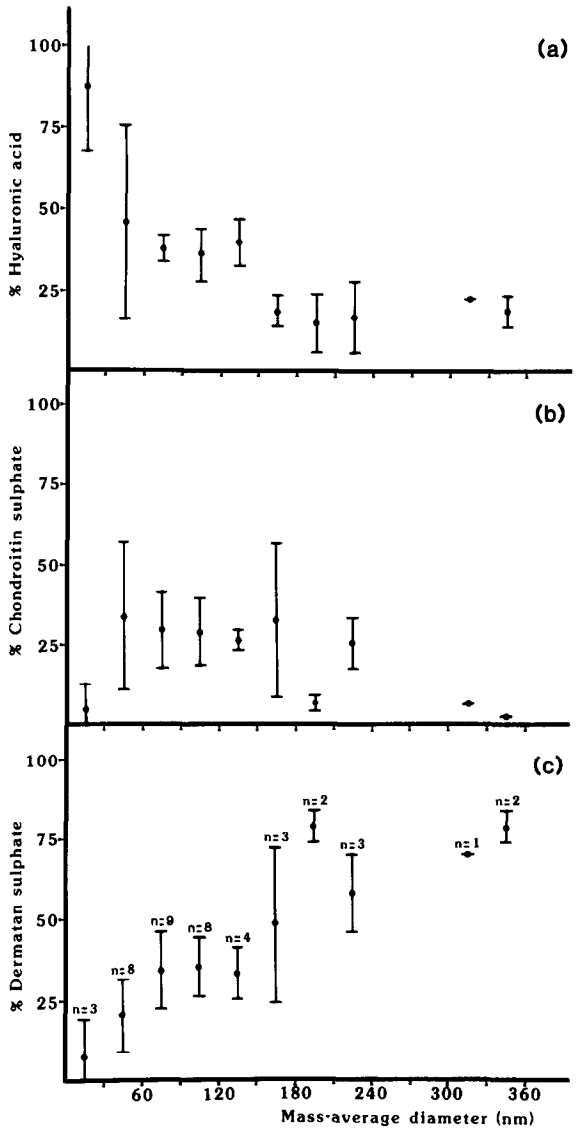

Fig. 1. The mean percentage contents of: (a) hyaluronic acid, (b) choindroitin sulphate, (c) dermatan sulphate from various tissues of diverse ages are plotted as a function of the mass-average diameter (nm) of the constitutent collagen fibrils. Such diameters have been grouped into 30 nm intervals, and the mean values of the corresponding GAG contents are shown with limits of ± 1 SD. The number of values (n) from which each mean value was determined is shown in (c). Two of the three data points for the interval 0–29 nm correspond to vitreous humour and synovial fluid which contain small diameter collagen fibrils (~10 nm diameter and ~100% hyaluronic acid (G.C. Gillard, personal communication).

of longitudinal growth during this initial stage of fibrillogenesis [33]. Thus the tissue may lengthen and grow without the inherent restriction imposed by the presence of thicker fibrils. This mechanism does not imply that hyaluronic acid has a direct role as a nucleating agent; indeed it has already been noted that neither hyaluronic acid nor the other GAGs are essential for initiating the formation of collagen fibrils in vitro.

During maturation, the hyaluronic acid content of many connective tissues tends to decrease quite rapidly whilst the chondroitin sulphate and dermatan sulphate contents tend to increase from the low levels normally present during early foetal development. We propose that during this second stage of fibril development the inhibition of the lateral growth of fibrils imposed by the hyaluronic acid is removed by the proportionate increase of chondroitin sulphate [34] and/or dermatan sulphate synthesised preferentially by the cells in response to their changing mechanical or microelectrical environment [35,36]. Chondroitin sulphate, unlike hyaluronic acid, establishes weak inonic interactions with collagen under physiological conditions. We postulate that although this allows the development of larger diameter collagen fibrils, chondroitin sulphate itself will have an inhibitory effect on fibril growth beyond a diameter of about 150 nm.

Other tissues, especially those which are subjected to high tensile stresses, experience a third stage of development in which the tissue is characterised by an increased percentage of dermatan sulphate and a marked increase in fibril diameter. We believe that as a result of the strong ionic interaction between dermatan sulphate and collagen under physiological conditions, an increasing proportion of dermatan sulphate may remove the inhibition of fibril growth imposed at the earlier stages of development and hence allow the collagen fibrils to grow laterally [33] to those sizes required for their increased tensile loading [37] (mass-average diameters > 150 nm and typically ~ 170–240 nm). Although dermatan sulphate and chondroitin sulphate have the same charge density, dermatan sulphate appears to have a greater charge availability probably due to the presence of L-iduronic acid [31] and this may explain why it is capable of forming a stronger interaction with collagen.

We envisage that the scheme of fibrillogenesis that we have postulated is sequential; the tissues containing the largest collagen fibrils (e.g., some tendons) must pass through a hyaluronic acid-rich stage, a chondroitin sulphate- and/or dermatan sulphate-rich stage but ultimately a dermatan sulphate rich stage. However, whereas tissues with collagen fibrils of mass-average diameter ~ 200 nm can be predicted to have a high dermatan sulphate content, the converse cannot necessarily be assumed; i.e., a dominance of dermatan sulphate does not automatically favour the deposition of large diameter collagen fibrils unless the hyaluronic acid rich and the chondroitin sulphate- and/or dermatan sulphate-rich stages have occurred previously and in that sequence.

A special case exists for cornea but one which might nevertheless be similar in most respects to the scheme already suggested. In cornea, both corneal keratan sulphate and chondroitin sulphate are synthesised after the initial hyaluronic acid rich stage of development. The maintenance of uniform and small diameter collagen fibrils in cornea (~ 17 or 25 nm; [38]), in spite of the presence of a significant proportion of chondroitin sulphate, is probably associated with the unique physico-chemical properties of corneal keratan sulphate, which would appear to restrict fibril growth absolutely. Corneal keratan sulphate is similar to hyaluronic acid in that neither interact with collagen under physiologocial conditions [30–32]. Corneal scars, in which the corneal keratan sulphate is largely replaced by dermatan sulphate, contain larger diameter collagen fibrils than occur in normal cornea [19,39].

3. DISCUSSION

Let us now consider some of the data which is not included in table 1 but which provides support for the hypothesis outlined. Vitreous humour, synovial fluid and umbilical cord (as well as paratenon) are tissues which always contain a very high proportion of hyaluronic acid ($\gtrsim 60\%$) and which, as predicted, contain only small diameter fibrils (mass-average diameters ~ 10–50 nm). We also note that cartilage and bone, which contain predominantly chondroitin sulphate, have small to moderately sized fibrils with mass-average diameters ~ 40–80 nm at maturity.

Establishing a correlation between collagen fibril diameter in skin and GAG composition is inherently difficult. It is known that GAG composition changes with depth in the dermis [40,41] and that differential functional loading across the body of the animal results in local variations in fibril diameter distribution. However, the superficial (papillary) layer of the dermis of many animals (calf [40], human and pig [41]) has a higher GAG content, finer diameter collagen fibrils and a greater ratio of hyaluronic acid to dermatan sulphate than the deeper dermis. Also the perivascular and perifollicular zones of the dermis, which are associated with an hyaluronic acid-rich matrix, contain only small diameter collagen fibrils. These observations are predictable within the framework of the hypothesis.

Dupuytren's contracture (a pathological condition of human palmar subdermis and fascia) contains only small diameter collagen fibrils (~40–50 nm diameter) even though both the chondroitin sulphate and dermatan sulphate contents are high. A similar situation is found in another human pathological condition: hypertrophic scarring [42]. In both conditions the majority of collagen fibrils are of small diameters and are associated with high levels of chondroitin sulphate and low levels of hyaluronic acid compared with adjacent normal tissue. We may speculate that the omission of the hyaluronate rich stage from the onset of the pathological process has effectively prevented the formation of collagen fibrils of diameters $\gtrsim 60$ nm.

Additional evidence in favour of the hypothesis comes from our observations [35,43] which showed that the rabbit flexor digitorum profundus tendon (which curves around the back of the ankle) has markedly different chemical and structural features on the concave and convex sides. On the concave (pressure) side the GAG content is ~2.3–3.5%, of which 60% is chondroitin sulphate, whilst the associated collagen fibrils have a mass-average diameter of ~150 nm; in the tensional parts of the tendon, the GAG content is ~0.2% (70% being dermatan sulphate) and the fibrils have a mass-average diameter ~200 nm [44]. It has also been found that if the tendon is translocated forward so that it is subjected only to tensional forces, profound biochemical and morphological changes occur [35,43,45]. In particular, the cartilaginous pressure-bearing sesamoid region normally containing small diameter collagen fibrils and high levels of chondroitin sulphate is gradually replaced by normal tension-transmitting tendon with closely packed thick collagen fibrils associated with small amounts of GAG, of which a major proportion is now dermatan sulphate. This process can be reversed by repositioning the tendon at an appropriate time after initial translocation. Thus there is a reversible and concomitant change in fibril diameter and GAG composition when the physical environment of the cells is altered. It would thus seem possible that the growing collagen fibril can only sustain a particular diameter provided that the appropriate GAG levels are maintained [46].

Although the data presented in this work are consistent with the notion that the GAG composition and the collagen fibril size are changing simultaneously (and not sequentially) as a result of (say) mechanical or microelectrical factors, we believe that this possibility is unlikely. It seems more probable to us that alterations in GAG composition will precede collagen fibril diameter changes since the glycosaminoglycans turn over at a much greater rate than collagen.

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