GLYCOSAMINOGLYCAN DISTRIBUTION AND FINE STRUCTURE IN THE TENDON FASCICLE: IMPLICATIONS FOR EXTRACELLULAR MATRIX STRUCTURE-FUNCTION

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Introduction Soft connective tissues such as tendon and ligament are composed of collagen fibrils embedded in a hydrated matrix of proteoglycans and other glycoproteins. It is believed that the relative abundance of these macromolecules and their organization in the tissues exist primarily to provide mechanical function. We have previously established and utilized the isolated tail tendon fascicle as an appropriate model to investigate macromolecular structure-function relationships in soft tissues (1). Our studies demonstrated a high correlation between CSDS GAG content and tissue stiffness (1), consistent with the suggested mechanical role for the chondroitin/dermatan sulfate GAGs of collagen binding PGs in soft tissues (e.g., 2). Based on this, we developed the hypothesis that not only the total PG/GAG content, but also the distribution and structure of the GAG, may influence tissue mechanical properties. As such, in the current study we quantitate the distribution of CSDS chains to specific proteoglycan core proteins as well as examine the CSDS fine structure in the tendon fascicles.

Materials and Methods Proteoglycans (PGs) were quantitatively extracted from ventral tail tendon fascicles of 3 wk C57 B1/6 (n=6), 8 wk C57 B1/6 (n=10), and 8 wk Mov13 (n=5) male mice with 4 M GdmHCl (w/ protease inhibitors) and then purified by Q-Sepharose ion exchange chromatography. PGs were identified and quantitated by SDS-PAGE on 4-20% gradient gels, with or without prior digestion with Ch ACII, ABC or B. Gels were stained with Coomassie and Alcian blue, and the relative proportions of decorin and biglycan estimated by densitometric scanning. The amount of total PG applied in each well represented mg collagen equivalents of intact fascicles.

Total GAGs were prepared from papain digests of fascicles from n=1 or 2 mice per group by CPC and EtOH precipitation (3). GAGs were digested with Ch ABC and sulfated Δdisaccharides and non-reducing terminal residues were separated and quantitated by fluorescence based ion exchange HPLC (4).

Results PG Identification and Quantitation Figure 1 demonstrates that decorin (DC) and biglycan (BG) were the predominant PGs in fascicles from all groups. In all samples, decorin and biglycan were largely substituted with DS, since only Ch ABC (Figure 1) and B, but not ACII (data not shown) were able to generate the expected 45 kDa cores (CP). Decorin and biglycan from 3 wk fascicles migrated slower than from mature fascicles, suggesting differences in GAG fine structure between the two ages. Further, biglycan from Mov13 migrated as a more polydisperse band, revealing a faster migrating biglycan species not detected in 3 or 8 wk controls.

Densitometric scans suggest that 3 wk fascicles contained approximately twice as many PG molecules per mg of collagen as 8 wk controls (Table 1). Decorin remained the predominant PG even after skeletal maturation, accounting for ~ 66% of CSDS chains in both 3 and 8 wk control fascicles, with the remaining ~ 34 % of CSDS chains present on biglycan. On a total GAG basis, the PG content of Mov13 fascicles was similar to that of adult controls (Table 1). Although the majority of CSDS in Mov13 was substituted on decorin (~ 57%), CSDS from biglycan represented a larger proportion (~ 43%) of the total than in either control group. Moreover, estimation of decorin and biglycan core protein content clearly demonstrated a significantly higher abundance of small PGs per mg collagen in Mov13 fascicles. Taken together with the observation in Figure 1 that Mov13 fascicles contained a faster migrating biglycan population, these data may indicate Mov13 are enriched in biglycan, substituted with either a single or two shorter CSDS chains.

CSDS GAG Fine Structure The number average length of chains was estimated from the ratio of non-reducing chain termini to the total internal sulfated fluorescent products (ΔDiS) and found to be ~50 repeating disacchar-ides in 3 wk fascicles and ~36 disaccharides in both adult groups (Table 2). Chain disaccharides in all groups were almost exclusively sulfated in the C4 position of the GalNAc residues (ΔDi4S), however, 3-7% were additionally sulfated in the C2 position of an adjacent uronic acid residue (ΔDiB). If disulfated residues are evenly distributed amongst all chains, each CSDS chain in immature fascicles would contain ~1.7 disulfated residues and this is increased to ~2.3 and 2.5 in adult control and Mov13 respectively (Table 2).

Discussion Collagen bound decorin is believed to provide an important mechanical role in soft tissues by regulating collagen fibril diameter (e.g., 5, 6), as well as forming 'interfibrillar bridges' via interaction of the CSDS chains (e.g., 2, 7, 8). Hence, we as well as others have investigated relationships between decorin or CSDS content and tissue mechanical properties (e.g., 1). However, quantifying the relative amount of a particular tissue component does not allow us to assess relationships between "biochemical structure" and mechanics. In the present study, a methodological approach was developed to quantitatively examine the fine structure of CSDS chains and their distribution on different core proteins in the tendon fascicle. Results suggest that fascicle maturation in control and transgenic animals is accompanied by unique adaptive changes in proteoglycan metabolism. In control mice, significant differences between immature and mature fascicles were detected in CSDS fine structure and GAG:collagen content. Whereas in adult Mov13 fascicles, CSDS was contributed more from biglycan, but GAG fine structure and GAG: collagen ratios were not significantly different than age-matched controls.

Taken together with our biomechanical observations (1), results from this study suggest that 'model-specific' adaptations could preclude the existence of global structure-function relationships. However, inclusion of additional experimental groups to these studies will allow us to quantitatively assess the existence of biochemical structure-function relationships across diverse experimental models. Future studies will seek to incorporate data from new experimental paradigms (e.g., immobilization, overuse, other transgenics) and also multiple groups within a particular model in an effort to assess both global and model-specific structure-function relationships in soft tissues.

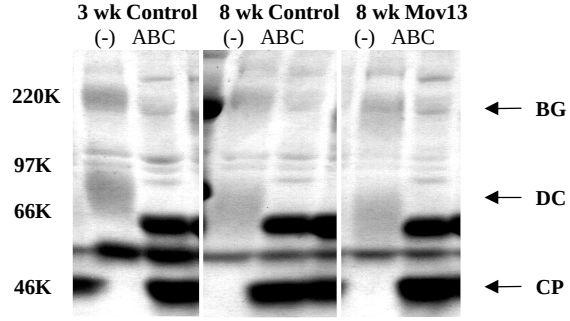

Figure 1. SDS-PAGE separation of intact PGs and their deglycosylated core proteins. (-), (ABC) = intact and Ch ABC digested PGs respectively.

Table 1. Densitometric scanning intensity of CSDS PGs (A).

	Biglycan GAG /Collagen	Decorin GAG /Collagen	(Biglycan + Decorin) GAG /Collagen	(Biglycan + Decorin) Core /Collagen
3 wk Control	845 (32%)	1776 (68%)	2621	2420
8 wk Control	479 (35%)	877 (65%)	1356	1744
8 wk Mov13	590 (43%)	773 (57%)	1363	2673

Table 2. Chain length and sulfation of CSDS chains

	Number average chain length (# of ΔDiS)	ΔDi4S (GlcA-GalNAc4S) per chain	ΔDiB (GlcA2S-GalNAc4S) per chain
3 wk Control	50	48.3	1.7
8 wk Control	36	33.7	2.3
8 wk Mov13	36	33.5	2.5

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