COMPOSITION AND PROPERTIES OF THE HAGFISH (*MYXINE GLUTINOSA*) NOTOCHORD SHEATH

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The notochord in adult hagfish (*Myxine glutinosa*) is a fiber wound cylinder lacking both recognizable cartilage and calcified structures. The core or lumen of the cylinder is comprised entirely of interconnected, epithelial-like cells with large intracellular vacuoles surrounded by dense arrays of intermediate filaments. A relatively high fixed charge density within the core produces a hydrostatic pressure that pressurizes the cylinder and governs notochord mechanical properties (Koob *et al.*, *Bull. MDIBL* 33; 5-8, 1994; Kielstein *et al.*, *Bull MDIBL* 35; 105-107, 1996; Sinwell *et al.*, *Bull. MDIBL* 38; 94-96, 1999; Czuwala *et al.*, *Bull. MDIBL* 39; 104-105, 2000). The core cells are bounded by the notochord sheath, and it is this sheath that delimits core dimensions. The acellular sheath (~ 50 μm thick) is composed of densely packed, thin fibrils 8 – 11 nm in diameter surrounded by 2 – 4 μm thick elastica externa (Welsch *et al.*, *The Biology of Hagfishes*, Ed: Jorgensen, Lomholt, Weber and Malte, pp. 145-159, 1998). Two questions were the focus of this investigation: 1) Are the thin fibrils in the sheath collagenous, and if so, what type of collagen forms the fibrils? 2) What are the material properties of the sheath? The latter question involves two related issues: the role of fiber orientation in the mechanical behavior of the notochord and the contribution of the mechanical properties to notochord function.

For analysis of fibril composition, notochord sheaths were isolated from three animals, the core contents were removed, the sheaths were extracted with 6 M guanidine to remove non-fibrillar components, and the insoluble fibrillar residue was then subjected to pepsin digestion according to established methods for isolation of fibrillar collagens. Following pepsin digestion (10 mg pepsin/100 mg dry weight sheath in 3% CH_3COOH, pH 2.5, 4°C, 24 hr), the pepsin digest was fractionated at 0.7, 1.2 and 2 M NaCl. Precipitates were collected by centrifugation at 27,000 x g for 30 min. The precipitates and 2 M NaCl supernate were redissolved in 3% CH_3COOH, dialyzed against 0.03% CH_3COOH and lyophilized. The NaCl fractions were analyzed by SDS/PAGE, and compared to standard mammalian type I (tendon) and type II (cartilage) collagens prepared with identical procedures. Over 95% of the pepsin solubilized protein from the notochord sheath precipitated at 1.2 M NaCl, whereas both types I and II collagen precipitate at 0.7 M NaCl. SDS/PAGE analysis revealed that the major protein in the 1.2 M precipitate migrated with an apparent molecular weight nearly identical to the alpha chains of types I and II collagen at ~120 kDa (Fig. 1). Only one major band was apparent in the sheath precipitate, similar to the single alpha chain that comprises cartilage type II collagen. In addition, a single β chain (two alpha chains covalently crosslinked) was evident in the sheath 1.2 M fraction, which would be expected if the collagen molecule was made up of three identical alpha chains (as opposed to type I collagen which contains 2 distinct alpha chains, α1 and α2, and, therefore 2 β chains). These observations are consistent with the conclusion that a type II, cartilage-like collagen comprises the fibrils in the notochord sheath. The difference in solubility between hagfish notochord sheath collagen and cartilage type II collagen could derive from differences in amino acid composition or carbohydrate substitution. Further analyses will be necessary to delineate the basis for the difference and its potential significance.

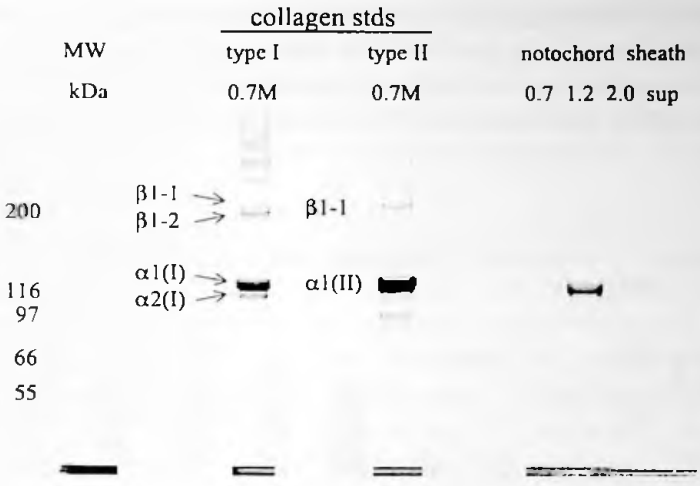

Figure 1. SDS/PAGE (3-8% Novex NuPage) analysis of NaCl fractionated pepsin digests of notochord sheath. Standard type I collagen was isolated from bovine tendon by pepsin digestion and precipitation with 0.7 M NaCl; type II collagen was purified from bovine articular cartilage by pepsin digestion and precipitation at 0.7 M NaCl. The pepsin digest of the notochord sheath was fractionated at 0.7 M, 1.2 M and 2.0 M NaCl, plus the 2 M NaCl supernate.

The mechanical properties of isolated notochord sheath were assessed with uniaxial tensile tests to failure at strain rates of 37%/sec. Specimens were cut at angles ranging from 0 to 90° relative to the long axis of the notochord. A standard clamp-to-clamp gauge length of 5.5 mm was used for all specimens. Force/deformation curves were recorded and subsequently normalized to initial length (to compute strain) and cross-sectional area (to compute stress). Figure 2 shows the stress/strain curves for representative specimens.

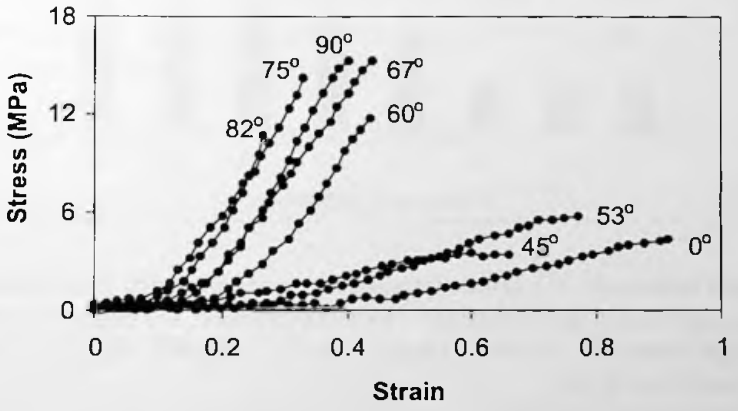

Figure 2. Stress/strain curves for representative specimens of hagfish notochord sheath tested in uniaxial tensile tests to failure. The angle of the specimen relative to the long axis is noted.

Three parameters were calculated from the stress/strain curves and are shown in Figure 3. Stiffness (Young's modulus) was calculated as the slope of linear portion of the stress/strain curve; maximum elongation at failure was calculated as strain; and tensile strength was calculated from the highest load attained before failure normalized to cross-sectional area.

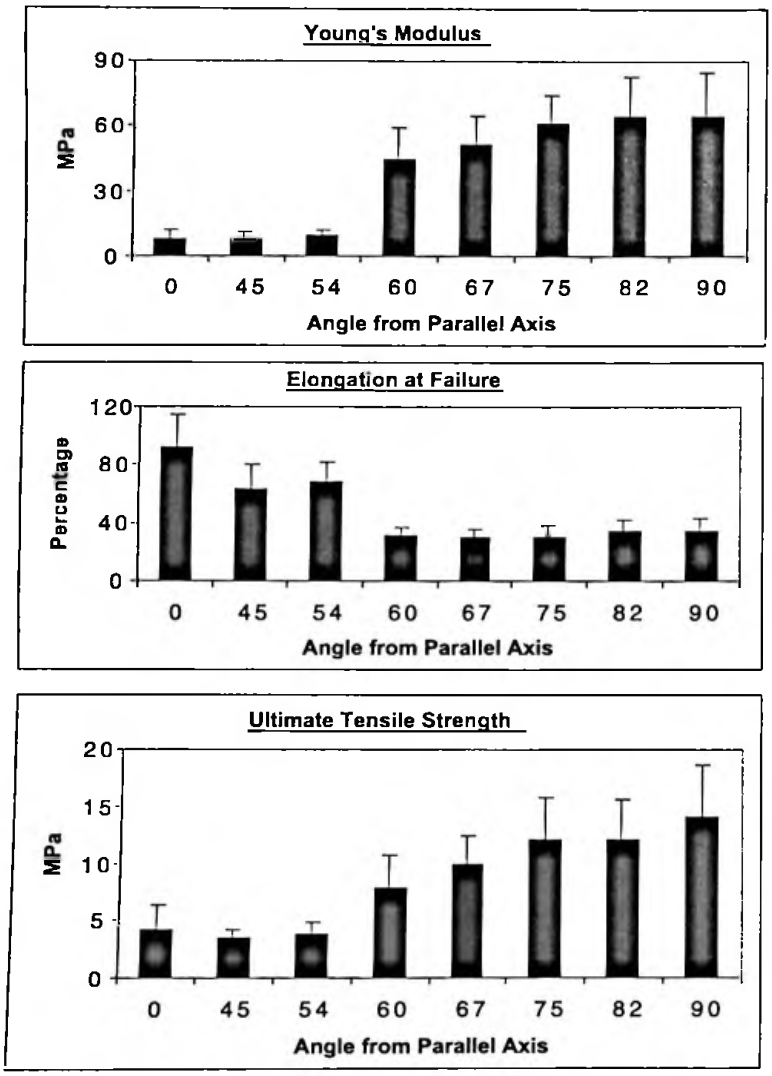

Figure 3. Material properties of hagfish notochord sheath calculated from uniaxial tensile tests to failure. Specimens were obtained from six animals; the number of specimens tested at each angle were: 0° – 11; 45° – 10; 55° – 9; 60° – 20; 67° – 21; 75° – 24; 82° – 4; 90° – 35. Error bars show S.D.

The material properties of the sheath specimens tested at differing angles segregated into two groups, those for specimens cut at angles at or below 54°, and those at 60° and above. The high angle specimens failed at smaller strains than the low angle

specimens, indicating that the sheath can undergo much larger longitudinal deformations than circumferential expansion. The tensile strength and stiffness of the high angle specimens were greater than those of the low angle specimens. Moreover, specimens cut between 75° and 90° were the strongest and stiffest. These tensile properties indicate that the bulk of the collagen fibrils in the sheath are oriented more or less circumferentially around the lumen of the notochord.

Shape changes in hydraulic systems such as the notochord are constrained by the orientation of the reinforcing fibers (Koehl *et al.*, *Amer. Zool.* 40; 28-41, 2000). Given the same magnitude of pressurization, cylinders with fiber angles below 54° are generally stiff in bending, whereas those with fiber angles greater than 54° are less stiff in bending. Larger angles result in less flexural stiffness. Tensile tests indicate that the fiber angle in the hagfish notochord sheath is very high, approaching 90°, indicating that its flexural stiffness would be as low as possible for the *in situ* hydrostatic pressure. Therefore, the collagen fiber angle in the notochord sheath is in large part responsible for allowing the high amplitude bending which is characteristic of hagfish swimming and burrowing (Long *et al.*, *Bull. MDIBL* 37; 114-116, 1998). In addition, the nearly circumferential orientation of the collagen fibrils would prevent localized bulging of the notochord during locomotory bending.

Fibrils in the hagfish notochord sheath appear to be composed of a type II, cartilage-like collagen. The presence of type II collagen in the sheath is remarkable not only because the sheath is not cartilaginous but also because recent analyses have demonstrated that the predominant structural proteins in hagfish cartilage are non-collagenous (Robson *et al.*, *Anat. Embryol.* 202; 281-290, 2000). Despite the fibrous nature of the sheath, in addition to type II collagen, it contains proteoglycans similar to those in vertebrate cartilage, including aggrecan, the predominant proteoglycan in articular cartilage (Koob *et al.*, *op. cit.*). By morphological criteria the sheath is not cartilage. Yet it contains the principal components of cartilage. Perhaps the composition of the sheath should not be surprising since the notochord is the embryonic precursor of intervertebral discs in vertebrates, which contain type II collagen and aggrecan. A cartilaginous composition may have appeared first in the chordate lineage in the form of fibrous tissue in the notochord of agnathans.

Funded by The Shriners of North America #8610.