BIOMECHANICAL PROPERTIES OF HAGFISH (MYXINE GLUTINOSA) NOTOCHORD

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In most vertebrates, the axial skeleton passes through three phases of development, the first of which is the embryonic notochord. However, in cyclostomes, the notochord remains the sole axial support throughout life. The notochord in adult cyclostomes is a fiber wound cylinder comprised of a relatively thin collagenous sheath enclosing a basement membrane delimited cellular core. Physicochemical tests previously demonstrated that the notochord of Myxine glutinosa is osmotically active and that fixed charge density contributes to the generation of this osmotic pressure (Koob et al., Bull. MDIBL 33, 5-8, 1994). These properties are similar to the physicochemical properties of the nucleus pulposus of the mammalian intervertebral disc, which derives in part from the embryonic notochord. The present report describes experiments on the isolated hagfish notochord examining the structural basis for this osmotic behavior and its contribution to specific biomechanical properties

Whole notochords were excised from propylene phenoxetol anesthetized hagfish (Myxine glutinosa). To measure osmotic properties, the core was removed by cutting open one end of the notochord and squeezing out the contents. Core tissue was then extracted with 6M guanidinium-HCl, 50 mM Na acetate, pH 6.5 for 24 hr at 4°C. The extracts were centrifuged at 27,000 x g for 30 min. The supernate was collected and dialyzed against 0.5 M NaCl, 1 mM NaH_2PO_4 for 24 hr at 4°C. One ml aliquots of the dialyzed extracts were placed in dialysis membranes. The membranes were blotted dry, weighed, and the contents were then dialyzed against 1 mM NaH_2PO_4, pH 7.0 containing NaCl concentrations varying between 0.15 M and 1.75 M for 24 hr, after which the dialysis bags were blotted and weighed again. Changes in wet weight of the dialyzed extracts greater than that of dialyzed control salt solutions are expressed as percentage change in wet weight compared to the weight of the initial 1 ml of extract.

Mechanical tests were performed on isolated notochord segments. Excised notochords were ligated with 00-silk suture at 4 cm intervals and transsected to produce uniform cylinders for tensile tests. Specimens were incubated in the neutral phosphate buffered salt solutions containing 0.15, 0.5 or 1.75 M NaCl for 24 hr at 4°C. Tensile tests were performed by attaching one end of the specimen with the suture to a fixed clamp. The other end was attached through the suture to a movable clamp mounted on nylon monofilament. The monofilament passed over a pulley and ended with a plastic beaker into which calibrated weights were added. Weights were added in 4 g increments, the tissue was allowed to elongate for 10 min at which time preliminary tests established it had reached equilibrium. Displacement was measured on graph paper mounted directly behind the beaker. Following the initial test, the suture at one end was carefully removed,

the core tissue was squeezed out and replaced with an equivalent volume of the neutral salt buffer in which the original test was performed. The specimen was then tested as above.

When equilibrated in solutions of varying ionic strength, dialyzed notochord core guanidine extracts changed volume in inverse proportion to the external salt concentration (Fig. 1). Core extracts increased volume in NaCl concentrations below 0.5 M and decreased volume in NaCl above 0.5 M. These results are nearly identical to previous experiments on bulk swelling of whole notochord specimens (see Koob et al., op cit.), indicating that the swelling properties of the notochord are determined to a large extent by the physicochemical properties of the core.

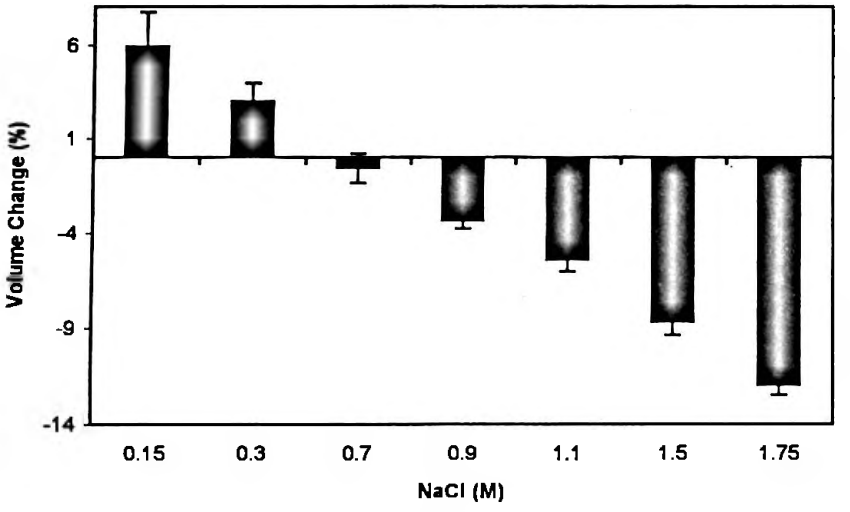

Figure 1. Volume change of notochord core extracts in dialysis tubing incubated for 24hr at 4°C in 1 mM NaH_2PO_4, pH 7.0, containing NaCl at the indicated concentrations. Volume change represents the relative change of weight over the starting weight. Values shown are means $\pm$ S.D. (n = 3/group).

Tensile tests revealed clear differences in the response of intact notochord segments to uniaxial load after equilibration in differing NaCl concentrations (Fig. 2A). The notochord was stiffest (i.e., least deformation per g load) when tested in 0.15 M NaCl. Specimens tested in 0.5 M NaCl, were less stiff than those tested in 0.15 M NaCl. Specimens tested in 1.5 M NaCl were significantly more extensible under load than those in the lower salt concentrations. When the core tissue was replaced with NaCl solutions equivalent to the original test solutions, the deformation/load curves of specimens tested in the three salt solutions were essentially identical (Fig. 2B).

These observations indicate that the physicochemical properties of the core tissue contribute significantly to the mechanical properties of the hagfish notochord. They suggest that, at the physiological osmolarity which is present in situ, the stiffness of the notochord and its ability to provide axial support are largely determined by the properties of the core macromolecules.

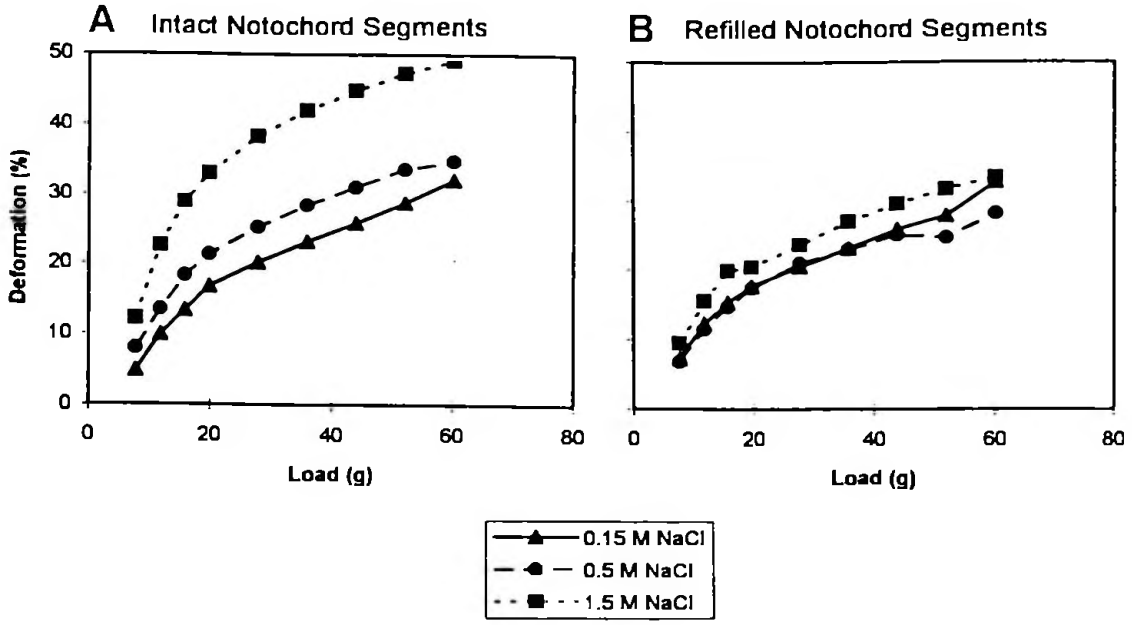

Figure 2. Tensile tests on isolated notochord segments with (A) and without (B) core tissue. Uniaxial tensile tests were performed in the indicated NaCl solutions containing 1 mM NaH_2PO_4, pH 7. Load was applied in 4 g increments, the specimen was allowed 10 min to reach equilibrium length after each addition of load. Deformation is expressed relative to the initial specimen length and values presented are means of three specimens.

Biochemical analyses of core extracts failed to detect proteoglycans (Koob et al., op. cit.) and we have been unable to find hyaluronic acid in the core, indicating that its composition is distinctly different from that in the mammalian nucleus pulposus. The principal protein found in extracts of the core is a molecule of apparent molecular weight of 45 kDa. It appears to be highly disulfide bonded since extraction done in the absence of reducing agents fails to solubilize it. Moreover, in order for the protein to enter a 4-20% linear SDS/PAGE gel, it must be reduced, suggesting that it exists in situ in large aggregates. We are currently characterizing this protein to determine the properties which contribute to the physicochemical properties of the notochord.

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