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# Glycosaminoglycan composition of tarpon (*Megalops atlanticus*) and ladyfish (*Elops saurus*) leptocephali

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Heparan sulphate was found to be the principal glycosaminoglycan (GAG) in tarpon leptocephali, with keratan sulphate also present. The main GAG in ladyfish leptocephali appeared to be a form of chondroitin sulphate (possibly undersulphated).

Key words: Elopiformes; biochemical composition, glycosaminoglycans; larvae.

Whole-body glycosaminoglycan (GAG) composition of eel (Anguilliformes) leptocephali has recently been shown to differ from that of bonefish [formerly Elopiformes but now recognized as a separate order, Albuliformes (Robins, 1989)] leptocephali (Pfeiler, 1991). Electrophoretic migration on cellulose acetate and susceptibility to GAG-degrading enzymes showed that the main GAG components in eight eel species were quite variable. The eel GAGs were tentatively identified as chondroitin sulphate (possibly of varying degrees of sulphation) and hyaluronic acid. However, the predominant GAG in bonefish, *Albula* sp. and *Albula vulpes* (Linnaeus), leptocephali is keratan sulphate (Pfeiler, 1984, 1991). We have recently obtained leptocephali from two additional species of elopiforms, the tarpon (Megalopidae: *Megalops atlanticus* Valenciennes) and the ladyfish (Elopidae: *Elops saurus* Linnaeus), and we report here the results of whole-body GAG analyses.

Tarpon leptocephali (premetamorphic) were collected in the Gulf of Mexico, near the Florida Keys and off the western coast of Florida, from 11–21 July, 1990. Collections were made during the night in the upper 50 m of the water column using a neuston net (1 × 2 m mouth opening) and hoop nets (1 and 2 m diameter mouth openings). Standard lengths of larvae used for GAG analysis ranged from 14·5–24·6 mm.

Ladyfish leptocephali (early metamorphic) were collected in Cross Bayou Channel, Pinellas County, Florida, on 7 February, 1991. The collection was made during the night (21.00–02.00 hours) on a flood tide using a stationary net (1 $m^2$ mouth opening; 0·75 mm mesh) secured to two pilings. Standard lengths of larvae used for GAG analysis ranged from 36·0–41·5 mm.

GAG composition was determined by cellulose acetate electrophoresis before and after treatment with specific GAG-degrading enzymes (keratanase, chondroitinase ABC, chondroitinase AC and testicular hyaluronidase) (Pfeiler, 1991). An additional enzyme, heparitinase (EC 4.2.2.8 from *Flavobacterium heparinum*) obtained from Seikagaku Kogyo Co., Tokyo, Japan, was also used. Heparitinase is specific for heparan sulphate. Approximately 60–120 μg of sample GAG was treated with 0·01 units of heparitinase. The mixture was incubated for 2 h at 37° C in 0·05 M Tris–HCl buffer (pH 7·3).

Total GAG yield from tarpon and ladyfish leptocephali (Table I) is comparable to that of bonefish, *Albula* spp., leptocephali (Pfeiler, 1991).

Cellulose acetate electrophoresis of the tarpon GAGs revealed a major spot in the position of standard heparan sulphate, with a smaller, but intensely stained, spot running slightly behind standard keratan sulphate (Fig. 1). The major spot was almost completely broken down by heparitinase, and was resistant to the other four enzymes, indicating the presence of heparan sulphate. The smaller spot, which co-migrated with keratan sulphate



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TABLE I. Glycosaminoglycan yield from tarpon and ladyfish leptocephali

| Species | No. of larvae | Wet wt (g) | GAG yield (% wet wt) |
|---|---|---|---|
| *Megalops atlanticus* | 35 | 0·5719 | 0·61 |
| *Elops saurus* | 12 | 1·3118 | 1·17 |

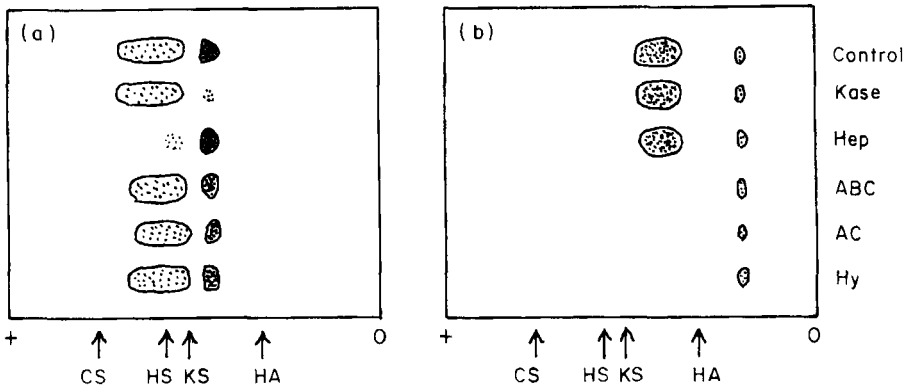


FIG. 1. Cellulose acetate electrophoresis of GAGs extracted from (a) tarpon and (b) ladyfish leptocephali before (control) and after treatment with GAG-degrading enzymes. Kase, Keratanase; Hep, heparitinase; ABC, chondroitinase ABC; AC, chondroitinase AC; Hy, testicular hyaluronidase; O, origin; +, anode; arrows show migration of standard GAGs: CS, chondroitin sulphate (mixed isomers from whale or shark cartilage); HS, heparan sulphate (from bovine kidney); KS, keratan sulphate (from bovine cornea); HA, hyaluronic acid (from human umbilical cord). Standard dermatan sulphate (from porcine skin) and heparin (from porcine intestinal mucosa) run with CS. Electrophoresis was carried out for 90 min at 100 V in 0·18 M Tris–$Na_2$EDTA–borate buffer (pH 8·4). Strips were stained with 0·5% (w/v) Alcian blue 8 GX and destained in 5% acetic acid.

from bonefish leptocephali (not shown), decreased in size and staining intensity after keratanase treatment, indicating that it is composed, at least in part, of keratan sulphate. Some staining of the minor spot was evident after keratanase treatment, and slight decreases in staining intensity of this spot were observed after treatment with both chondroitinases and testicular hyaluronidase, suggesting the presence of an undersulphated chondroitin sulphate that co-migrates with keratan sulphate (see below).

Cellulose acetate electrophoresis of the ladyfish GAGs showed a different pattern from that seen in tarpon (Fig. 1). The major spot migrated between standard keratan sulphate and hyaluronic acid. This spot was resistant to keratanase and heparitinase but broken down by both chondroitinases and testicular hyaluronidase. Enzyme susceptibility of this spot suggests that it is a form of chondroitin sulphate A and/or C. The slow migration of this spot compared with standard chondroitin sulphate may be due to a low sulphate content. Both electrophoretic migration and enzyme susceptibility of this spot suggest that it is similar to the major GAG of several species of anguilliform leptocephali, including *Ophichthus* sp. (Ophichthidae), *Gymnothorax* sp. (Muraenidae) and an unidentified species of Congridae (Pfeiler, 1991). The minor spot of ladyfish larvae was resistant to all enzymes (Fig. 1) and remains unidentified. Similar results were obtained for the slowly migrating, unidentified spots from leptocephali of the congrid eel, *Hildebrandia flava* (Goode & Bean) (Pfeiler, 1991).

The results presented here agree with, and extend, previous observations (Pfeiler, 1991) showing the high degree of variability in GAG composition of leptocephali. This variability

is especially evident in the four species of elopiform (including Albuliformes) leptocephali that have now been analysed. The principal GAGs include keratan sulphate in both species of bonefish (*Albula* sp. and *A. vulpes*), heparan sulphate in the tarpon and a form of chondroitin sulphate A and/or C (possibly undersulphated) in the ladyfish. None of the eight species of anguilliform leptocephali previously studied utilizes keratan sulphate or heparan sulphate as the major GAG (Pfeiler, 1991). The GAG composition of ladyfish is more typical of several species of eels than it is to either bonefish or tarpon.

Both premetamorphic leptocephali (eels and tarpon) and early metamorphic leptocephali (bonefish and ladyfish) have been used in these studies on larval GAG composition. The size and morphology of the premetamorphic larvae indicated that they were all well-developed and not recently hatched. Although differences in rates of synthesis of the component GAGs during development could affect the patterns observed, we are assuming that once the GAG pattern of the gelatinous body matrix of premetamorphic leptocephali is established, it remains largely unchanged until metamorphosis, and the corresponding breakdown of the matrix, is well underway. For example, in advanced metamorphic bonefish (*Albula* sp.) leptocephali, the principal GAG is chondroitin sulphate, rather than keratan sulphate, since the latter is specifically broken down during metamorphosis (Pfeiler, 1984).

It has been argued that the leptocephalus is a primitive character which is of little value in determining relationships between the species that possess it (Hulet & Robins, 1989). However, the between species variability in whole-body GAG composition may prove to be a useful trait for establishing phylogenetic relationships. At the very least it points to the extent of biochemical diversity that has occurred in this supposedly primitive larval group.

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