THE NATURE OF THE POLYELECTROLYTE GEL IN THE AMPULLAE OF LORENZINI OF *SQUALUS ACANTHIAS*

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The electrosensory system in elasmobranchs, the ampullae of Lorenzini, is used primarily to detect and capture prey. It is composed of innervated ampullae that communicate with the external environment through gel filled canals terminating in open pores. The function of the ampullary gel is not clear. Others have speculated that it could maintain the geometry of the canals, prevent infection, or facilitate transduction of electrochemical signals through the relatively long canals (Brown *et al.*, Physical. Rev. E 65, 061903, 2002). Recent evidence suggests that it could play a role in detecting small temperature gradients (Brown, Nature 421, 495-496, 2003). Previous analyses have shown that the gel contains proteins ranging in size from 20 to 200 kDa and a large but uncharacterized, charged macromolecule tentatively identified as a sulfated glycoprotein (Doyle, Biochem. J. 103, 325 - 330, 1967; Brown *et al.*, *op. cit.*). The objectives of the present study were to identify the functional macromolecular component of the gel and to develop methods to purify the gel polyelectrolyte for biochemical analyses and functional studies.

Ampullae were resected en bloc from the posterior supraorbital cluster of male spiny dogfish. The ampullary gel was expressed from the canals, the volume was determined by weight (~ 3 ml/shark), and an equal volume of 6 M guanidine-HCl, 0.1 M Na acetate, pH 6.0 was added. The extract was agitated for 16 h at 4°C, after which the gel had dissolved. The extract was centrifuged at 14,000 x g for 10 min to remove cellular debris. The resulting extract was a homogeneous, viscous fluid that was used as the starting material for purification.

Initial analysis of the extracted/solubilized gel on 4 – 12% SDS/PAGE showed the presence of proteins ranging in apparent molecular weight from less than 20 kDa to over 400 kDa. However, by far the predominant component of the gel was large molecular weight material that did not enter the resolving gel and stained with Alcian blue. Upon dilution of the gel sample and electrophoresis on 4% SDS/PAGE, a single Alcian blue staining diffuse band was the only visible component and migrated with an apparent molecular weight of over 1000 kDa. Since the material stained with Alcian blue, which selectively stains negatively charged polymers at acid pH, it was analyzed by anion exchange chromatography.

In order to purify the macromolecular constituents, the gel extract was exchanged by dialysis into 0.2 M NaCl, 0.02 M Tris-HCl, pH 8.0 for anion exchange chromatography on a 5 ml HiTrap Q column (Pharmacia). Due to the viscosity of the dialyzed gel, it was diluted with an equal volume of 7 M urea, 0.2 M NaCl, 0.02 M Tris-HCl, pH 8.0 before application to the column. The column was eluted with a linear counterion gradient from 0.2 to 2.0 M NaCl. Flow-through fractions containing material that did not bind to the resin, and fractions from the NaCl gradient were monitored for protein by absorbance at 280 nm and indirectly for negatively charged polymers by reaction with dimethylmethylene blue (DMMB).

The flow-through fraction contained protein but no DMMB reactive material. The NaCl gradient eluted both protein and DMMB positive material (Fig. 1 – left panel). DMMB reactive material eluted in a single broad peak between 0.76 and 1.5 M NaCl (fractions 8 – 15), indicating a high negative charge density. Protein co-eluted with the DMMB reactive material.

To determine whether the charged polymer could be depolymerized by proteolysis, the original gel material was digested with papain (40 units/ml gel, pH 6.0) for 18 h at 60°C (Worthington Biochemical Corp.) and chromatographed on the HiTrap Q column. Papain digestion did not alter the elution position of the major DMMB reactive material, indicating that proteolysis did not depolymerize the charged material and thereby establishing that it is a polysaccharide polymer (Fig 1, right panel).

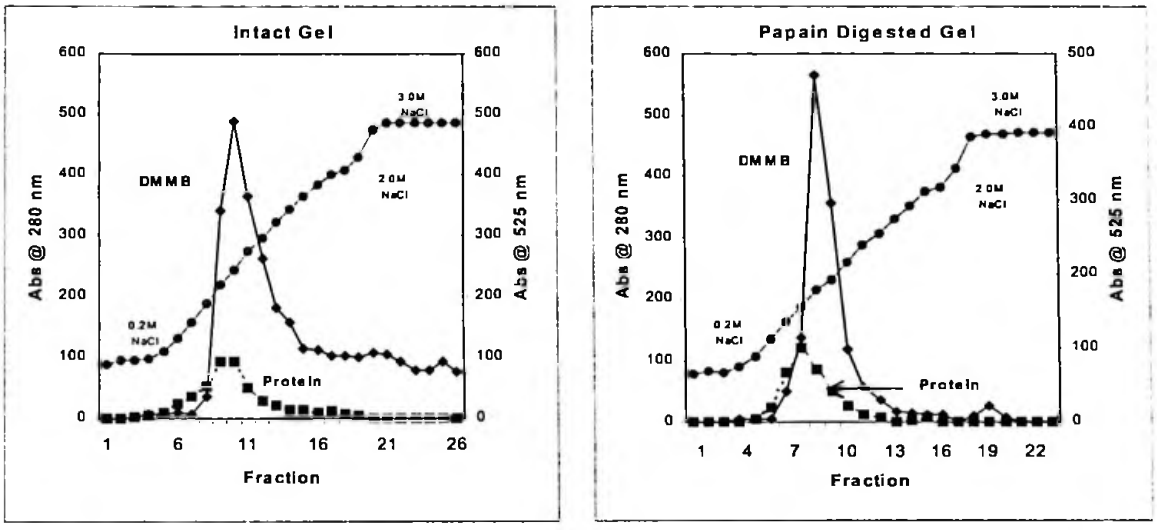

Figure 1. HiTrap Q anion exchange chromatography of gel from the ampullae of Lorenzini of *Squalus acanthias*. The left panel shows the chromatogram for the intact gel. The right panel shows the chromatogram from gel that had been digested with papain. Protein content of each fraction was measured by absorbance at 280 nm; GAG content was measured with dimethylmethylene blue (DMMB) (Abs @ 525 nm). NaCl concentration was measured indirectly by conductivity.

The DMMB reactive material from the HiTrap Q column of the intact gel was chromatographed on a Sephacryl S-300 HR gel filtration column (fractionation range: 1.5×10^6 – 10^4; Pharmacia) under denaturing conditions to determine its hydrodynamic size and mass distribution. The DMMB reactive macromolecule eluted as a sharp peak near the void volume of the column (Fig. 2, left panel). The elution position and narrow size distribution indicate that the negatively charged polymer is a single entity with an apparent molecular weight over 1000 kDa.

The HiTrap Q purified macromolecule was digested with papain, dialyzed to remove digestion products and chromatographed on the S-300 column. Proteolysis substantially altered the size distribution of the DMMB reactive material, all of which retained a molecular size greater than 300 kDa (Fig. 2, right panel).

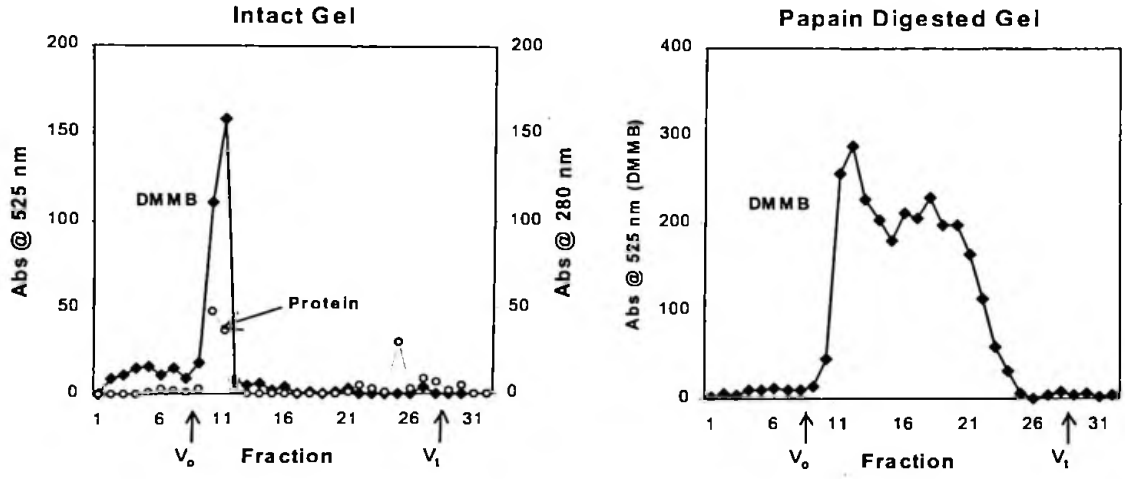

Figure 2. Gel filtration chromatography on Sephacryl S-300 HR in 1 M guanidine-HCl of the negatively charged ampullary gel macromolecule isolated from the HiTrap Q separation shown in Figure 1. Fractions 8 – 14 from the HiTrap Q column were combined, dialyzed against water and lyophilized. Left Panel: The dried sample was redissolved in 1 M guanidine-HCl, 0.1 M Na acetate, pH 6.0 and chromatographed on a 16 x 60 Sephacryl HR S-300 column. Fractions were analyzed for protein by absorbance at 280 nm and for negatively charged polymers by DMMB. Right panel: The dried sample was digested with papain, dialyzed against the guanidine buffer and chromatographed on the S-300 column. Fractions were analyzed for negatively charged polymers with DMMB.

These results support previous analyses (Doyle, *op. cit.*) indicating the ampullary gel in *Squalus acanthias* contains a glycoprotein with substantial oligosaccharide side chain substitution. We have shown that it is an extremely large macromolecule whose physicochemical properties are governed by the high fixed negative charge density of the variably sized polysaccharide constituents. Both the negative charge and size disparity are likely important for the structural and functional properties of the gel.

The ability to purify the macromolecule with simple chromatographic steps will allow future in-depth biochemical analyses of the composition of the macromolecule. Moreover, it will facilitate purification of substantial amounts of material in order to examine the physicochemical properties of the gel as they relate to function. Ultimately, delineation of the physiological function of the gel will rely on testing the pure material.

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