PHYSICOCHEMICAL PROPERTIES OF THE JELLY IN *LEUCORAJA ERINACEA* EGG CAPSULES

Thomas J. Koob
Skeletal Biology, Shriners Hospital for Children, Tampa, FL 33612

The nidamental gland in oviparous elasmobranchs produces not only the egg capsule but also a hydrated egg jelly that surrounds the fertilized ovum and fills the lumen of the capsule. During the first third of incubation, the jelly is transformed from a gel to a liquid, starting near the ovum and proceeding outward, thereby providing the developing embryo a fluid environment in which to move and respire. Eventually the dense jelly in the horns is liquefied; as a result, the slits near the ends of the horns open for embryo assisted movement of water through the capsule (Long and Koob, *Bull. MDIBL* 36; 117-119, 1997). Previous analyses of this material from *Leucoraja erinacea* capsules established that it is a polyanionic, carbohydrate-rich polymer and that liquefaction involves depolymerization without bulk compositional changes (Koob and Straus, *Bull. MDIBL* 37; 117-119, 1998). These observations suggest that the jelly functions primarily as a structural and hydrodynamic support for the egg and developing embryo. The generation of and basis for the jelly's physicochemical properties are therefore of paramount importance for successful development to hatching. Experiments investigating the role of fixed charge density and divalent cations in mediating gel properties are described here.

Lumen jelly was collected from fully formed egg capsules excised from the uterus, capsules removed from the urogenital sinus, capsules within 12 hr of ovipostion, at 24 and 48 hr after oviposition, and after 30 days of incubation. For measurement of cation concentration, the entire jelly sample from the lumen of each capsule was diluted to 1 N HCl by addition of an equal volume of 2 N HCl. Calcium and magnesium concentrations in serially diluted, acidified jelly samples were measured by atomic absorption spectroscopy (AAS). To determine the effects of cations on the gel properties of the jelly from freshly oviposited capsules, osmotic swelling tests were performed. Jelly samples were loaded into dialysis bags and changes in volume after dialysis against solutions of defined ion composition were measured. Calcium and magnesium in one set of these samples were measured by AAS as above. The viscosity of other experimental jelly samples subjected to dialysis was measured with falling ball viscometer.

Jelly from fully formed egg capsules from the uterus contained both calcium and magnesium at levels below those of the maternal body fluids and much lower than seawater (Fig. 1). Calcium and magnesium concentrations were significantly higher in capsules removed from the urogenital sinus, where the capsule is carried for about 12 hours prior to oviposition and is first exposed to seawater. After oviposition, the levels of both cations increased, attaining by 48 hr concentrations comparable to those in seawater. Calcium concentration increased over 4-fold that in utero, while magnesium levels increased by nearly 18-fold. Thirty days later, calcium and magnesium concentrations were slightly higher than at 48 hr, but remained close to those of seawater.

The response of the jelly from capsules collected at oviposition to removal of counter ions was assessed by changes in volume after dialysis as shown in Fig. 2A. Removal of mobile ions by dialysis against water resulted in an average increase in weight of the jelly by 18.3 +/- 1.8%.

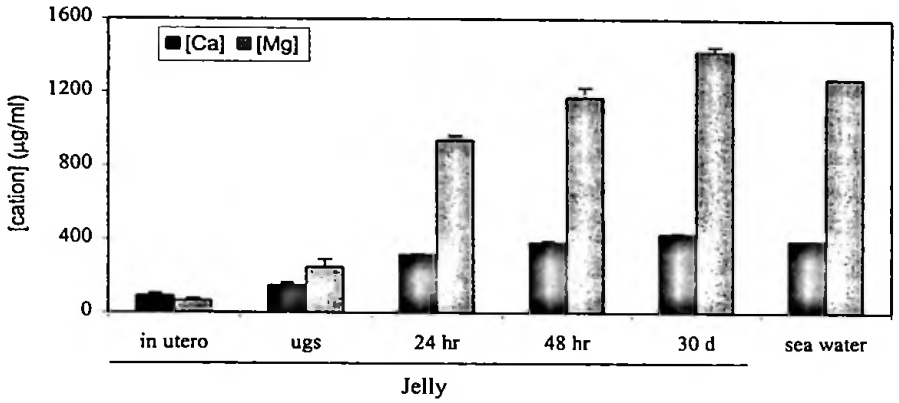

Figure 1. Calcium and magnesium concentrations in egg jelly from capsules in utero, capsules from the urogenital sinus (ugs), capsules 24 hr, 48 hr and 30 days after oviposition. Values shown are means +/- S.D. for the entire lumen jelly from the following number of capsules: in utero – 5; ugs – 3; 24 hr – 11; 48 hr – 9; 30 d - 5.

Ninety-three % of the Ca and 96% of the Mg were lost from the jelly after dialysis against water (Fig. 2B). The remaining divalent cations were bound to the jelly. Elimination of divalent cations by chelation with 0.1 M EDTA followed by dialysis against water caused an average weight increase of 92.2 +/- 28.2%. The latter was limited by the volume of the dialysis tubing.

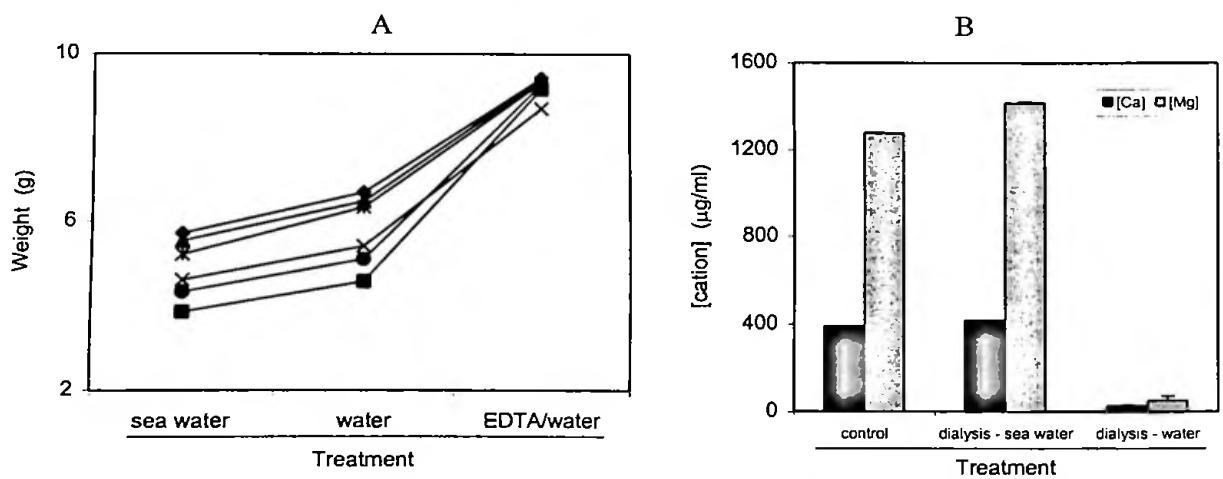

Figure 2. A. Changes in weight of egg jelly after dialysis against water and treatment with EDTA. Jelly samples were first dialyzed against sterile filtered seawater and weighed in order to normalize the starting ionic composition. These samples were then dialyzed exhaustively against de-ionized water and weighed. Samples were next dialyzed overnight against 0.1 M EDTA, pH 7.96, followed by exhaustive dialysis against water. The values shown are weights for individual jelly samples from 6 egg capsules; variations in the starting weight reflect natural variations in capsule jelly volume. B. Calcium and magnesium concentrations in jelly dialyzed against seawater and de-ionized water. Jelly from freshly oviposited capsules was exhaustively dialyzed against filtered sea water or de-ionized water, the jelly was diluted to 1 N HCl, and the concentrations of calcium and magnesium were measured in serially diluted aliquots by AAS. Values shown are means of 3 jelly samples +/- S.D.

The viscosity of the jelly was altered by experimental manipulation of the cation concentration (Fig. 3). Removal of mobile ions by dialysis against water resulted in an increase in viscosity. Removal of bound calcium and magnesium by treatment with EDTA caused a further significant rise in gel viscosity. Incremental addition of calcium to divalent ion depleted (EDTA-treated) jelly caused a concentration dependent decrease in relative viscosity (Fig. 3).

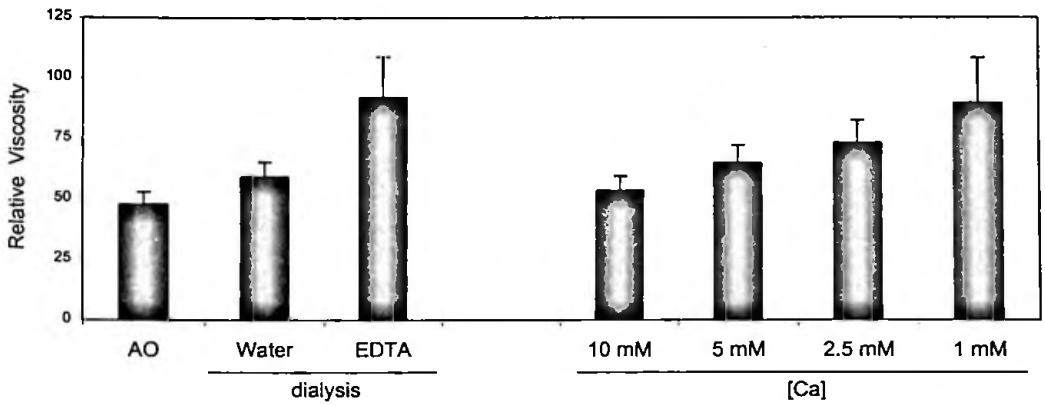

Figure 3. Relative viscosity of egg jelly from capsules at oviposition (AO), and before and after removal of mobile ions (water) and bound divalent cations (EDTA/water). Jelly samples were treated as described in Fig. 2 above. Viscosity was measured by a modified falling ball viscometric procedure. Values presented are means +/- S.D., n = 6.

Together with previous compositional analyses, these results indicate that the jelly in *Leucoraja erinacea* egg capsules is a hydrophilic, polyanionic, high fixed charge density, polymeric gel stabilized by divalent cationic crosslinks. Formation of the jelly involves secretion from the nidamental gland in a condensed state, addition of water resulting in swelling to fill the lumen of the capsule, and gel stabilization through the introduction of divalent cations. The source of the imbibed water may be the region of the uterus immediately caudal to the shell gland since the structure of the uterine wall in this region is predominated by a loose, broadly dilated, hydrated connective tissue matrix with an epithelium typical of transporting tissues (Koob and Hamlett, J. Exp. Zool. 282, 421-437, 1998). Calcium and magnesium are incorporated into the jelly during formation in utero, and these cations may also derive from uterine sources (Koob, *Copeia* 1991(2), 339-347, 1991). Based on the swelling data, one or both of these divalent cations forms ionic crosslinks within the anionic polymer and thereby coalesces the gel and regulates its viscosity. Other polymerization mechanisms may also contribute to gel stabilization since the jelly does not dissolve in choatropic agents, detergents (Koob and Straus, op.cit.), or after elimination of cations by dialysis and treatment with EDTA. Future analyses will be necessary to determine whether the jelly is polymerized by a quinone tanning process similar to that which sclerotizes the capsule wall. Nevertheless, the present observations indicate that the negative fixed charge density of the jelly coupled with divalent cation stabilization governs the viscosity of the jelly and thereby the functional properties of the gel. These results further support the conclusion that the egg jelly is a biomechanical element and functions jointly with the egg capsule to envelop and protect the egg and embryo during the initial developmental stages. Supported by The Shriners of North America (#8610).