RAJA ERINACEA EGG CAPSULE: A MODEL FOR INTERACTIONS OF METALS WITH MEMBRANE-BOUND QUINONES

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Raja erinacea egg capsule is stabilized by a quinone tanning process that involves catechols and catechol oxidase. Catechols are introduced into the capsular material after secretion from the nidamental gland; they are then oxidized to quinones forming dark pigments which sclerotize the capsular matrix (Koob & Cox, The Bulletin 26, 109-116, 1986). This tanning mechanism resembles the biosynthetic pathway of melanin in which tyrosinase oxidizes catecholic derivatives of tyrosine to semiquinones. The tanned egg capsule of the little skate displays chemical properties similar to melanin, most notably chemical oxidation by metals (Koob, The Bulletin 25, 123-125, 1985).

Heavy metals avidly bind to quinone tanned scleroproteins. The byssus threads of Mytilus edulis, which are stabilized by a tanning mechanism similar to that in Raja erinacea egg capsule, bind iron, copper and zinc (Coombs & Keller, Aquat. Toxicol. 1, 291-300, 1981). Metals also bind to melanin. ESR studies have shown that both di- and tripositive metal ions bind to o-semiquinones within natural and synthetic melanins forming chelate complexes and increasing the concentration of free radicals. Since Raja erinacea egg capsule is a quinone tanned scleroprotein displaying melanin-like chemistry, we predicted that metals would bind to capsule generating free radicals and thereby influence the physicochemical properties of the capsule.

Metal Binding

Body walls of recently oviposited capsules were dissected into 1cm x 4mm strips and incubated in concentrations of $FeCl_3$ or $CuCl_2$ from 0.5mM to 100mM in water for 24hr at ambient sea water temperature. Strips were then washed with water, dried and weighed and the amount of bound metal was measured in 1M HCl eluates by atomic absorption spectrophotometry. Preliminary experiments determined that equilibrium binding occurred within 24hr and that 1M HCl quantitatively eluted bound metals. Significant amounts of Fe and Cu bound to capsule strips at all concentrations. Binding was concentration dependent with saturating levels attained in 100mM metal chlorides. Strips incubated in 100mM $FeCl_3$ bound 5.3 $\pm$ 1.0 ug Fe/mg dry weight. Strips incubated in 100mM $CuCl_2$ bound 19.0 $\pm$ 0.3 ug Cu/mg dry weight. In order to assess relative binding strength, capsule strips were incubated in 10mM $FeCl_3$ or $CuCl_2$ for 24hr, washed with water and subsequently exposed to increasing concentrations of HCl. HCl concentrations below 0.1M failed to elute Fe from the capsule; 68 $\pm$ 4% of bound Fe was eluted with 0.1M HCl; the remaining Fe eluted in 1.0M HCl. Bound Cu eluted at lower HCl concentrations; HCl at 0.01M removed 21 $\pm$ 2% of bound Cu and 0.1M HCl eluted the remainder.

Capsule Catechol Content

Capsule strips were treated for 24hr with 100mM $FeCl_3$ or $CuCl_2$ in water or incubated in water alone. Following incubation strips were washed with water, weighed and hydrolyzed in 6N HCl at 108°C for 24hr. Catechol contents were measured in diluted hydrolyzates by the Arnow method (J. Biol. Chem. 118, 531-537, 1937). Both iron and copper significantly reduced capsule catechol

content. Control specimens contained 21.4 ± 0.4 ug catechol/mg wet weight. Specimens treated with $FeCl_3$ contained 12.9 ± 0.4 ug catechol, and specimens treated with $CuCl_2$ contained 8.2 ± 0.4 ug catechol/mg wet weight. $ZnCl_2$ at 100mM did not affect capsule catechol content. Concomitant with metal salt induced reductions in catechol content was an increase in optical absorbance at wavelengths above 460nm. These results indicate that metal binding is accompanied by oxidation of membrane-bound catechols to quinones.

Capsule Permeability

In order to assess the effect of bound Fe or Cu on capsule permeability specimens were pre-treated in saturating concentrations of $FeCl_3$ or $CuCl_2$ for 24hr. Metal chlorides were not included in the solutions used during permeability measurements. The only metal present was that bound to the capsule. Permeability of control and treated specimens was measured by the method of Picheny and Grodzinsky (Biopolymers 15, 1845-1851, 1976). Capsule specimens were mounted between two chambers containing 0.03M NaCl with $2cm^2$ of capsule as the active membrane area separating the two chambers. $Ag/AgCl_2$ electrodes in each chamber measured transmembrane potential which was continuously monitored with a Keithley model 614 electrometer after the NaCl concentration in one chamber was increased to 0.06M. Control specimens exhibited transmembrane potentials averaging 20.4 ± 0.6 mVolts. Specimens treated for 24hr with 100mM $FeCl_3$ showed potentials of 2.0 ± 0.6 mVolts. Specimens treated with 100mM $CuCl_2$ had potentials of 1.1 ± 0.1 mVolts.

Permeability to oxygen was measured by the method of Diez and Davenport (J. mar. biol. Ass. U.K. 67, 249-261, 1987) using a polarographic oxygen electrode. Two cm^2 of capsule was the sole area available for gas exchange between two chambers one of which was filled with deaerated sea water into which was placed the oxygen electrode. The second chamber was filled with oxygen saturated sea water and oxygen tension within the deaerated chamber was continuously monitored as oxygen diffused across the capsule specimen. Oxygen diffusivity of control specimens averaged 23.1 ± 9.4 nmoles x min^{-1} x cm^{-2}; specimens treated with 100mM $FeCl_3$ for 24 hr prior to permeability measurements showed mean oxygen diffusivity of 9.7 ± 4.0 nmoles x min^{-1} x cm^{-2}.

Conclusions

These studies provide evidence that metals will affect the physicochemical properties of biological membranes containing quinones. The results suggest a mechanism which involves metal binding, generation of semi-quinones and an increase in the concentration of membrane free radicals. In the case of skate egg capsule, these events result in an alteration in membrane permeability. Since we found a differential effect of metals on capsule permeability to NaCl and oxygen, the extent and direction of changes in membrane permeability will likely depend on the chemistry of the diffusing solute.

The results also indicate that the skate egg capsule may provide a model for investigating the interactions of metals with membrane-bound quinones. Further experiments will be necessary to determine whether concentrations of metals encountered in the environment affect quinone-mediated properties of biologically active membranes.

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