STUDIES ON SKATE (RAJA ERINACEA) EGG CAPSULE FORMATION

T.J. Koob and D.L. Cox*
Mount Desert Island Biological Laboratory, Salsbury Cove, Maine and *Department of Biology, Vassar College, Poughkeepsie, New York

The mechanical and chemical stability of skate egg capsules together provide the embryo protection from extrinsic attack over long developmental periods in the open marine environment. These inherent physico-chemical properties must derive in part from events occurring during assembly and maturation of secreted capsule precursors. When secreted from the nidamental gland capsular material is white and lacks strength, but it gradually darkens to a deep greenish-brown and coincidentally stiffens with time in utero. Brown histochemically identified a polyphenoloxidase in the nidamental gland and tanning capsule of Raja clavata and suggested this enzyme was instrumental in the formation of quinones during capsule tanning (Quart. J. micro. Sci. 96, 483-488, 1955). Further information regarding capsule formation is lacking. We have therefore begun examining the underlying chemical basis for the development of specific physical properties in Raja erinacea egg capsules and here report on the identification and partial purification of proteins involved in capsule formation.

Materials and Methods

Oviducts of Raja erinacea containing newly secreted egg capsules (the anterior horns being fully formed but untanned) were ligated at the cervix and just cephalad to the nidamental gland. Ten ml of cold 1.0M NaCl, 0.05M NaH_2PO_4, pH 7.5 were introduced into each uterine lumen with a syringe via the cervical canal. After manipulating the buffer to thoroughly wash the uterine contents, it was collected through the opened upper oviduct. The uterine flush contained much susparticulate which was removed by centrifugation at 3000 rpm and 4°C for ten min.

Catechol oxidase activity was demonstrated directly in the uterine flush by incubating 100ul aliquots with 1mM substrate in 0.5M NaCl, 0.025M NaH_2PO_4, pH 7.5 according to Waite (personal communication) for 5 hours at ambient temperature. Substrates tested were 4-methylcatechol, l-dihydroxyphenylalanine (l-dopa), N-acetyldopamine and l-tyrosine. Substrate specific absorbance was monitored over the incubation period and compared to controls which had been boiled for 1 min prior to incubation.

In order to obtain capsule precursors, Raja erinacea shell glands were dissected free of exogenous tissue and homogenized in 10% (v/v) acetic acid (five volumes) for 15 min at 4°C using a glass homogenizer. Newly secreted, untanned capsular material was likewise homogenized. Acid soluble shell gland proteins were subjected to differential salt precipitation at 0.25M, 0.5M, 1.0M and 2.0M NaCl. Each salted homogenate was held on ice for 1 hour and was then centrifuged at 15,000 rpm at 4°C for 15 min. Precipitates were resuspended in cold 10% acetic acid and stored frozen.

Electrophoresis of shell gland and capsule proteins was by an acid/urea gel system adapted from Panyim and Chalkley (Arch. Biochem. Biophys. 130, 337-346, 1969). Acrylamide and N,N'-methylenebisacrylamide concentrations were 6% and 0.15% (w/v) respectively, while Temed concentration was routinely 5% (v/v). Electrode buffer was 5% acetic acid and the sample buffer contained 5% acetic acid, 8M urea at pH 4.2. Migration was toward the cathode. Gels were pre-electrophoresced at 2.5mA per slab and 4°C for 12 hours. Sample electrophoresis was performed at 4°C using 25mA per slab. Gels were stained with Coomassie Brilliant

Blue G-250 for 2 hr at ambient temperature, and were subsequently destained first in methanol:acetic acid:water (45:10:45) and then in 5% acetic acid.

Results

The uterine flush was found to contain an activity which catalyzed the conversion of the O-dihydroxyphenols to their corresponding O-quinones. This activity was destroyed by heating to 100°C indicating its enzymic nature. The enzyme was identified as a catechol oxidase on the basis of its ability to oxidize 4-methylcatechol, l-dopa and N-acetyldopamine. Oxidation rates however differed for the three catechols examined and depended on the nature of the para-substituted group (Figure 1). Based on extinction coefficients (see Figure 1 and Waite, Analyt. Biochem. 75, 211-218, 1975), 4-methylcatechol was oxidized at the greatest rate, 128 uM/min, while l-dopa and N-acetyldopamine were oxidized at slower rates, 100 and 21 uM/min respectively. Tyrosine was little affected by the enzyme (Figure 1).

As judged by acid/urea gel electrophoresis, differential salt precipitation effected partial purification of several acid soluble shell gland proteins (Figure 2). Substantially more protein was precipitated at 1.0M NaCl than at any other salt concentration. Newly secreted, untanned capsular material is comprised of at least three distinct proteins. Results of electrophoresis furthermore demonstrate that salt insoluble precipitates from the acid soluble fraction of shell glands contain proteins that co-migrate with untanned capsule proteins.

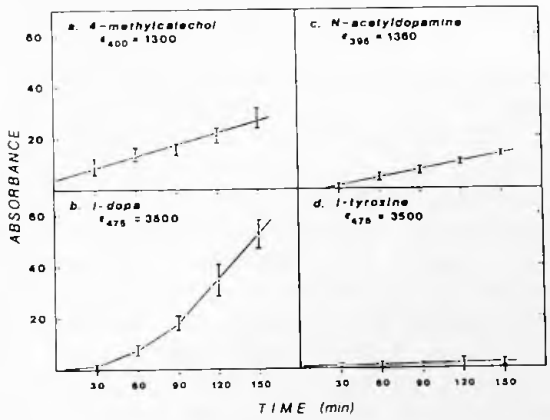

Figure 1. Oxidation of catechols by the uterine flush. Absorbance was monitored at the indicated wavelengths.

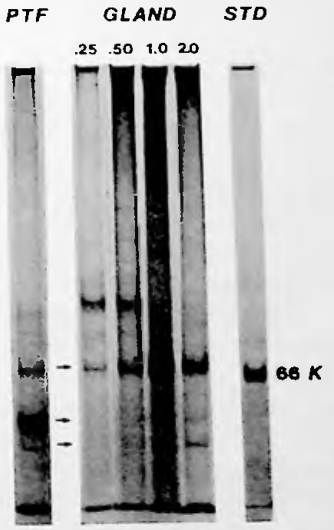

Figure 2. Acid/urea gel electrophoresis of proteins isolated from the nidamental gland (GLAND) and newly secreted pretanned capsular material (PTF). Arrows indicate proteins common to the gland and PTF extracts. The standard contained bovine serum albumin.

Discussion

These results confirm the presence of active catechol oxidase in tanning capsules of Raja erinacea. While the natural substrate of the enzyme is not known, we have found substantial amounts of l-dopa in hydrolyzates of tanning and oviposited egg capsules (unpublished). Electrophoretic analysis of proteins from tanning capsules demonstrated the presence of at least three proteins which are also extractable from shell glands. Analysis of these purified proteins will determine whether l-dopa is present in capsule precursors isolated from the nidamental gland or is added later during capsule formation in

utero. Further studies will delineate at what stage of capsule formation catechol oxidation occurs. The fate of the enzymatically produced O-quinone is currently unknown. It could remain in the oxidized form or interact with other reactive groups. We believe, however, that catechol oxidase is catalyzing a crucial step in the development of capsule properties which are ultimately important for embryogenesis and fetal development. We wish to thank Mr. Hal Church for sharing his considerable photographic expertise. This work was supported by a Mellon Grant to D.L. Cox and by NSF PCM 8104144.