EFFECTS OF SALINITY, pH AND UREA ON SHELL GLAND CATECHOL OXIDASE OF RAJA ERINACEA

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Catechol oxidase plays a pivotal role during the formation of skate egg capsules. It catalyzes the oxidation of catechols to highly reactive quinones forming dark pigments which tan the capsular matrix. Both the introduction and oxidation of catechols appear to occur after secretion and assembly of capsular precursors from the nidamental gland while the formed capsule resides in the uterine lumen (Koob & Cox, The Bulletin 26, 1986). The present report describes studies which examined the sensitivity of shell gland catechol oxidase to salinity, pH and urea in order to gain insight into the conditions which exist within the capsular material during tanning.

Materials and Methods

Initial salt extraction of shell glands from spawning Raja erinacea was performed as previously described (Koob & Cox, The Bulletin 25,132-134,1985). This 1.0M NaCl extract was subsequently fractionated with ammonium sulfate at neutral pH by sequential precipitation at 5, 10, 20, 30 and 40% $(NH_4)_2SO_4$. Precipitates were collected by centrifugation at 17,500rpm for thirty minutes at 4°C and redissolved in 1.0M NaCl, 0.05M NaH_2PO_4, pH 7.0. Not all the precipitate formed at low ammonium sulfate concentrations dissolved in the buffer and therefore these solutions were clarified by centrifugation at 10,000rpm and 4°C for fifteen minutes. Protein determinations were performed on the original extract and ammonium sulfate fractions (Lowry et al., J. Biol. Chem. 193, 265-273, 1951).

Catechol oxidase assays were performed as previously described (Koob & Cox, The Bulletin 25, 132-134, 1985). Diluted aliquots of the initial extract and ammonium sulfate fractions were incubated with 1mM 4-methylcatechol at a final concentration of 0.05M NaH_2PO_4, pH 7.0. The reaction was initiated by adding substrate and the absorbance at 400nm was recorded over the next six minutes. All assays were performed at room temperature.

For experiments examining the effects of salinity, pH and urea on enzyme activity, the desired concentrations were effected by dilution of the enzyme with stock solutions. NaCl was varied between 0.25M and 1.0M in 0.05M sodium phosphate, pH 7.0. pH was varied from 4.5 to 9.5 using three buffers: pH 4.5 to 6.0 in 0.05 sodium acetate, pH 6.0 to 7.5 in 0.05M sodium phosphate and pH 7.5 to 9.5 in 0.05M TrisHCl. Urea concentration was varied from 0 to 4.8M in 0.5M NaCl and 0.05M NaH_2PO_4, pH 7.0.

Results

Ammonium sulfate fractionation of the 1.0M NaCl extract of shell glands resulted in a significant enrichment of enzyme specific activity (Table 1). Catechol oxidase activity was found predominantly in precipitates formed at 5 and 10% $(NH_4)_2SO_4$ while most of the protein precipitated at higher concentrations. Based on specific activity, a fifteen- to twenty-fold purification of the enzyme was accomplished. For characterization of enzyme properties

the 5% and 10% fractions were combined.

Table 1. Catechol oxidase activity in ammonium sulfate fractions of the 1.0M NaCl extract of Raja erinacea shell glands.

SAMPLE		OXIDASE ACTIVITY (uM/min/ml)	PROTEIN (mg/ml)	SPECIFIC ACTIVITY (uM/min/mg)
1.0M NaCl extract		26.4 ± 0.8	12.5	2.1 ± 0.1
$(NH_4)_2SO_4$ fractions:				
	5%	152.4 ± 7.2	3.8	39.7 ± 1.9
	10%	160.4 ± 5.1	5.1	31.4 ± 0.2
	20%	40.4 ± 1.2	19.0	2.1 ± 0.1
	30%	trace	9.0	-
	40%	-	5.5	-

The concentration of NaCl in the catechol oxidase assay was varied from 0.25M to 1.0M in 0.05M NaH_2PO_4, pH 7.0. The enzyme activity at NaCl concentrations below 0.25M could not be measured accurately due to the substantial increase in turbidity resulting from protein precipitation. Rates of oxidation were little affected by NaCl concentrations above 0.25M (Figure 1). A slight increase in the oxidation rate of 4-methylcatechol was found at 0.5M and this was statistically different from that at 0.4M or 1.0M.

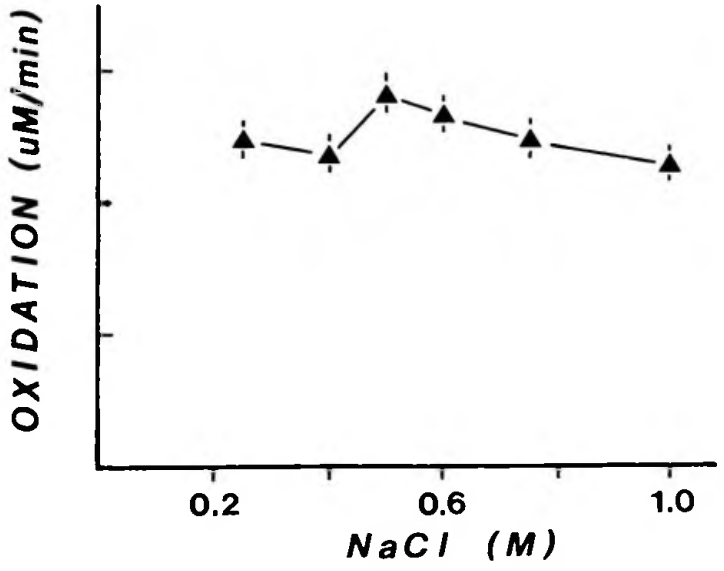

Figure 1. Effects of NaCl on catechol oxidase activity.

Enzymatic activity was sensitive to the pH of the reaction mixture (Figure 2). Maximal oxidation rate occurred at pH 7.5. The enzyme displayed little activity at a pH of 5.0 or less. Rates of oxidation at higher pH were substantially above the natural oxidation rate of 4-methylcatechol. At pH 9.5 the enzyme retained approximately 70% of its maximal activity.

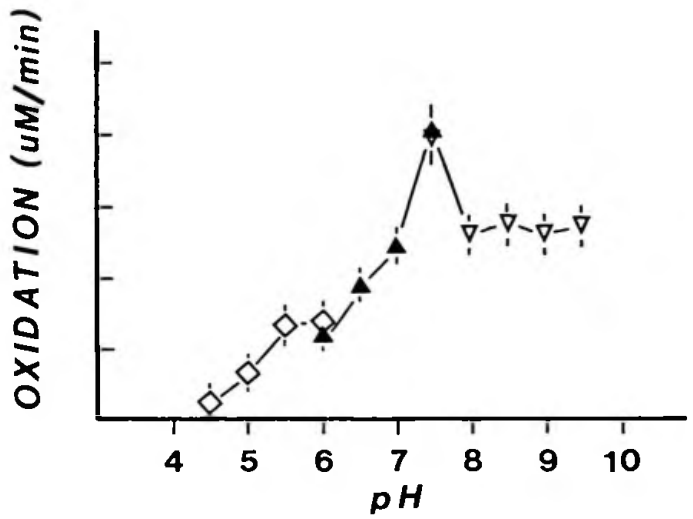

Figure 2. Effects of pH on catechol oxidase activity. □ - sodium acetate; ▲- sodium phosphate; ▽ - trisHCl.

Urea inhibited catechol oxidase in a concentration dependent manner (Figure 3). At the lowest concentration examined, 0.15M, a slight reduction in enzyme activity was observed. Fifty percent inhibition occurred at approximately 4.0M urea. Oxidation rates were reduced by approximately 10% at the concentration of urea normally maintained in elasmobranch tissues.

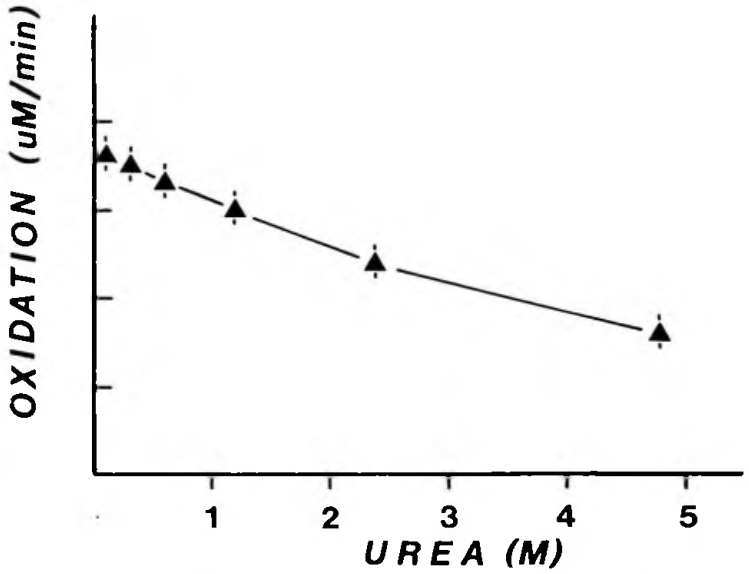

Figure 3. Effects of urea on catechol oxidase activity.

Discussion

These experiments establish the optimal conditions for assay of catechol oxidase from Raja erinacea nidamental glands. The partially purified enzyme exhibited maximal activity at 0.5M NaCl and pH 7.5. Whether these conditions obtain in capsular material during tanning is not known, however, they closely approximate the osmolality and pH generally maintained in elasmobranch tissues. The chemical conditions within the capsular matrix could be established during secretion of capsular precursors or alternatively result from regulation of the intrauterine milieu. The sensitivity of the enzyme to urea is not unexpected as renal and branchial enzymes from other elasmobranchs show identical inhibition by urea (Malyusz & Thiemann, Comp. Biochem. Physiol. 54B, 177-179, 1976). We do not know whether urea is present in fluid bathing the tanning capsule or in the capsular material itself.

The wide tolerance of shell gland catechol oxidase to alkaline pH and elevated salt concentrations provides evidence that it might remain active in sea water following oviposition of the capsule. Egg capsules of the little skate continue to tan during incubation by a process which might involve catechol oxidation (Koob, The Bulletin 25, 123-125, 1985). While it appears from the data presented here that catechol oxidase operates principally during capsular tanning in utero, it could also play a role in post-ovipositional tanning of capsular material. Future experiments will determine whether this enzyme remains active following oviposition during incubation in sea water.