REDOX POTENTIAL IN THE EGG CAPSULE OF THE LITTLE SKATE RAJA ERINACEA

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The egg capsule of the little skate at oviposition can be oxidized or reduced by a variety of chemical agents including ferric chloride, cupric chloride, potassium persulfate, sodium metaperiodate, ascorbic acid and sodium borohydride (Koob, J. Exp. Mar. Biol. Ecol. 113:155-166, 1987; Koob & Cox, The Bulletin 27:16-17, 1987-88; Koob, unpublished). Reduction with sodium borohydride resulted in a loss of color and an increase in transmitted light. In contrast, oxidation by ferric chloride caused a blackening of the capsule and a loss of transparency, which coincided with oxidation of catechols to quinones. This latter effect of oxidation mimicked the natural darkening of the capsule which occurs normally during incubation. These observations led to the hypothesis that the egg capsule contains an inherent redox potential at oviposition and that the incubation-related change in capsule chemistry results from oxidation (Koob, op.cit.). The experiments described here were undertaken in order to characterize the redox potential of the skate egg capsule at oviposition and to determine whether changes in redox potential occur during incubation.

Capsule specimens (10mm x 5mm) were dissected from the dorsal and ventral walls of the following capsules: fully formed capsules removed from the uterus, capsules collected within two days of oviposition, capsules which had incubated three months and capsules which had incubated one year (all of which contained live embryos). Specimens were thoroughly washed with deionized water and equilibrated in 5mM sodium acetate, pH 7 at 4°C. To determine the oxidation-reduction potential specimens were placed in a 1ml solution containing one of a series of standard redox indicator dyes (100μM in 5mM sodium acetate, pH 7). Dyes of principal interest were resazurin, resorufin, 2,6-dichlorophenol indophenol, indigo monosulfonate, methyl viologen, methyl thymol blue, bromophenol red, methyl red, cresol red, thymol blue and pyrocatechol sulfonephthalein. Several redox indicator dyes could not be used because they bound to the capsule specimens thereby reducing dye content in the solution (methylene blue, toluidine blue, Janus green, Nile blue, phenosafranin, safranin-T and neutral red). Specimens were left in the dye solution at room temperature for twenty four hours. The optical absorbance spectra of the solutions were then obtained with a Beckman scanning spectrophotometer. Solutions exposed to capsule specimens were compared to control solutions which were treated in parallel without a specimen. Capsule mediated oxidation or reduction changed the dye solution in one of two ways. In the first case, the dye became colorless. In the second case, the dye changed its absorbance maximum. Measurements were made on replicate specimens from three capsules at each time point.

The following dyes were reduced by specimens from capsules in utero and capsules collected at oviposition: resazurin, resorufin, 2,6-dichlorophenol indophenol and indigo monosulfonate. Dyes that were oxidized by specimens from these capsules were cresol red, bromophenol red, cresol purple, and pyrocatechol sulfonephthalein. Methyl viologen and methyl red were not affected by these specimens.

Capsular redox potential slowly decreased during incubation. Specimens from capsules incubated three months exhibited diminished capacity to oxidize or reduce the indicator dyes as compared to specimens from newly oviposited capsules. Specimens from capsules incubated one year affected the dyes even less than did the three month specimens. This decrease in redox potential can be illustrated by the effects of capsule specimens on resazurin. Resazurin undergoes a two step reduction. The first step is the reduction of resazurin (blue) to resorufin (pink). The second step renders resorufin colorless. Specimens from capsules at oviposition reduced resazurin to the colorless dihydroresorufin. Three month specimens produced a mixed color product with some resorufin remaining. Specimens from one year were able to accomplish only the first reduction step since the dye changed from blue to pink but retained the same color intensity. Since resazurin and resorufin have redox potentials of +70mV and -51mV, respectively, it is clear that the redox potential of the capsule decreased during incubation.

These results establish that the egg capsule of the little skate contains redox potential at oviposition. The capacity to reduce resorufin (-51mV) and indigo monosulfonate (-160mV) indicates that the capsule has a substantial reducing potential. The chemical basis for redox potential in the capsule is uncertain. However, it seems likely that it derives from the introduction of catechols and their subsequent oxidation to quinones during the tanning and sclerotization of the forming capsule in the uterus (Koob & Cox, The Bulletin 26:109-112, 1986). At oviposition the capsule contains catechols, quinones and catechol/quinone condensation products, which could provide redox capabilities (Koob, op.cit.). These results also demonstrate that capsule redox potential decreases during incubation, supporting the hypothesis that the capsule slowly oxidizes in concert with embryonic development. The function of an oxidation-reduction system in the capsule and the change in this redox potential during incubation remains enigmatic.

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