SKATE (RAJA ERINACEA) EGG CAPSULE PROTEINS

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Early chemical studies of elasmobranch egg capsules suggested that the capsular matrix is composed predominantly of keratin. More recent ultrasructural (X-ray diffraction and electron microscopy) and compositional analyses have been interpreted as evidence that collagen is the major capsule protein in the fibrils of these capsules. The presence of elevated levels of tyrosine in capsule hydrolyzates and histochemical identification of tyrosine-rich protein in the shell gland have led to the hypothesis that a polyphenolic protein which participates in the tanning process is mixed with collagen during formation in the shell gland. However, the situation appears to be somewhat more complex. We here present evidence for at least six major proteins comprising the egg capsule of the little skate (Raja erinacea), none of which is a typical interstitial collagen.

Newly secreted, untanned capsular material was removed from the nidamental gland and solubilized in 3.3% lauryl sulfate, 0.1M Tris-HCl, 15% glycerol, 50mM dithiothreitol, pH 6.8 (gel sample buffer, GSB). Electrophoresis on 4-20% linear gradient polyacrylamide gels of solubized untanned egg capsule showed six major proteins with the following apparent molecular weights: 95, 70, 38, 27, 23 and 20 kDa. When dithiothreitol was omitted from the GSB extraction, the 95 and 27 kDa proteins were not solubilized, indicating that an early event in stabilization of these proteins involves formation of disulfide bonds. Omission of DTT did not affect solubilization of the other major proteins or their electrophoretic migration. In addition, at least ten other proteins occurring in relatively minor amounts were detected by SDS/PAGE.

Amino acid compositions of the six major capsule proteins are shown in Table 1. All of these proteins contain elevated levels of glycine, serine, proline and tyrosine. Glycine content varied from just under 200 residues to over 500 residues per thousand (RPT). Serine content averaged approximately 100 RPT with the 70kDa protein being especially serine rich at 156 RPT. Significant amounts of proline were found in the 95, 70, 38 and 27 kDa proteins. Hydroxyproline was present in significant amounts in the large proteins. Tyrosine levels ranged from 86 to over 200 RPT. Tyrosine is especially abundant in the 23 and 20 kDa proteins that also contain unusually high levels of glycine. Approximately three quarters of the amino acids in these proteins are glycine and tyrosine.

Table 1. Amino acid composition of Raja erinacea egg capsule proteins (values in residues/thousand).

	95 kDa	70 kDa	38 kDa	27 kDa	23 kDa	20 kDa
ASX	111	173	115	106	53	47
GLX	56	24	50	49	9	5
HYP	19	2	11	14	1	0
SER	106	152	108	94	81	85
GLY	258	196	229	279	516	522
HIS	2	11	19	12	28	39
ARG	59	11	42	36	4	3
THR	51	62	46	39	9	0
ALA	58	29	53	47	5	0
PRO	79	65	72	69	25	23
TYR	86	177	110	115	204	218
VAL	25	6	24	24	3	9
MET	2	10	8	13	1	1
ILE	14	5	22	22	6	3
LEU	15	6	24	24	7	3
PHE	10	5	21	21	2	1
LYS	35	65	45	38	45	41

Solubility of these capsule proteins in GSB coordinately declined with time after secretion from the shell gland and this decreasing solubility directly correlated with increasing catechol contents. Greatest solubilization occurred with newly secreted, untanned specimens containing no catechol. Little solubilization occurred with tanning specimens containing highest catechol contents. These results together with previous analyses (Koob & Cox, J. mar. biol. Assoc. U.K. 70, 395-411, 1990) suggest that stabilization of the capsular proteins during tanning relies on the introduction of catechols and their subsequent oxidation to quinones by catechol oxidase.

The egg capsule of the little skate is composed of six major proteins ranging in apparent molecular weight from 95kDa to 20kDa. Although none of these proteins have an amino acid composition typical of interstitial collagens, they may be elasmobranch minor collagens or have collagenous domains. Further studies will be necessary in order to identify collagens, if present, in skate egg capsules. Nevertheless, our results clearly show that the little skate egg capsule contains proteins other than collagen that are important for capsule matrix formation and stabilization. The 23 and 20 kDa proteins that are unusually rich in tyrosine likely participate in the tanning mechanism by providing tyrosine residues that can be converted to catechols and subsequently oxidized to quinones. Since quinones form adducts with side chains of alanine, lysine, cysteine and methionine, it seems likely that amino acid-quinone condensation products readily form during the tanning process. If the reactive quinones are themselves side chains of structural proteins produced from tyrosine in the 23 and 20 kDa proteins, then quinone condensation would result in a highly crosslinked protein polymer. Whether this pathway or another operates during sclerotization of skate egg capsule is currently being explored.

These studies were funded by the investigators.