EGG CAPSULE PROTEINS FROM FOUR SPECIES OF NORTHWESTERN ATLANTIC SKATES

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Oviparity has evolved at least four times in cartilaginous fish (Dulvy and Reynolds, Proc. Royal Soc. Lond. B 264, 1309-1315, 1997). All chimaeroids are oviparous, species in six orders of sharks are oviparous, and all species in the order Rajiformes, the skates, are oviparous. Common to all cartilaginous fish is an oviducal or shell gland that produces materials that encapsulate fertilized eggs. The basic morphology of shell glands is remarkably similar in cartilaginous fish, although the relative size and complexity depends on the reproductive mode (Hamlett *et al.*, J. Exp. Zool. 282, 399-420, 1998). However, what comprises capsules in these diverse taxa is not known. In fact, capsule composition has been studied in only two species, *Leucoraja erinacea* (Koob and Cox, Environ. Biol. Fishes 38, 151-157, 1993) and *Scyliorhinus canicula* (Luong et al., Biochem. Biophys. Res. Comm. 250, 657-663, 1998). The present study was undertaken to compare the protein composition of egg capsules from four skate species.

Partially formed egg capsules were collected from *Leucoraja erinacea* (little skate), *Leucoraja ocellata* (winter skate), *Malacoraja senta* (smooth skate), and *Amblyraja radiata* (thorny skate). The recently secreted, untanned region of each capsule was solubilized in SDS/PAGE gel sample buffer containing β-mercaptoethanol (GSB). We have previously shown that this method completely solubilizes the untanned capsule proteins (Koob and Cox, Environ. Biol. Fishes 38, 151-157, 1993). The extracts were analyzed by electrophoresis on 4-20% linear gradient SDS/PAGE gels. In order to determine whether the mechanism responsible for capsule sclerotization involving catechol based polymerization similar in *Leucoraja erinacea* (Koob and Cox, *op.cit.*) occurs in other species, tyrosine and Dopa contents in specimens from a partially formed *M. senta* capsule were measured by amino acid analysis. Changes in tyrosine and Dopa contents were correlated with the extent of capsule polymerization by assessing solubility of the capsule proteins in GSB from specimens adjacent to those in which tyrosine and Dopa were measured.

Freshly oviposited egg capsules of the four species differ in size, form and color (Fig. 1). *L. erinacea* capsules are brownish green with golden hues. *L. ocellata* capsules are deep green. Both *M. senta* and *A. radiata* are dark brown. Differences in color indicate distinct chemistries associated with composition or the polymerization mechanism.

Egg capsules of the four species are composed of a similar array of structural proteins ranging in size from 50 to 5 kDa (Fig. 2). However, the apparent molecular weights and the relative amounts of the major proteins differ among the capsules. Common to three of the capsules (*L.erinacea*, *M. senta* and *A. radiata*) is a group of major proteins of relatively small size: a doublet at approximately 11 kDa and a smaller protein of lesser relative abundance. Other similarities include a 40 – 42 kDa protein present in all capsules and several proteins between 21 and 36 kDa. The protein composition of *L. ocellata* capsule differs significantly from that of the others. The major low molecular weight proteins (10 – 11 kDa) are absent or significantly larger (15 to 20 kDa).

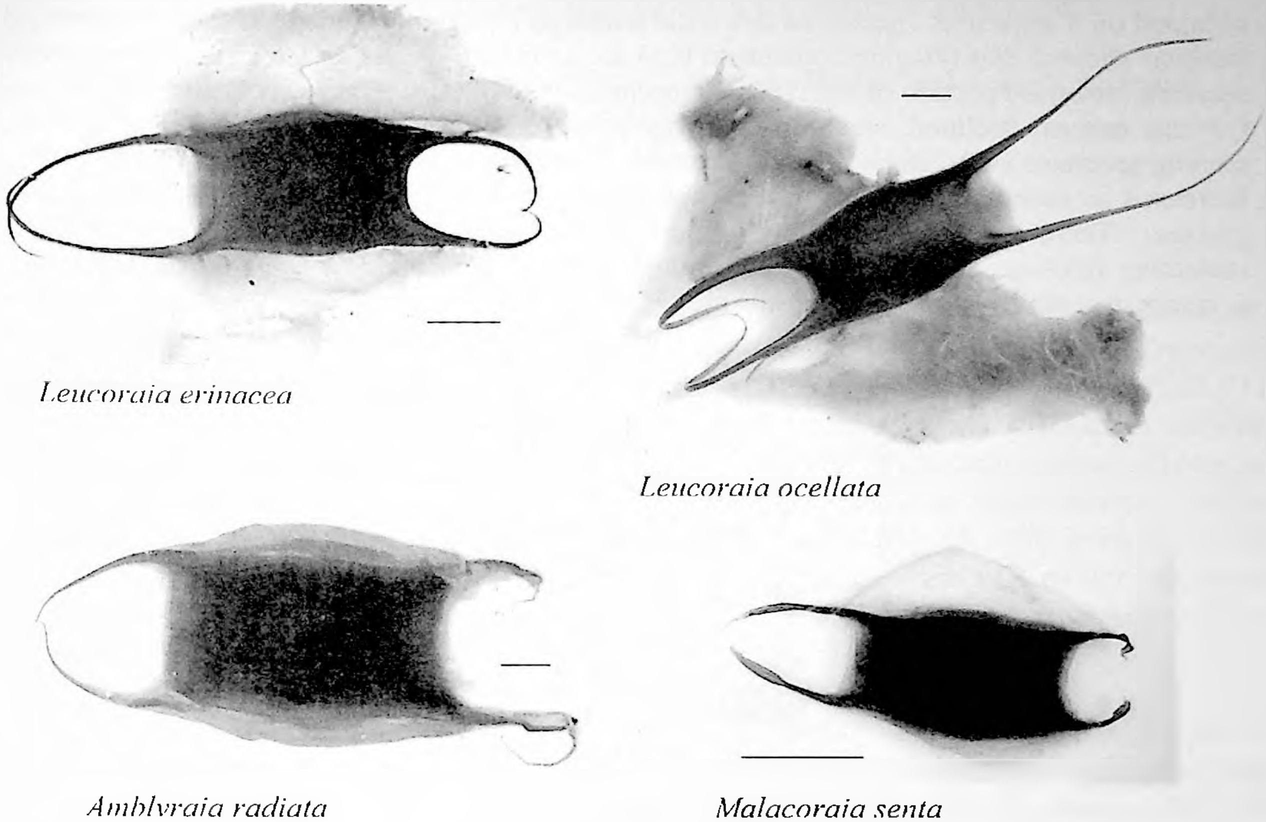

Figure 1. Egg capsules of the little skate (*Leucoraja erinacea*), winter skate (*Leucoraja ocellata*), thorny skate (*Amblyraja radiata*), and smooth skate (*Malacoraja senta*). The scale bar in each photograph represents 2 cm.

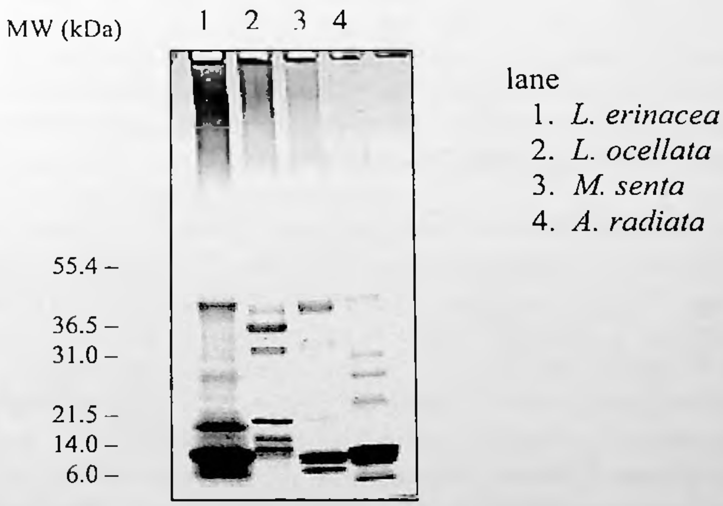

Figure 2. SDS/PAGE analysis of egg capsule proteins solubilized from newly secreted, untanned egg capsule removed from the lumen of the shell gland in four skate species.

To examine the polymerization mechanism in *M. senta*, amino acid compositions were obtained on 8 sequential specimens down the length of a partially formed capsule. Amino acid analysis showed that tyrosine contents at 0.54 moles/mole glycine were highest in the freshly secreted, untanned portion of the capsule (specimens 1 and 2; Fig. 3). In successive specimens, tyrosine content declined, reaching its lowest level (0.39 moles /mole glycine) in the oldest, tanning specimen (#8). Dopa was not detectable in untanned specimens 1 and 2. Dopa contents increased in successive specimens, reaching highest levels in specimen 8 (0.12 moles/mole glycine). There was a direct relationship between the loss in tyrosine and the increase in Dopa, indicating tyrosine was hydroxylated forming Dopa. The increase in Dopa correlated with a decrease in solubility of the egg capsule proteins (Fig. 3).

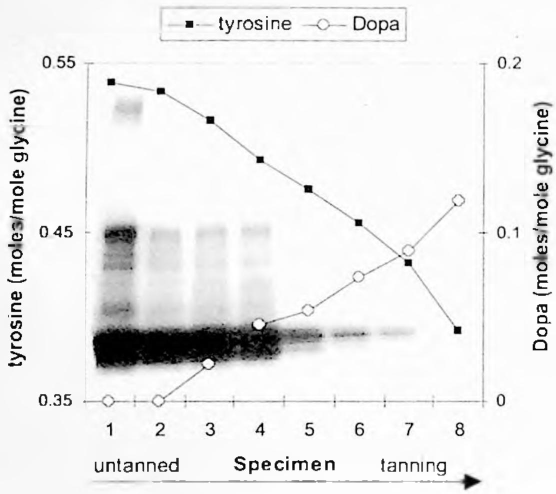

Figure 3. Tyrosine and Dopa contents in a partially formed egg capsule of *Malacoraja senta*. The dorsal wall of the capsule was sectioned into eight equivalent specimens from the newly secreted, white portion in the lumen of the shell gland (#1 specimen) to the oldest, tanning region (#8) in the uterus. Specimens were lyophilized, weighed, hydrolyzed in 6 N HCl at 108°C for 24 hr, dried *in vacuo*, and the amino acid composition determined. The amount of tyrosine and Dopa in each specimen was normalized to moles of glycine. The inset SDS/PAGE gel shows the relative solubility of the capsule proteins. Adjacent specimens of the capsule were placed directly in GSB containing β-mercaptoethanol, extracted for 24 hr, then electrophoresed on 4-20% SDS/PAGE and stained with Coomassie blue.

Further characterization of the egg capsule proteins in these species will be necessary to establish chemical and structural relationships. Nevertheless, these observations suggest similarities in several major structural proteins, particularly the small proteins which we have previously shown in *L. erinacea* capsules are unusual in that they are composed of 50% glycine and 20 –25% tyrosine. Differences in the protein composition of the capsules may reflect functional adaptation related to capsule properties. Current studies are underway to determine whether the mechanical properties, permeability, and durability in the marine environment differ. Despite the difference in protein composition, the polymerization chemistry appears similar in *L. erinacea* and *A. senta*, and therefore may be analogous in all skate egg capsules. Funded by the Shriners Hospitals for Children, project 8610.