TELEOST AND ELASMOBRANCH EYE LENS PROTEINS AND PIGMENTS

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Human senile cataract, the loss of transparency of the eye lens with aging, is due to either excessive light absorption or light scattering within the lens. Absorption arises from an increase in the pigmentation of the lens, making it yellowish or brown; scattering arises from increased aggregation and insolubilization of the highly concentrated proteins (crystallins) within lens cells.

We are now studying fish lenses as models to elucidate the molecular mechanisms of lens protein and pigment aging. Unlike most mammals, lenses of many fish also contain pigments. These absorb ultraviolet light either in the 320 nm or 360 nm wavelength ranges, or both (Kennedy and Milkman, Biol. Bull 111, 1956, 375-386; Bon et al, Exp. Eye Res. 7, 1968, 603-610). Several functions have been ascribed to the pigments in human fish lenses, such as elimination of chromatic aberration to provide sharper vision, and protection of the retina against photochemical damage. While some human pigment structures have been determined, the structures of fish pigments are unknown.

Lens size vs. lens weight

Fish have a hard, spherical eye lens. We measured lens weight and diameter of 66 lenses from dogfish (*Squalus acanthias*), cod (*Gadus morhua*) and haddock (*Gadus aeglefinus*), and found a universal linear relationship between weight (range 0.3-2.3 gram) and volume (0.25-2.05 cm^3):

$$\text{lens weight (gram)} = 1.19(\pm 0.01) \times \text{volume } (cm^3)$$

Thus, although there is a density and refractive index gradient from lens periphery to center, the overall density of the lens is found to remain constant at 1.19 ($\pm$0.01) g/cm^3 during growth.

Presence of UV-pigments in lenses of various fish

Whole lenses were extracted in either Na-phosphate buffer pH=7 (containing EDTA, dithiothreitol and sodium azide) or 96% ethanol. Ultraviolet absorption spectra of the buffer soluble fractions (Fig. 1) show that cod and dogfish have only the 360 nm pigment, alewife (*Alosa pseudoharengus*) has only the 320 nm pigment, and no or little pigment is extracted from whiting (*Gadus merlangus*), sole and skate (*Raja erinacea*). The 280 nm peak represents lens proteins extracted in buffer; no protein is extracted in 96% ethanol. The UV-pigments are both water- and ethanol-soluble. Purified pigment from cod has maxima at 260 nm and 360 nm, which is similar to the kynurenine derivatives found in human lenses.

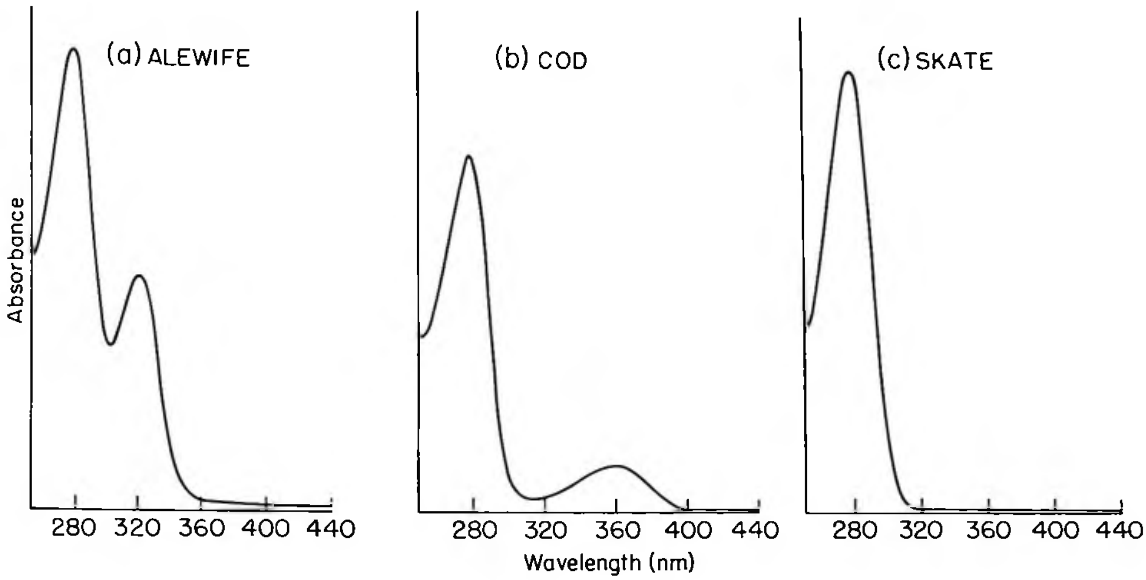
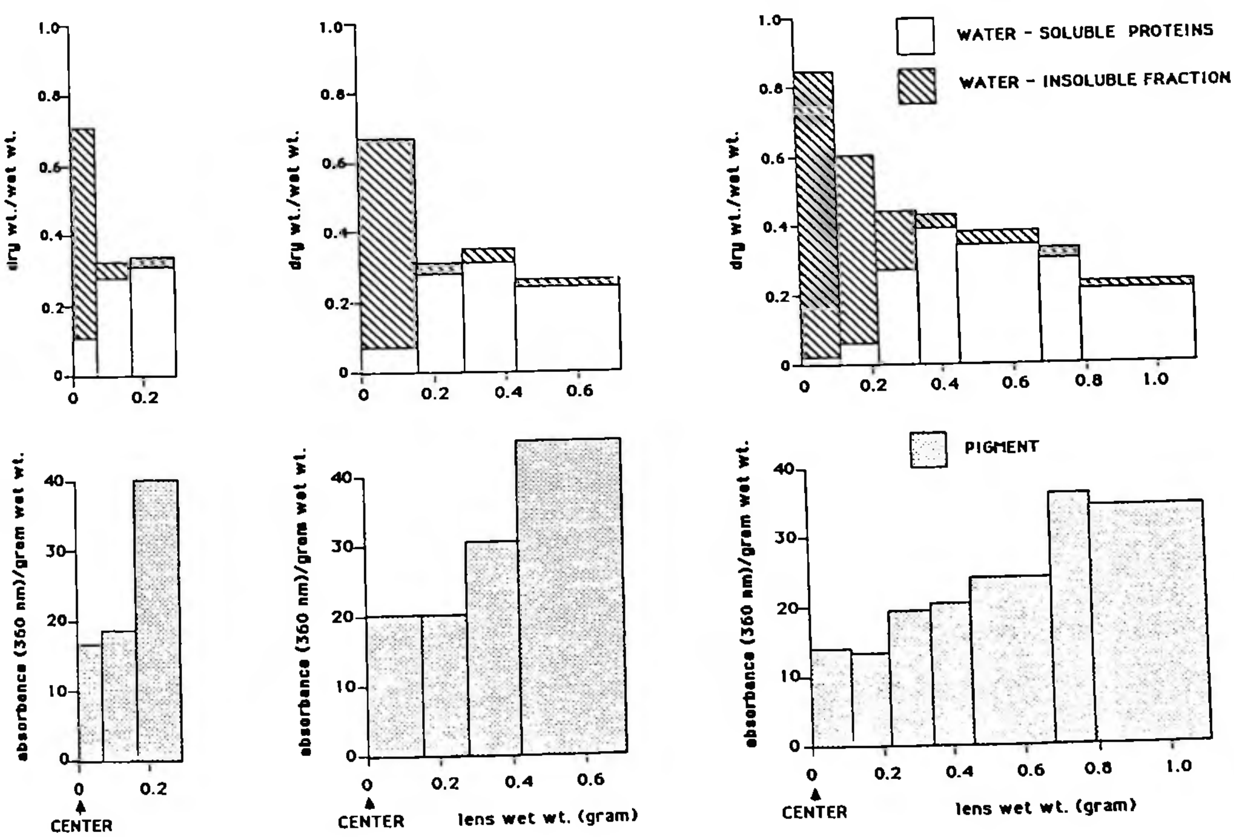


Fig. 1. Ultraviolet adsorption spectra of phosphate buffer extracts of eye lenses from (a) alewife, (b) cod, (c) skate.

Fig. 2. Distribution of 360 nm pigment (lower histograms), and dry weight fraction (upper histograms), in concentric layers of cod lenses of 0.29 g (left), 0.71 g (middle) and 1.11 g (right) wet weight.



Spatial and temporal distribution of protein and pigment in cod lenses

Three cod lenses of widely different age (weights 0.29, 0.71 and 1.11 gram) were extracted in Na-phosphate/NaCl buffer pH = 7.0 ionic strength 0.5. Concentric layers of the lenses were extracted. Figure 2 illustrates the distribution of 360 nm pigment, soluble protein and insoluble material in these layers.

In each lens, the dry wt./wet wt. ratio increases from lens periphery to center, producing the refractive index gradient. This ratio ranges from about 0.2 - 0.8, but averages 0.40 (±0.02) in each lens irrespective of age. At the same time, the soluble/insoluble ratio diminishes towards the center, more so in the old lens than in the younger one. The protein composition in these layers will be analyzed by HPLC and SDS-polyacrylamide electrophoresis. Pigment is found throughout the lenses. Its concentration appears to decrease towards the center; little age variation is observed.

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