OCCURRENCE OF A RESILIN-LIKE PROTEIN IN THE LENS CUTICLE OF THE DRAGONFLY MESOGOMPHUS LINEATUS (SELYS) (ANISOPTERA: GOMPHIDAE)

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The occurrence of a protein similar to resilin in the lens cuticle of adult *M. lineatus* is recorded for the first time.

INTRODUCTION

The rubber-like elastomer protein, resilin, was discovered in the ligaments of the flight system of the dragonfly Anax by WEIS-FOGH (1960). Since then many workers have recorded resilin from several species of all the different classes of Arthropoda (NEVILLE, 1963; SANNASI, 1970; SUNDARA RAJULU & GOVINDA RAJAN, 1974). This paper describes the first record of the occurrence of a protein similar to resilin in the lens cuticle of a dragonfly Mesogomphus lineatus.

MATERIAL AND METHODS

Adult M. lineatus, available in and around Erode, Tamil Nadu, India were collected and fixed (VARADARAJ, 1978). The compound eyes were dissected out and left in fresh fixative for 48 hrs. Three to five micrometre sections of the eyes were prepared by double embedding in celloidin and wax. The toluidine-blue/light-green stain at different pH was employed for staining sections following ANDERSEN & WEIS-FOGH (1964). Unstained and stained sections were examined with a light microscope. Fluorescence in the cuticle was observed in a microscope equipped with a high pressure mercury lamp and an excitation UV-filter (360 µm wave length). For barrier filters in fluorescent examinations, filters GG9/OGI were fixed to the binocular tubes.

For chromatographic analyses of the amino acids, the lens cuticles from about 300 dragonflies were obtained, hydrolyzed with 6N HCl and analyzed by paper chromatography according to the

method of BAILEY & WEIS-FOGH (1961). The amino acids in the chromatogram were visualized by exposing to ultraviolet light and identified by their Rf. values and also by comparison with authentic samples of amino acids run on chromatograms under identical conditions.

RESULTS AND DISCUSSION

Examinations of unstained sections under a light microscope revealed that the lens cuticle is colourless and highly transparent. It is composed of an outer epicuticle and an inner procuticle. The procuticle comprises an outer colourless hyaline exocuticle (corneal lens), the inner border of which is elongated into a number of conical processes and corresponds to the mesocuticle of other insects with regards to staining and histochemical reactions. The structural protein, resilin is selectively coloured sapphire blue with toluidine-blue/light-green at pH 4-7 and swells in organic solvents (ANDERSEN & WEIS-FOGH, 1964). With the toluidine-blue/light-green dye the lens cuticle stained deep sapphire blue at pH 4-7 and the material swelled considerably in cupric ethylene-diamine, formamide, formic acid, lithium thiocyanate and phenol.

Chromatographic analyses for amino acids of the lens cuticle revealed the presence of alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine and valine. In addition, they showed the presence of two amino acids with Rf. values 0.05 and 0.18. The fluorescence of these two spots increased when the chromatograms were exposed to ammonia vapour, whereas vapour from hydrocholic acid suppressed the fluorescence completely. These features are said to be characteristic of dityrosine and trityrosine. The presence of di- and trityrosine is the most diagnostic feature of resilin (BAILEY & WEIS-FOGH, 1961).

Examination of the unstained sections with a fluorescence microscope showed that the entire cuticle fluoresced vividly blue. The fluorescence disappeared after treatment with N- bromosuccinamide. A similar result was observed after incubation of the material with pepsin. These results indicate unequivocal evidence for the presence of resilin in the eye lens of *M. lineatus*.

Since resilin does not flow nor suffer any permanent deformation, THURM (1963) and ANDERSEN & WEIS-FOGH (1964) have suggested that resilin would be an absolute frame of reference for a mechanical sense organ. Resilin is remarkably stable and no major changes take place. Its physiological properties are very much unchangeable. Since the lens cuticle, which is transparent, is also mechanically resistant, and resilin has been reported to occur in some nonelastic regions (RAVEN, et al., 1971), it is reasonable to presume that the hardening of the cuticle in the photoreceptors forming the lens may involve cross linking by diand trityrosine, which are characteristically autofluorescent. However, the hyaline transparency of the lens cuticle may not only be due to the occurrence of resilin but also to other factors such as absence of phenolically tanned protein or

melanin pigments which normally owe their amber or black colour to the cuticle. Moreover, the characteristic amino acids di- and trityrosine of resilin are uniquely autofluorescent.

In view of the above it is likely that the lens cuticle, which is patched with resilin with the property of autofluorescence, may aid in absorbing and filtering the suitable light rays of the required spectral range and thereby allow the harmless light rays into the interior of the compound eyes to be utilized for subsequent photoactivation and perception of objects by the dragonfly.

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REFERENCES

- ANDERSEN, S.O. & T. WEIS-FOGH, 1964. Resilin-A rubber-like protein in arthropod cuticle.

 Adv. Insect Physiol. 2: 1-66.
- BAILEY, K. & T. WEIS-FOGH, 1961. Amino acid composition of a new rubber-like protein-resilin. *Biochem. biophys. Acta* 48: 452-459.
- NEVILLE, A.C., 1963. Growth and deposition of resilin and chitin in locust rubber-like cuticle. J. Insect Physiol. 9: 265-278.
- RAVEN, D.J., C. EARLAND & M. LITTLE, 1971. Occurrence of dityrosine in Tussah silk fibroin and keratin. *Biochem. biophys. Acta* 251: 96-99.
- SANNASI, A., 1970. Resilin in the lens cuticle of the firefly Photinus pyralis Linnaeus. *Experientia* 26: 154-155.
- SUNDARA RAJULU, G. & S. GOVINDARAJAN, 1974. Presence of resilin in a scorpion Palamnaeus swammerdami and its role in the food capturing and sound-producing mechanisms. Experientia 30: 908
- THURM, U., 1963. Die Beziehungen zwischen mechanischen Reizgrössen und stationären Erregungszustanden bei Borstenfeldsensillen von Bienen. Z. vergl. Physiol. 46: 351-382.
- VARADARAJ, G., 1978. Studies on the haemolymph and cuticle of a dragonfly Anax immaculifrons (Anisoptera: Aeshnidae). Ph.D. thesis, Univ. Madras.
- WEIS-FOGH, T., 1960. A rubber-like protein in insect cuticle. J. exp. Biol. 37: 889-907.