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Secondary structure of chorion proteins of the Lepidoptera *Pericallia ricini* and *Ariadne merione* by ATR FT-IR and micro-Raman spectroscopy

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ABSTRACT

The gross morphological features of the eggs and eggshells (chorions) of two Lepidoptera species, *Pericallia ricini* and *Ariadne merione* were revealed for the first time by scanning and transmission electron microscopy. These two insect pests are extremely serious threats for many crops, mainly in India, but also in several other regions of the world. Micro-Raman and ATR FT-IR spectroscopy were also applied to study in detail the secondary structure of the eggshell (chorion) proteins of these Lepidoptera species. Both techniques indicate that the two species have nearly identical conformations of their chorion proteins with abundant antiparallel β-pleated sheet. These results are in support of our previous findings that the helicoidal architecture of the proteinaceous chorion of Lepidoptera and fishes is dictated by a common molecular denominator, the antiparallel β-pleated sheet secondary structure.

1. Introduction

Pericallia ricini (Arctiidae), a.k.a. Castor wooly bear, is a polyphagous pest with a wide range of hosts in India and many parts of the world. Its eggs are laid in clusters on the leaves of various plants (Castor, Sunflower, Sesame, Brinjal, Pumpkin, Banana, Sweet potato, Radish, etc.) and its larvae feed on them causing complete defoliation [1]. *Ariadne merione* (Nymphalidae), a.k.a. Common Castor butterfly, is an orange butterfly flying among the foliage of Castor plants (*Ricinus communis*) and its larvae feed almost exclusively on their leaves [2]. Both, *P. ricini* and *A. merione* have proved to be extremely serious threats for many crops in many regions of the world, including India [1,2].

Besides the economic impact of these insect species, there are several other reasons for the systematic structural study of their eggs and eggshells: the morphological study could provide new and useful information for taxonomic and phylogenetic purposes, as well as about their physiology and/or demography. Also, useful clues for insect pest control could be anticipated if detailed studies at the eggshell (chorion) protein secondary structure level are provided [3].

In this work, the overall morphology of laid eggs and eggshells from both species was studied by scanning and transmission electron microscopy. In addition, detailed ATR FT-IR and micro-Raman structural studies of the eggshell (chorion) proteins were performed, to provide information about the secondary structure and composition of their eggshell (chorion) constituent proteins.

2. Materials and methods

2.1. Sample preparation

All the eggs of *P. ricini* and *A. merione* examined in this study were obtained from cultures set up from 2nd and 3rd instar larvae trapped from castor plants at the vicinity of the Zoology Department, Allahabad University, India, and reared in the laboratory on castor leaves in troughs placed in an incubator at 28 ± 1 °C. The eggs were allowed to pupate and emerge. Freshly emerged adults were shifted to glass chimneys whose top was covered with thin cotton cloth impregnated with a 5% sucrose solution for feeding. Each chimney contained a single pair of insects and fresh castor leaves with stem for egg laying. After copulation, the females laid eggs in clusters on leaf lamina, initially by scrapping epidermal layers

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and later by cutting the blades. Eggs were easily detached from the leaves with the help of a brush or a needle, fixed in Karnovsky's fixative [4] overnight at 4 °C for electron microscopy measurements and then shifted in a 0.1 M phosphate buffer kept at 4 °C.

For IR and Raman spectroscopic measurements, eggs were cut in half with fine needles and forceps and washed thoroughly several times with distilled water and sonicated to remove the oocyte and/or other remnants. The remaining, 'cleaned' eggshells (chorions) were then thoroughly dried in air.

2.2. Scanning electron microscopy

The eggs kept in 0.1 M phosphate buffer were transferred to alcohol and dehydrated in a graded alcohol series up to absolute alcohol and dried in a critical point drier apparatus (Polaron E 3000) at 20 °C using liquid CO_2 as a transitional fluid. Further, the eggs were mounted on copper stubs, coated with 35 nm gold in a sputter coater (Balzers SCD 020 Sputter Device) and viewed in a LEO 435 VP [UK] scanning electron microscope, operating at 15 kV.

2.3. Transmission electron microscopy

The eggs from 0.1 M phosphate buffer were transferred and dehydrated in a graded acetone series, infiltrated and embedded in araldite CY 212. Thin sections were cut by using glass knives in an ultramicrotome and mounted on 300 mesh copper grids. The sections were then stained by uranyl acetate (1%) and lead acetate (1%) and viewed under a Morgagni 268D transmission electron microscope (Fei Company, The Netherlands), operating at 80 kV. Digital images were acquired using a CCD camera (Megaview III, Fei Company, The Netherlands) and the software supplied with the microscope (Soft Imaging System, Munster, Germany).

2.4. Micro-Raman

Micro-Raman spectra were recorded on a dispersive confocal Raman microscope (Renishaw InVia Reflex, 1200 l/mm). Data were collected through a 50× lens using the 785 nm diode laser line for excitation. Several spectra were collected per sample and averaged. The total acquisition time was of the order of 1 h per sample.

2.5. Attenuated total reflectance infrared (ATR FT-IR) spectroscopy

Infrared spectra were recorded on a Fourier transform instrument (Equinox 55, Bruker Optics) equipped with a single-reflection diamond Attenuated Total Reflectance (ATR) accessory (DuraSamplIR II by SensIR). Both the spectrometer and the ATR accessory were purged by dry N_2 to reduce the effect of H_2O vapors on the spectra. Clusters of chorions were held in contact with the diamond element by means of a suitable press. With a penetration depth of the order of a few μm, ATR provides spectra free of optical saturation effects. Several spectra were measured, each representing the average of 100 scans at a resolution of 4 cm^{-1}. The measurement of each sample was bracketed between two background measurements to allow for the complete compensation of H_2O vapor. Average spectra are shown in the ATR absorption formalism, i.e., after correction for the wavelength-dependence of the penetration depth ($d_p \propto \lambda$).

2.6. Post-run computations of the spectra

In order to enhance the resolution of sharp features overlapping with broad bands, ATR FT-IR absorption band maxima were determined from the minima in the second derivative of the corresponding spectra. Derivatives were computed analytically by the Savitsky–Golay algorithm [5] using routines of the OPUS software (Bruker Optics) and included a 13-point smoothing. Given the zero-filling factor employed for the Fourier transform of the data, this smoothing range corresponds to ±12 cm^{-1}. Smoothing over narrower ranges resulted to a deterioration of the *S*/*N* ratio and did not increase the number of minima that could be determined with confidence.

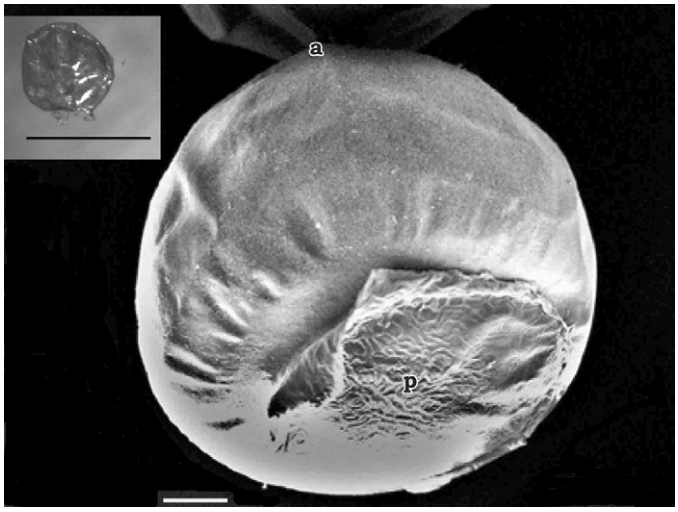

Fig. 1. Scanning electron micrograph of a laid egg of *Pericallia ricini*. The eggshell surface structure showing the anterior pole (a) and the posterior pole (p) is seen. Bar is 100 μm. Inset, shows a photograph taken under a dissecting microscope (stereo-microscope) of a 'cleaned' eggshell of *P. ricini*, used for IR and Raman measurements (see Section 2). Bar is 1 mm.

3. Results

3.1. Eggshell (chorion) morphology

The follicular cells surrounding the oocyte synthesize and secrete the proteins forming the eggshell (chorion) ([6] and Refs. therein). Fig. 1 is a scanning electron micrograph of an egg of *P. ricini*. The eggs have a spherical shape with a diameter ca. 720 μm. Fig. 1, inset, shows a photograph taken under a dissecting microscope (stereo-microscope) of a 'cleaned' eggshell of *P. ricini*, used for IR and Raman measurements (see Section 2). Fig. 2 shows a transmission electron micrograph taken from a thin, transverse (perpendicular to the surface of chorion) section through the chorion of *P. ricini*. The lamellar ultrastructure of chorion suggests a helicoidal architecture [3,6–8]. A similar lamellar ultrastructure is seen for the chorions of *A. merione* (data not shown).

Fig. 3 is a scanning electron micrograph of a laid egg of *A. merione*. The eggs seem to have a spherical shape, ca. 600 μm in diameter. The anterior pole of chorion contains the micropyle (M, white arrow) through which sperm entry occurs [9]. Respiratory appendages or respiratory filaments [9] (RF, white arrow), ca. 200–300 μm in length, protrude away from the surface of chorion, reminiscent of the spines (quills) of a porcupine or the spines of a sea urchin. The respiratory functions are facilitated by these long appendages arising from egg chorion as extrachorionic tubular projections [9]. Gravid females lay eggs in substrates and these respiratory appendages or filaments extend above the substrate surface thereby facilitating oxygen supply to the embryo and at the same time expulsion of carbon dioxide. The large numbers of appendages are there to meet the high oxygen requirements of the developing embryo/oocyte and to provide simultaneously protection from predators. Fig. 2, inset, shows a photograph taken under