A COMPARATIVE STUDY OF EGGSHELL PROTEINS IN LEPIDOPTERA

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Summary

As a first step in the study of chorion composition, biochemical development and morphogenesis, we have studied the proteins of moth chorions (eggshells). We draw attention to the extensive similarities of these proteins in a variety of species. We also report that the eggshell proteins are deposited in succession, each with its characteristic time table. This phenomenon may be related to the morphogenesis of chorion.

The shell or chorion of insect eggs is a structure of unusual interest. In terrestrial species, it has to meet the dual, apparently conflicting requirements of preventing the desiccation of the embryo and permitting gaseous exchange. Since it may serve as a barrier to penetration of ovicides, including third generation pesticides (1), its study could have considerable practical importance.

In saturniid moths, the chorion is a massive structure, approximately 33% of the dry weight of the laid eggs (2). The chorion is synthesized by a single layer of follicular epithelial cells surrounding the oocyte, and is deposited extracellularly between follicular cells and oocyte; in the silkmoths *Hyalophora cecropia* and *Antheraea polyphemus* the secretory period lasts approximately two days (2,3). Electron microscopic studies reveal that the silkmoth chorion is intricately constructed (4). The layer deposited first (i.e. closest to the oocyte) is a porous "collonade," whereas the remaining 90-95% consists of lamellae of helicoidal microfibrils (4,5). The lamellar chorion is further differentiated in time and space. For example, characteristic air spaces appear (4,6), and the microfibrils are obscured late in the secretory phase perhaps because of permeation by a matrix material (5). Moth eggshells consist almost exclusively of protein (4,7). For an understanding of chorion structure and development, parallel morphological and biochemical studies are needed. In another publication (3) we report studies on characterization and biosynthesis of the Polyphemus chorion. [We have adopted the common usage of the specific names as trivial names for the saturniid silkmoths.] Here we compare the pattern and development of chorion proteins in a variety of moth species.

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Chorion proteins are unusually small and also quite insoluble, presumably because of their high content of glycine and other hydrophobic amino acids and because of extensive cross-linking by disulfide bonds (7). The proteins (Fig. 1) can be analyzed conveniently by SDS-acrylamide electrophoresis on highly cross-linked gels, following dissolution with mercaptoethanol and urea and carboxamidomethylation with iodoacetamide (3). Chorions are obtained either from follicles dissected from the animal or from laid eggs. Dissected follicles are washed in Weevers' saline (8) and then in distilled water (either with or without 10^{-3} M phenylmethylsulfonylfluoride in 7% n-propanol). The follicles are cut in half with fine scissors and the oocyte washed away; the follicular epithelium separates from the chorion in distilled water and is removed by agitation or, in young follicles, by dissection. Laid hatched eggs are crushed in a mortar, transferred to a test tube, Vortex agitated with 5 changes of 0.05 M Tris-HCl, pH 7.0 (5 ml each) and 5 changes of distilled water (5 ml each), and then lyophilized. Chorions are dissolved in sample buffer and S-carboxamidomethylated (see Legend to Fig. 1) prior to electrophoresis. Optimal results are obtained with 10 to 30 μg of protein per gel, i.e. about one-half (*Bombyx* and *Manduca*) to one-thirtieth (e.g. Mylitta) of a mature chorion.

Probably because of the low molecular weight of the proteins and their tendency to interact, the electrophoretic conditions are crucial for resolution. With optimal resolution, 15 to 20 bands are apparent in mature chorion preparations. Two or more are located near the top of the gel, and may be derived from the vitelline membrane (3). The remaining bands fall within three major groups, named A, B, and C in order of increasing molecular weight. For convenience, individual bands to be discussed within each group are numbered with Roman numerals (Fig. 1). This terminology is provisional, since it omits a number of bands. By reference to the top gel in Fig. 1 (Pernyi), it can be seen that group A consists of four bands, two of them prominent (AI and AII). Group B begins with a faint band (BI), and includes the two most prominent chorion proteins, BII and BIII. BIV is a diffuse double band, sometimes separated into its two components (e.g.see Mylitta and *Bombyx* in Fig. 1). An additional, unnumbered component can often be resolved between BII and BIII. Group C consists of a number of minor bands, prominent among which is CIII. By reference to protein molecular weight standards, it has been determined that the apparent MW is 7,000 to 8,000 for AI, 10,000 to 12,000 for BII and BIII, and 18,000 for CIII (3).

Chorion protein profiles (Fig. 1) are quite constant within the superfamily Bombycoidea, including the families Bombycidae (*Bombyx mori*) and Saturniidae

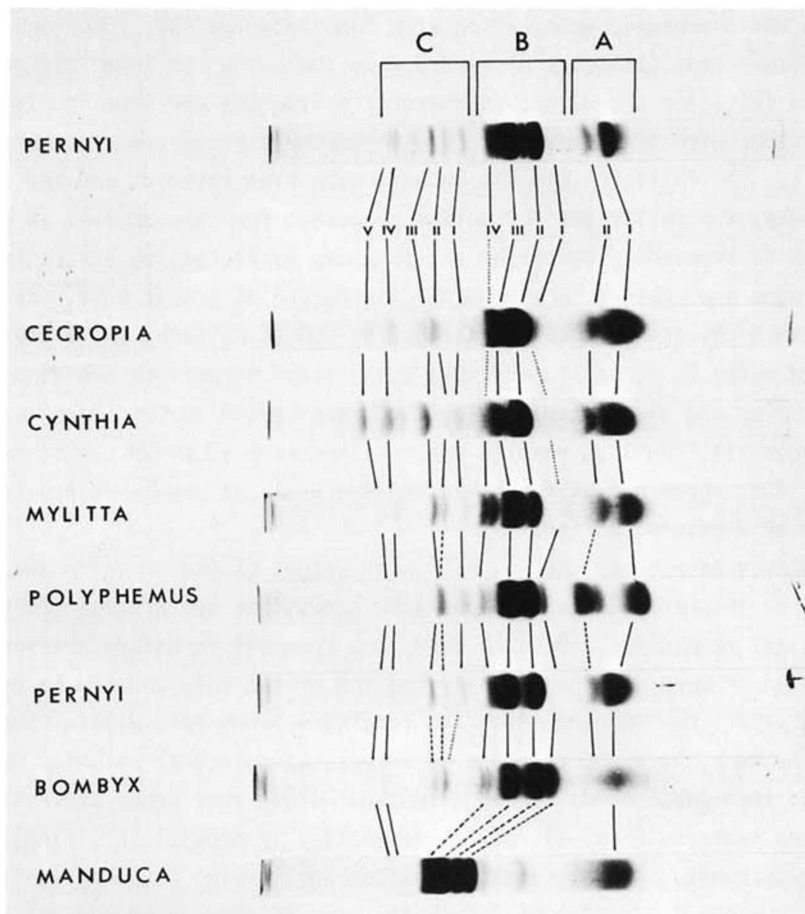

Fig. 1. Electropherograms of moth eggshell proteins. Chorions of laid hatched eggs (Cecropia and Cynthia) or mature ovarian follicles were dissolved by heating at 100°C for 10 minutes in sample buffer (6 M urea, 1% SDS, 1% mercaptoethanol and 0.01 M Tris-HCl, pH 8.4),carboxamidomethylated with 0.33 M iodoacetamide and finally made 1% in mercaptoethanol (excess over iodoacetamide). Similar profiles were obtained when chorions were dissolved at 4°C,room temperature or 65°C (3). Electrophoresis was performed on 9 to 9.5 cm gels containing 13% acrylamide, 1% N, N'-methylenebisacrylamide, 6 M urea and 0.5% SDS. Gels were stained with Coomassie brilliant blue. Confident protein identifications are indicated by ———, whereas - - - -shows tentative identifications, and ...... the hypothetical location of components which are not visible. The origin is on the left; the markings on the opposite end are codes for identification of the gels.

(Antheraea pernyi, A. mylitta, A. or Telea polyphemus, Hyalophora cecropia and Samia cynthia). The pattern of A proteins is uniform, except for Polyphemus, in which AI and AII seem widely separated and the two fainter bands have intermediate mobility. In group B, BI has variable mobility as well as intensity; it is most abundant in Polyphemus and Bombyx. BII and BIII are quite uniform in mobility, but their relative abundance varies in different species. These two proteins are of approximately equal abundance in Pernyi, Polyphemus, and Bombyx; BIII is relatively more abundant in Mylitta, but less abundant in Cynthia and Cecropia. The BIV complex is variable in intensity and mobility; it is not seen in Cecropia, possibly because it overlaps with BIII. In group C, CI is either preceded by a faint band (Pernyi) or is itself double (Polyphemus, where two different hues are apparent in the original gel). CII is variable in mobility and intensity, and in Polyphemus apparently joins with CIII into a broad unresolved band. CIII is quite constant in migration and intensity. CIV and CV are consistent landmarks, most abundant in Cynthia. Two faint unequal bands are present between CIII and CIV.

At first sight, the profile of the tobacco hornworm, Manduca sexta (superfamily Sphingoidea, family Sphingidae) appears quite different from the bombycoid profile. Nevertheless, similarities are apparent in A proteins and in CIV and CV. Moreover, the most intense bands are reminiscent of bombycoid group B proteins; the difference may be primarily in molecular weight. However, it is also possible that the major bands in Manduca are C proteins; perhaps different structural elements are emphasized in the two types of chorion. It should be emphasized that our identification of proteins depends on a subjective judgment of relative intensities and mobilities; in some cases staining hue is useful for confirmation. Definitive phylogenetic comparisons can only be made after the proteins are purified and characterized further.

Moth ovarioles consist of a string of follicles in a timed sequence of progressively older developmental stages (2). In Polyphemus and Pernyi, a developmental landmark (3) is the abrupt increase in size as the last vitellogenic follicle (follicle 0) becomes hydrated (follicle 1); older follicles can be staged relative to this transition. In both species, dissectable chorion is first obtained from follicle 3. Figure 2 shows the proteins of progressively older chorions in both species. It is apparent that each chorion protein has a characteristic pattern of accumulation. In general, C proteins accumulate early during chorion production, although in Pernyi the highest molecular weight proteins, including CIV and CV, accumulate late. Accumulation of A proteins occurs throughout, and AI accumulates primarily towards the end of chorion formation. Group B proteins are very minor in the first dissectable

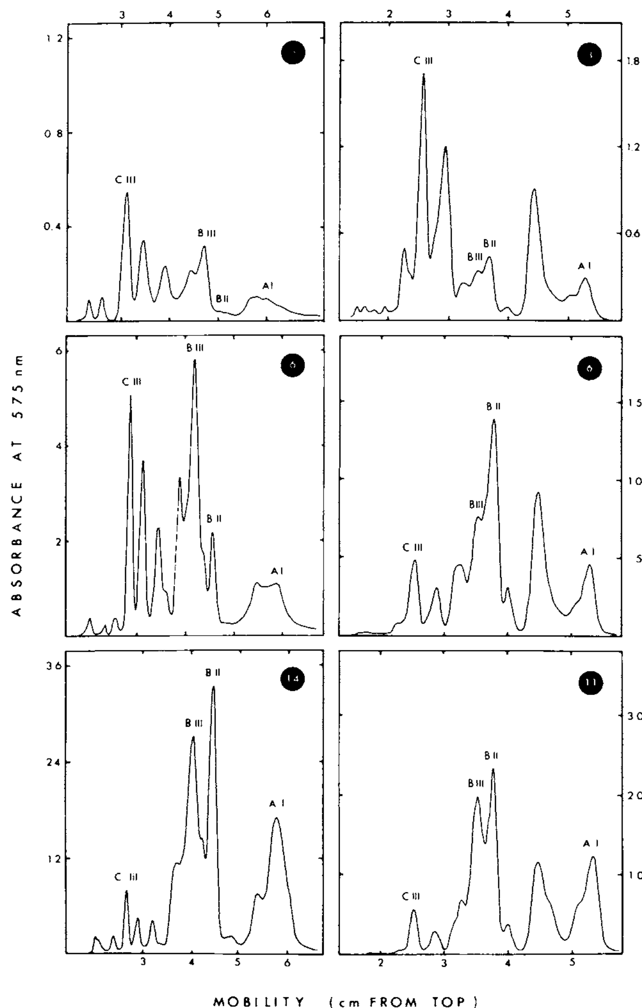

Fig. 2. Gilford scans of electrophoretically analyzed proteins from two developmental series of chorions (left, Pernyi; right, Polyphemus). The stage of the ovarian follicle from which each chorion was obtained is indicated by a number on the upper right-hand corner of the scan. After dissolution of the entire chorion, carboxamidomethylated aliquots containing 10 to 20 μg of protein (1/2 to 1/30 of each chorion) were electrophoresed as described in Fig. 1 on 8 cm (Polyphemus) or 9 cm (Pernyi) gels. The gels were stained with Coomassie brilliant blue and scanned at 575 nm. The absorbance scale in each case was adjusted to indicate the absorbance of a whole chorion equivalent. Chorion 3 is the first dissectable chorion, and chorion 11 or 14, respectively, is nearly mature in terms of dry weight.

chorions (and, in the earliest specimens obtained, absent), but become predominant as chorion formation proceeds. In Pernyi, BIII is the dominant B component in early chorions, and BII becomes prominent only later; in Polyphemus this developmental sequence is reversed.

We have shown that chorion proteins are not interconverted; each is synthesized and secreted as a distinct component (3). Thus, it is clear that production and subsequent deposition of specific chorion proteins is a developmentally controlled process. This process is undoubtedly related to the morphogenesis of chorion and is also of major interest for studies on control of protein biosynthesis during development.

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