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CHARACTERIZATION OF YOLK PROTEINS FROM THE EGGS OF THE INDIAN MEAL MOTH, *PLODIA INTERPUNCTELLA**

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Abstract—The eggs of the Indian meal moth, *Plodia interpunctella* (Hübner), contain four major yolk polypeptides (YPs). The four YPs were associated as two proteins that lacked immunocrossreactivity either as native proteins or as individual polypeptide subunits. Vitellin was found to be a glycolipoprotein composed of YP1 (153 kDa) and YP3 (43 kDa) and had an apparent molecular mass ranging from 398 to 475 kDa as established by various methods. The other major yolk protein was composed of glycosylated polypeptides YP2 and YP4 that were produced by ovarian tissues. Two forms of YP2/YP4 were observed under native conditions that had molecular masses of 93 and 235 kDa in pore-limited gel electrophoresis. The heterogeneity of the protein may be due to proteolytic cleavage of YP2 by endogenous proteases, since polypeptides of 50 and 60 kDa appeared in solutions containing partially purified YP2 YP4, and these smaller polypeptides were shown to be related to YP2 by peptide mapping. Thus, the yolk of *P. interpunctella* was found to contain two major yolk proteins; vitellin was identified as well as a large multiple subunit protein produced within the ovary that was unlike any previously described yolk protein from moths.

Key Word Index: Indian meal moth, *Plodia interpunctella*, vitellin, egg proteins, yolk proteins

INTRODUCTION

The production of yolk in lepidopterans requires the synthesis of proteins in both the fat body and the ovaries (Ono *et al.*, 1975). For the species examined, the fat body produces a vitellogenin that is packaged in the oocytes as vitellin. Vitellin has a native molecular mass ranging from 440 to 500 kDa and consists of large (180–230 kDa) and small (45–55 kDa) polypeptide subunits (*cf.* Kunkel and Nordin, 1985; Osir *et al.*, 1986). In addition to vitellin, a small polypeptide of approx. 30 kDa, designated microvitellin, has been identified in the hemolymph and oocytes of *H. cecropia* (Telfer *et al.*, 1981) and *M. sexta* (Kawooya and Law, 1983). Further characterization of vitellins has shown these proteins to be glycolipoproteins that undergo little post-translational modification during uptake and deposition in the yolk granules (Izumi *et al.*, 1980; Telfer *et al.*, 1981).

The yolk proteins produced within the ovaries have shown more variability in subunit composition between species than have the vitellins. Egg specific protein (ESP) in *B. mori* was described originally as a glycolipoprotein consisting of two subunits of a 55 kDa polypeptide (Ono *et al.*, 1975; Irie and Yamashita, 1983). Further characterization of ESP showed that the 55 kDa polypeptide originated from a phosphorylated 72 kDa polypeptide that formed a trimer (Takahashi, 1987, Zhu *et al.*, 1986). Paravitellin in *H. cecropia* had a molecular mass of 70 kDa and remained as a monomer during purification (Telfer *et al.*, 1981).

The proteinaceous yolk from the Indian meal moth, *Plodia interpunctella* (Hübner), consists of four major yolk polypeptides (YPs) when resolved by denaturing gel electrophoresis (Shirk *et al.*, 1984). The YPs were designated YP1, YP2, YP3, and YP4 in descending order of molecular mass. YP1 and YP3 were synthesized by the fat body and secreted into the hemolymph. In contrast, YP2 and YP4 were synthesized in the ovaries and were found only in the oocytes *in situ*. The evidence presented suggested that YP1 and YP3 were subunits of a 462 kDa protein analogous to vitellin described in other lepidopterans. However, YP2 and YP4 also appeared to associate as a protein since they remained associated as a 264 kDa protein after purification by ammonium sulfate precipitation, gel filtration, and ion exchange chromatography. This complex of YP2 and YP4 deserves special interest since it is a large, multiple subunit protein produced in the ovaries and is unlike any of the other described yolk proteins.

Before investigating the deposition and utilization of yolk during embryogenesis in *P. interpunctella*, we sought additional information on the relationships and structures of the yolk proteins and the four polypeptide subunits. In the following, we further describe the structures and subunit associations of the yolk proteins in their native states.

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RESULTS

Partial purification of yolk proteins

The yolk proteins were fractionated essentially as reported previously (Shirk *et al.*, 1984) except that the column chromatography separations were reversed and ion exchange was accomplished with a continuous salt gradient instead of a step gradient. This procedure produced final preparations of the yolk proteins that were purer as evidenced by silver staining (Fig. 1). Of the final concentrates prepared after ion exchange chromatography, the only significant protein contamination appeared in the combined fractions of YP2/YP4 (Fig. 1C lanes c and h); YP1/YP3 concentrates were relatively free of contamination at this level of detection (Fig. 1C lanes d and i). To determine the degree of purity of the YPs after ion exchange chromatography, the silver stained lanes in Fig. 1C were quantified by dentistometry. Since there is variability in the sensitivity of some proteins to silver stain, which can influence the proportion of the protein observed, we consider the observed percentages to be relative and not absolute values. YP1 represented 53% of the DEAE purified YP1/YP3 fraction and YP3 and 25% of the sample. Together, YP1 and YP3 were approx. 78% of the total protein in the sample. The purity of YP2/YP4 was not as great as the purity of YP1/YP3 since together YP2/YP4 were only 59% of the total protein in the purified fraction: YP2 represented 22% of the purified fraction and YP4 represented 37%. As a consequence of the ion exchange chromatography, a polypeptide of 86 kDa was brought to a reasonable level of purity as were the YPs (Fig. 1C, lanes e and j). However, the identity of the material in the other peaks appearing in the eluate from the ion exchange column was not determined.

Immunological properties of the YPs

The immunoprecipitation lines from partially purified YP1/YP3 and YP2/YP4 from ion exchange chromatography were compared with those from the proteins in an egg homogenate by radial double immunodiffusion against antiserum to total yolk (Shirk *et al.*, 1984). Two precipitation lines developed from the egg homogenate (Fig. 2A). The inner line was continuous with the precipitation line from YP1/YP3, and the outer line was continuous with the precipitation line from YP2/YP4. Where precipitation lines from YP1/YP3 and YP2/YP4 crossed, spurs were formed (identified by arrows in Fig. 2A) indicating there were unique antibodies to each protein. The presence of spurs demonstrated that there was no immunocrossreactivity between YP1/YP3 and YP2/YP4 protein species.

Antisera were raised against purified YP1, YP2, and YP3 as described in the Materials and Methods. Although material was prepared for YP4, the development of antisera to YP4 was never successful. Antiserum for each YP was reacted with yolk proteins electroblotted to nitrocellulose and a conjugated horseradish peroxidase color assay was used to identify the crossreacting material. Each of the antisera raised to a purified YP crossreacted specifically with only that YP (Fig. 2B): antiserum raised to YP1 reacted only with YP1, antiserum raised to YP2 reacted only with YP2, and antiserum raised to YP3 reacted only with YP3. This demonstrates that the YPs are antigenically distinct polypeptides. We conclude that YP4 also is antigenically distinct since none of the antisera for the other YPs showed any crossreactivity with YP4.

Determination of the molecular mass for the yolk proteins

From our previous S-300 Sepharcryl column chromatography, the molecular masses of YP1/YP3 and YP2/YP4 were estimated to be 462 and 264 kDa, respectively (Shirk *et al.*, 1984). The S-300 sephacryl column chromatography from this work showed the molecular mass of YP1/YP3 to be 456 kDa and YP2/YP4 to be 237 kDa (Table 1). To further characterize the two yolk proteins they were subjected to pore-limited gel electrophoresis and ultracentrifugation. In pore-limited electrophoresis, purified YP1/YP3 migrated corresponding to 475 kDa (Sd = 9.4; Table 1). Purified YP2/YP4 migrated as two bands in the pore-limiting electrophoresis. Under these conditions, the molecular mass of YP2/YP4 was estimated to be 235 kDa (Sd = 9.8) for the largest, and 93.4 kDa (Sd = 3.2) for the smallest. The appearance of two separate bands may be a function of the proteolytic cleavage of YP2 in the complex (see below) or the result of progressive association/dissociation of multimeric subunits.

When resolved by ultracentrifugation on sucrose gradients, YP1/YP3 migrated as a single peak and had a sedimentation coefficient of 13.7 S and an apparent molecular mass of 398 kDa (Table 1). YP2/YP4 had a sedimentation coefficient of 6.5 S with an apparent molecular mass of 98 kDa.

By following these isolation conditions, we also observed a protein that co-purified with YP2/YP4 and had a molecular mass of approx. 500 kDa. The protein was composed of YP2 and YP4 as well as two polypeptides of 15–25 kDa that were not related to either YP2 or YP4. Further characterization of this protein is being conducted.

Table 1. Molecular mass determination of yolk proteins from *Plodia interpunctella*

Yolk proteins	S-300 (kDa)	Pore-limited electrophoresis (kDa)	Sucrose gradient	
			Mass (kDa)	$S_{W/20}$
YP1/YP3 (vitellin)	456 (462)*	475	398	13.7
YP2/YP4 (large form)	237 (264)*	235	NP†	
YP2/YP4 (small form)	NP	93.4	98	6.5

*From Skirk *et al.* (1984).
†NP—not present.

Identification of YP2 proteolytic fragments

In many samples containing semipurified YP2/YP4, we found the appearance of several new polypeptide bands with molecular mass between 50 and 60 kDa that were not seen when the YP2/YP4 samples were denatured immediately. To test whether these new polypeptides were proteolytic cleavage fragments of YP2, bands containing a 50 and a 60 kDa polypeptide were excised from Coomassie Blue stained SDS-PAGE as were bands of YP2, and all were subjected to peptide mapping. After staining with silver, the peptide maps showed that the two smaller polypeptides shared nearly identical fragmenting patterns with YP2 (Fig. 3). From these digestion patterns, we conclude that these polypeptides are derived through specific proteolytic cleavage of YP2 by endogenous proteases.

Identification of attached moieties on the YPs

To determine whether the YPs were glycosylated, they were resolved by SDS-PAGE, electroblotted onto nitrocellulose, and then reacted with con-A conjugated to gold. YP1, YP2, and YP4 all bound with con-A (Fig. 4). The lack of crossreactivity of con-A with YP3 does not rule out the possibility of sugar moieties on YP3, since the appropriate sugar moieties may not be available on the surface of the polypeptide.

When stained for lipid moieties with Sudan Black, YP1/YP3 was identified as a lipoprotein, whereas YP2/YP4 did not stain (Fig. 5). When the gel was counter-stained with Coomassie Blue, YP2/YP4 was found at the appropriate position on the gel although it was not stained by the Sudan Black. We conclude that either YP2/YP4 does not contain lipid moieties or that the lipoprotein complex breaks down under these electrophoretic conditions.

DISCUSSION

Eggs of the Indian meal moth, *P. interpunctella*, contain two major yolk proteins. Vitellin was shown to be composed of two subunits, YP1 and YP3, that combined to form a glycolipoprotein that had a native molecular mass of 462 kDa by gel permeation chromatography as reported previously (Shirk *et al.*, 1984). The native molecular mass of YP1/YP3 determined here was 456 kDa by gel permeation, 475 kDa by pore-limiting gel electrophoresis and 398 kDa with a sedimentation coefficient of 13.7 S, by ultracentrifugation (Table 1). The smaller size of YP1/YP3

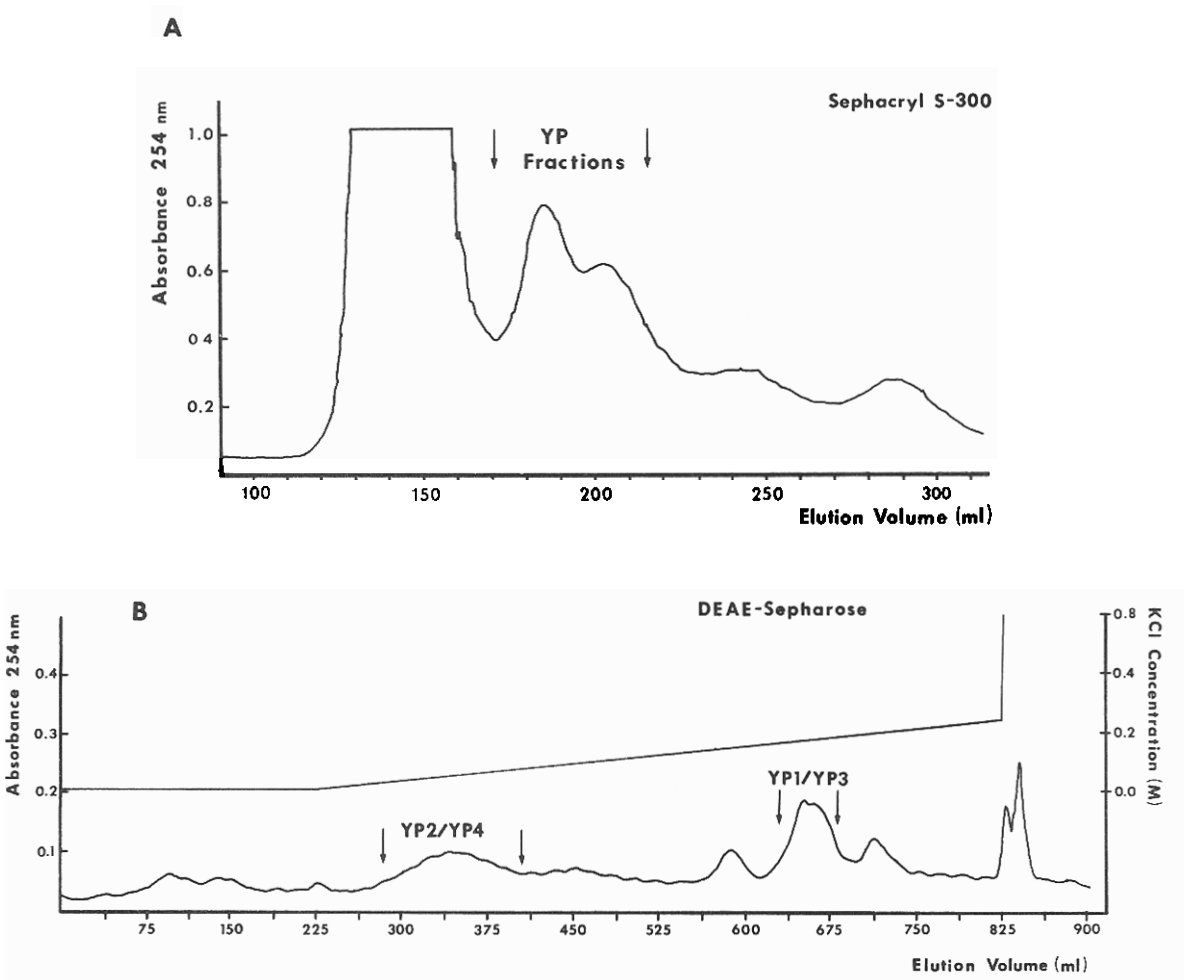

Fig. 1—*continued opposite.*

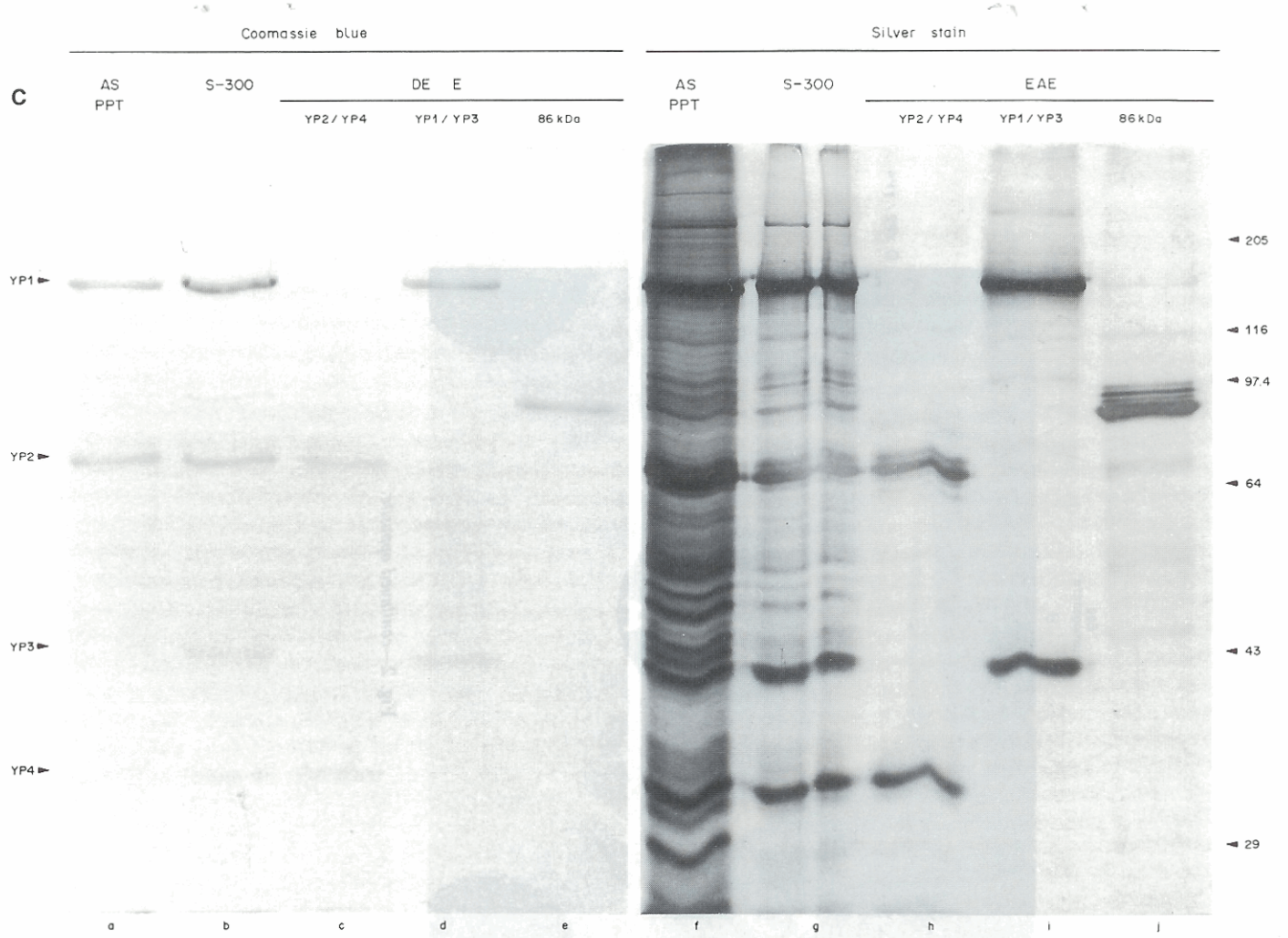

Fig. 1. Partial purification of the yolk proteins from *P. interpunctella*. (A) The elution profile of ammonium sulfate precipitated YPs from gel permeation. The fractions containing YPs (marked between the arrows) were pooled and concentrated by dialysis (see lanes b and g below). (B) The elution profile of the pooled S-300 YP fractions from ion exchange chromatography. The linear ionic gradient ranged from 5 to 250 mM KCl and is marked in the upper part of the graph. The YP2/YP4 and YP1/YP3 containing fractions (marked between the arrows) were pooled and concentrated by dialysis (see lanes c, d, h and i below). (C) Minigel SDS-PAGE of the various YP containing fractions (5 μg per lane) stained with either Coomassie Brilliant Blue (lanes a–e) or silver (lanes f–j). Lane designations: (a) and (f) = ammonium sulfate precipitate of egg homogenate; (b) and (g) = pooled YP fractions from S-300 gel permeation; (c) and (h) = pooled YP2/YP4 fractions from ion exchange chromatography; (d) and (i) = pooled YP1/YP3 fractions from ion exchange chromatography; (e) and (j) = pooled fractions of an 86 kDa uncharacterized yolk protein from ion exchange chromatography. Positions of the molecular mass markers (in kDa) are designated on the right, and positions of the four YPs are designated on the left. Abbreviations: AS PPT—ammonium sulfate precipitate; S-300—S-300 gel permeation fractions; DEAE—DEAE-sepharose ion exchange fractions.