*Insect Biochem.* Vol. 18, No. 2, pp. 199–210, 1988
Printed in Great Britain

0020-1790/88 $3.00 + 0.00
Pergamon Press plc

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

DANIEL W. BEAN,[1] PAUL D. SHIRK[1] and VICTOR J. BROOKES[2]

[1]Insect Attractants, Behavior, and Basic Biology Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL 32604, U.S.A.

[2]Department of Entomology, Oregon State University, Corvallis, OR 97331, U.S.A.

(*Received* 22 *June* 1987; *revised and accepted* 17 *September* 1987)

**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.

*The research was funded in part by a grant from the Department of Defense (No. DAAG 29-81-K-0124) to V.J.B. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or the recommendation for its use by the U.S. Department of Agriculture.

## MATERIALS AND METHODS

*Egg homogenization*

*P. interpunctella* were reared as described previously (Shirk *et al.*, 1984). Newly eclosed adults were allowed to mate and the females to oviposit through a screen into a petri dish bottom. Eggs were collected daily and frozen at −20°C. For homogenization, 2–7 g of eggs were thawed, washed five times in distilled water, once in bleach:water (1:2 v/v), once in Triton X-100® (0.1% w/v in water) and five times in phosphate buffered saline (PBS; 50 mM $NaPO_4$, 150 mM KCl, 5 mM EDTA and 0.02% $NaN_3$, pH 7.6). The washed eggs were homogenized in 10 ml of PBS that contained 1 mM phenylmethylsulfonylfluoride (PMSF) in a Dounce homogenizer. The homogenate was centrifuged 20 min at 10,000 *g* and 4°C. The infranatant between the floating layer and pellet was removed, brought to 20 ml with PBS and then ammonium sulfate was added to 75% saturation to precipitate the proteins. The solution was kept 14–18 h at 0°C and then centrifuged at 10,000 *g*. The pellet was redissolved in 2–3 ml PBS and then dialyzed (Spectrapore 15 kDa cutoff dialysis tubing) exhaustively against PBS. The resulting solution was used for gel permeation chromatography.

*Gel permeation chromatography*

The ammonium sulfate precipitate of the egg homogenate was layered onto an S-300 Sephacryl® (Pharmacia) column (95 × 2.5 cm) and eluted with PBS. The column was run at 20 ml/h at 5°C, and the absorbance (280 nm) of the eluate was measured continuously using an ISCO UA-5 absorbance monitor. Thyroglobulin (669 kDa, Sigma), apoferritin (443 kDa, Sigma) and β-amylase (200 kDa, Sigma) were chromatographed as molecular mass standards.

*Ion exchange chromatography*

Fractions that contained the major protein peak from the S-300 column (elution volume of 170–220 ml), were pooled and dialyzed against 10% polyethylene glycol (15–20 kDa, Sigma) in 5 mM KCl PBS to concentrate. The dialysate was applied to a DEAE Sepharose Cl-6B® (Pharmacia) column (40 × 2.5 cm) as described previously (Shirk *et al.*, 1984) and eluted with a linear gradient of increasing KCl from 5 to 250 mM. The change in salt concentration was monitored by measuring the refractive index of each fraction, while the absorbance (280 nm) was measured continuously using an ISCO UA-5 absorbance monitor.

*Ultracentrifugation*

Preparations of YP1/YP3 protein or YP2/YP4 protein (500 μg each) were centrifuged through linear sucrose gradients (5–20%) at 4°C for 1 h at 45,000 rpm in a VTi 80® rotor (Beckman). Absorbance (280 nm) of the eluted gradient fractions was monitored with an ISCO UA-5 absorbance monitor. Thyroglobulin (19.4 S, 669 kDa, Sigma); urease (18.6 S, 575 kDa, Sigma) and β-amylase (8.9 S, 200 kDa, Sigma) were centrifuged as standards. Sedimentation coefficients of the *P. interpunctella* yolk proteins were calculated from the migration of the standards according to Martin and Ames (1961).

*Polyacrylamide gel electrophoresis*

Native proteins were electrophoresed in nondenaturing conditions using a 5–20% polyacrylamide gel and allowed to attain a terminal migration distance that was limited by pore size (Slater, 1969). Molecular masses of the yolk proteins were computed by comparison to the migration distances of standards—thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), lactate dehydrogenase (140 kDa), and albumin (47 kDa) (Pharmacia)—using the 2400 GelScan® XL software (LKB). Proteins were electrophoresed in denaturing conditions by gradient gel SDS-PAGE as described previously (O'Farrell *et al.*, 1977, Shirk *et al.*, 1984), or by SDS-PAGE on 10% minigels (5 cm × 0.75 mm).

*Immunological properties of YPs*

Monospecific antisera were raised against YP1, YP2 and YP3 in New Zealand white rabbits. Partially purified YPs from ion exchange chromatography were resolved by SDS-PAGE, the gels were placed in 1 M KCl, and the individual YP bands were cut from the gel.

Approximately 1.5 mg of protein from each band was emulsified thoroughly in Freund's complete adjuvant (Miles) and injected subcutaneously and intramuscularly into separate rabbits. Four weeks later, the rabbits were boosted with injections of 0.5 mg of the protein in Freund's incomplete adjuvant. The specificity of each serum was determined by binding to yolk proteins electroblotted to nitrocellulose. S-300 purified YPs were resolved by SDS-PAGE and electroblotted to nitrocellulose (BA-85, Schleicher & Schuell) in transfer buffer (25 mM Tris, pH 8.3, 192 mM glycine, 20% methanol) at 20 V for 12 h using a Trans-blot cell (Bio-Rad). After the transfer, the remaining active sites on the nitrocellulose were blocked with 3% gelatin in 20 mM Tris (pH 7.5), 500 mM NaCl. The binding activity of each serum was visualized by color development using an Immun-Blot assay (Bio-Rad) after reacting the electroblotted membrane with horseradish peroxidase linked to goat antirabbit IgG.

*Ouchterlony double immunodiffusion*

Diffusion wells were cut in a 1% Noble agar gel in 150 mM NaCl and 10 mM phosphate, pH 7.2 (Ouchterlony and Nilsson, 1973). Antiyolk antiserum (Shirk *et al.*, 1984) was placed in the middle well and either a 12,000 *g* supernatant of an egg homogenate, purified YP2/YP4 from ion exchange chromatography, or purified YP1/YP3 from ion exchange chromatography were arrayed in the surrounding wells for the cross-diffusion. The diffusion plates were kept at 4°C for 4 days to establish the precipitation lines. The precipitation lines were photographed in the gel using dark field illumination.

*Protein staining*

Proteins were stained with either Coomassie Brilliant Blue or silver (Wray *et al.*, 1981). The gels were scanned using a computer-linked 2222 Ultrascan XL® laser densitometer (LKB), and the scans were analyzed using 2400 GelScan XL® software (LKB).

The presence of sugar moieties on the YPs was determined by reacting the proteins with colloidal gold labeled concanavalin A (con-A; Sigma). Purified YP1/YP3 and purified YP2/YP4 were resolved by SDS-PAGE and electroblotted onto nitrocellulose as above. After the transfer, the remaining active sites on the nitrocellulose were blocked with 3% BSA (Sigma) in 20 mM Tris (pH 7.5) plus 500 mM NaCl. The con-A was diluted in 1% BSA in 20 mM Tris (pH 7.5), 500 mM NaCl, with 0.05% Tween-20. After binding with the gold labeled con-A, the staining was enhanced with silver (IntenSE®, Janssen Life Sciences). Lipoproteins were identified by staining with Sudan Black B and then electrophoresis through 5% polyacrylamide gels under nondenaturing conditions (Natio *et al.*, 1973).

*Peptide mapping*

Polypeptides were characterized by partial peptide mapping as described previously (Cleveland *et al.*, 1977; Shirk *et al.*, 1984). The proteins contained in each band were digested with 0.05 μg of *Staphylococcus aureus* V8 protease in 125 mM Tris/HCl pH 6.8, 0.1% SDS and 1 mM EDTA. To visualize the cleavage fragments, the gels were stained with silver (Wray *et al.*, 1981).

## 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) | Sucrose gradient $S_{W/20}$ |
|---|---|---|---|---|
| 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.

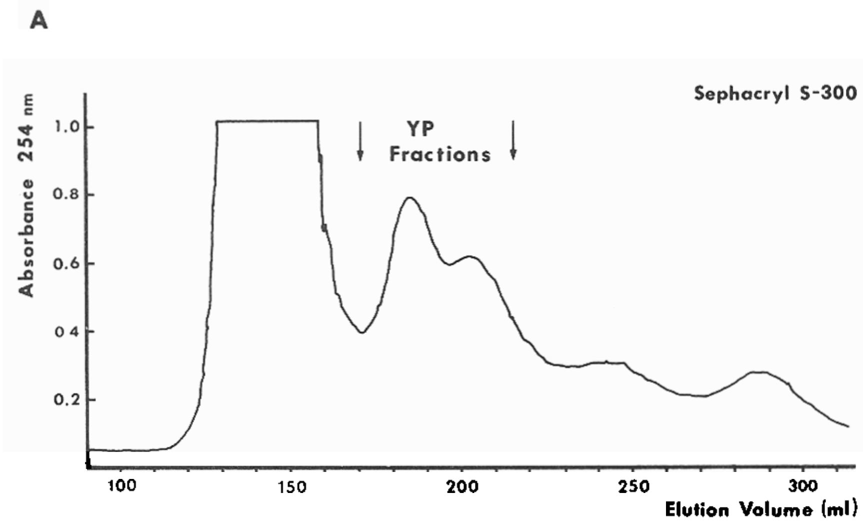
*Identification of attached moieties on the YPs*

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



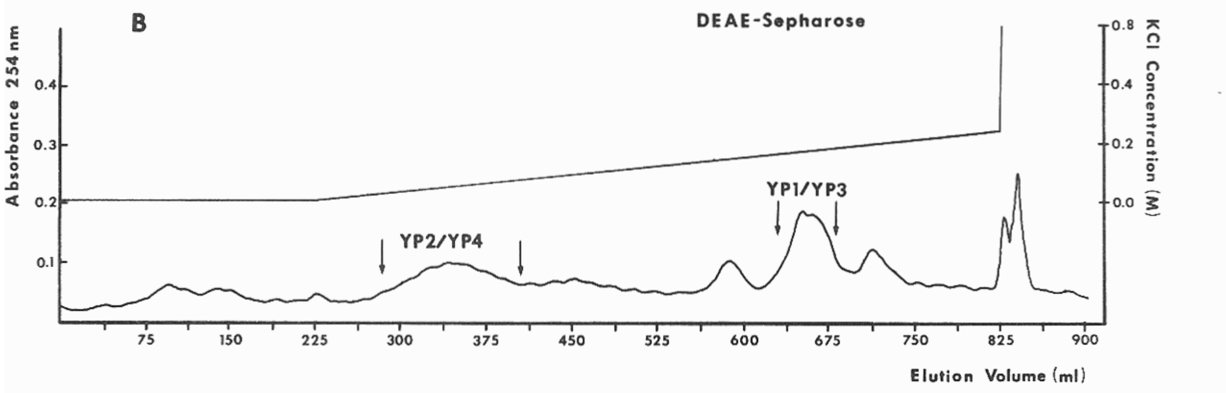




Fig. 1—*continued opposite.*

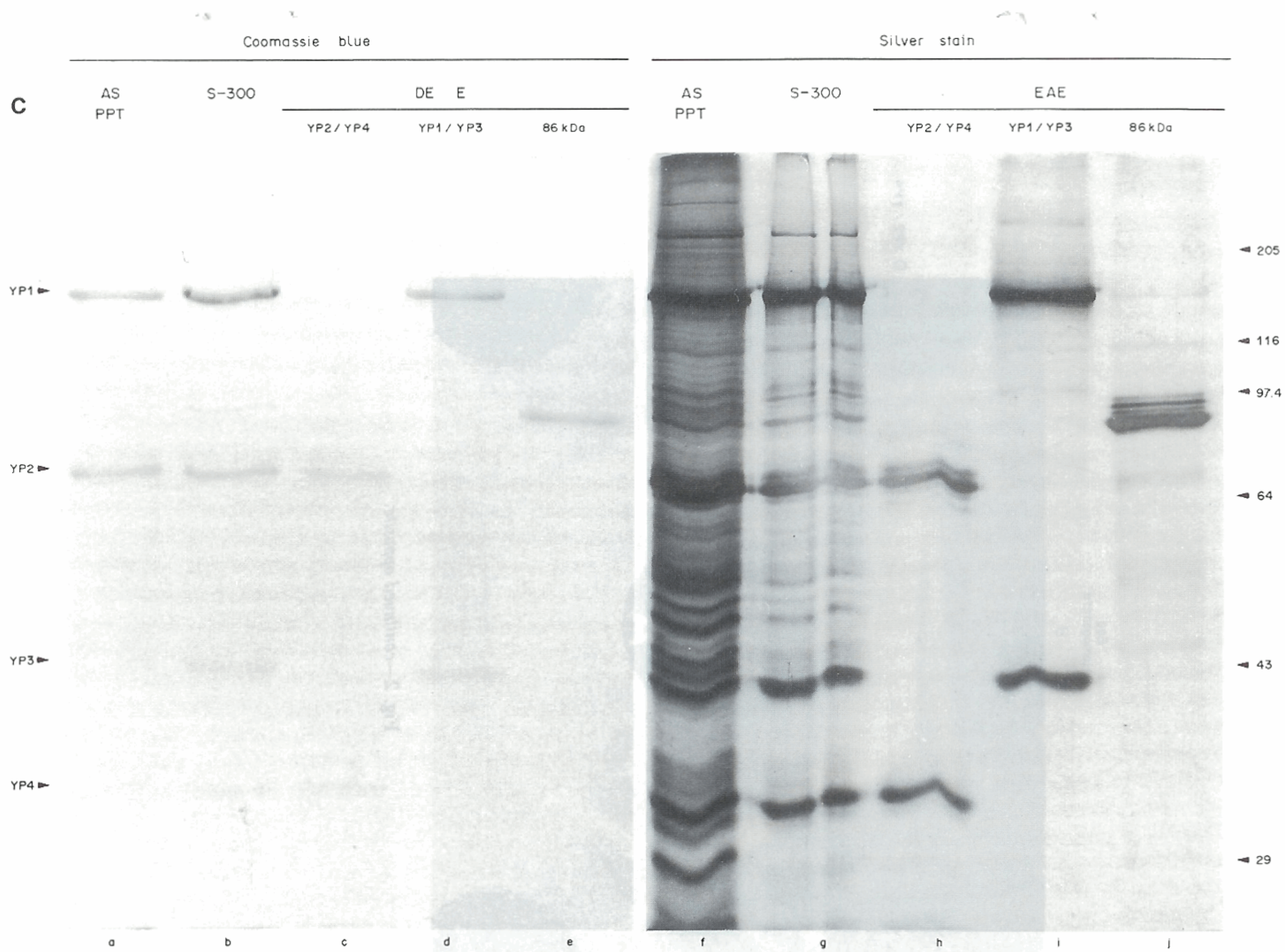



Fig. 1. Partial purification of the yolk proteins from *P. interpunctella.* (A) The elution profile of ammonium sulfate precipitated YPs from gel permeation. The fractions containing YPs (marked between the arrows) were pooled and concentrated by dialysis (see lanes b and g below). (B) The elution profile 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 $\mu$g 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.

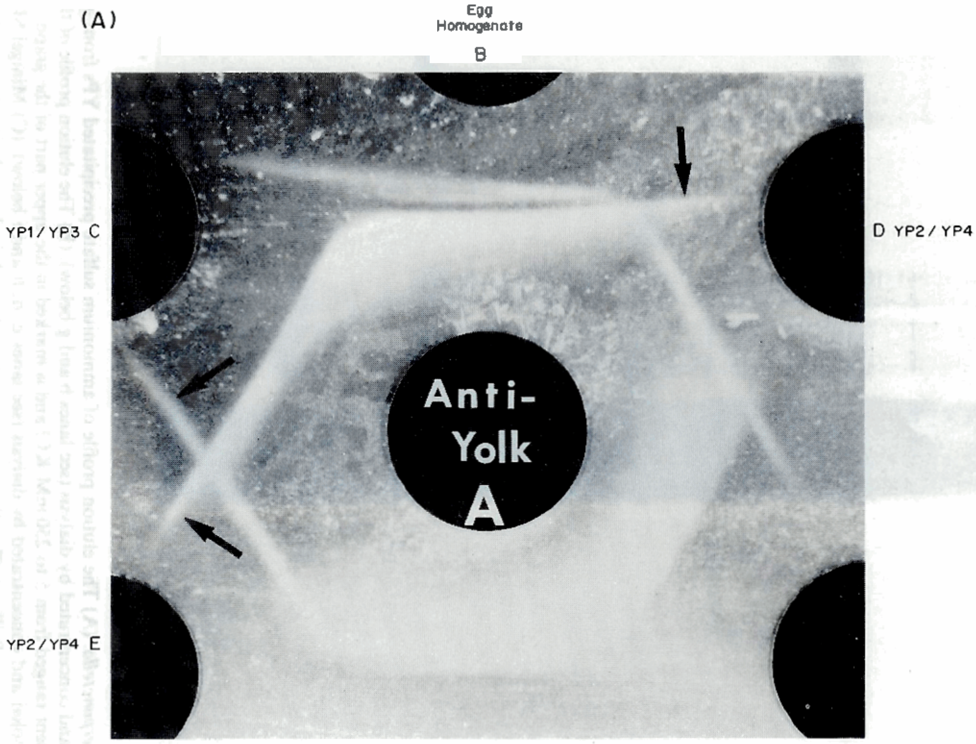



Fig. 2—*continued opposite.*

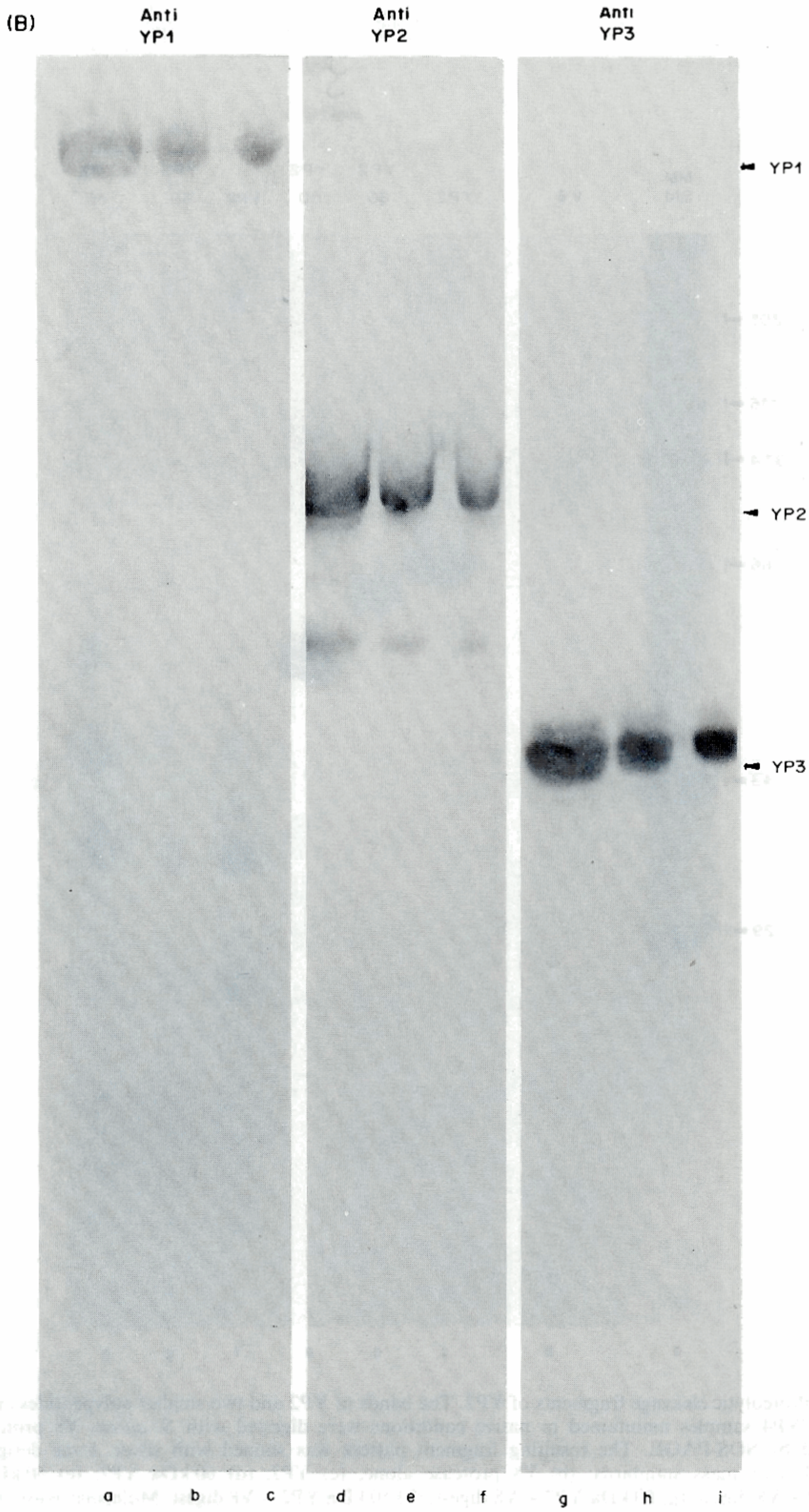



Fig. 2. Immunological identity of the yolk proteins. (A) Ouchterlony double immunodiffusion of yolk proteins against antiyolk antiserum. Well designations: (a) antiyolk antiserum; (b) 12,000 *g* supernatant of egg homogenate; (c) purified YP1/YP3 from ion exchange chromatography; (d) purified YP2/YP4 from ion exchange chromatography; (e) purified YP2/YP4 from ion exchange chromatography. The arrows designate spurs that indicate lack of immunocrossreactivity between the two precipitation lines. (B) Immunoblot of yolk proteins with antisera specific for YP1, YP2, and YP3. Pooled S-300 YPs were loaded at 5, 1, and 0.5 $\mu g$ of protein per lane and crossreacted with antisera raised to YP1 (lanes a, b and c), YP2 (lanes d, e and f), and YP3 (lanes g, h and i).

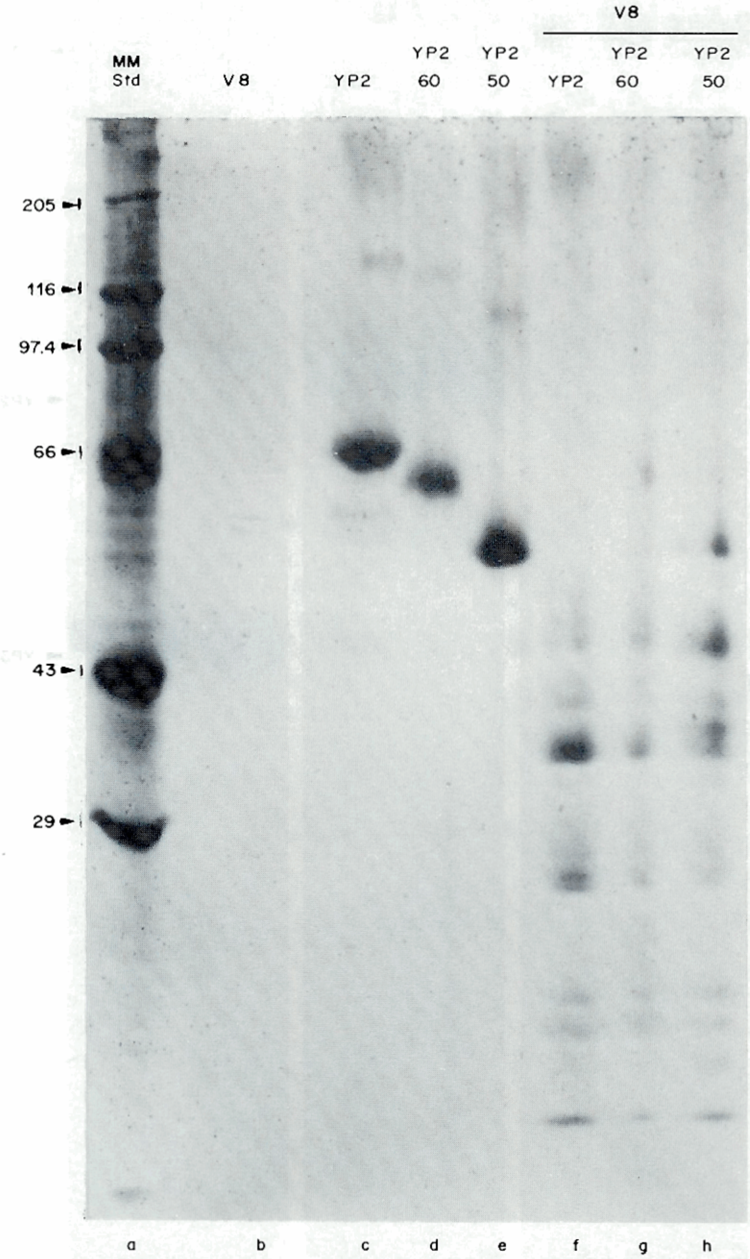



Fig. 3. Proteolytic cleavage fragments of YP2. The bands of YP2 and two smaller polypeptides appearing in YP2/YP4 samples maintained in native conditions were digested with *S. aureus* V8 protease and resolved by SDS-PAGE. The resulting fragment pattern was stained with silver. Lane designations: (a) molecular mass standards; (b) V8 protease alone; (c) YP2; (d) 60 kDa YP2; (e) 50 kDa YP2; (f) YP2 + V8 digest; (g) 60 kDa YP2 + V8 digest; (h) 50 kDa YP2 + V8 digest. Molecular mass standards are listed on the left (kDa). Abbreviations: MM Std—molecular mass standard; V8—*S. aureus* V8 protease; YP2 60—60 kDa YP2; YP2 50—50 kDa YP2.

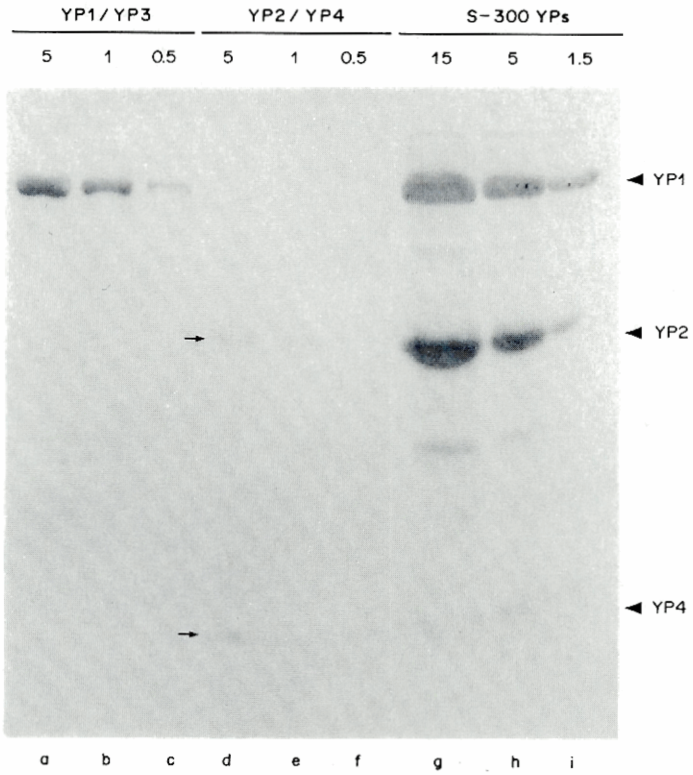



Fig. 4. Yolk proteins stained for sugar moieties with gold-conjugated con-A. Purified yolk proteins were immunoblotted to nitrocellulose, crossreacted with gold-conjugated con-A, and enhanced with silver. Lane designations: (a) 5 $\mu$g YP1/YP3; (b) 1 $\mu$g YP1/YP3; (c) 0.5 $\mu$g YP1/YP3; (d) 5 $\mu$g YP2/YP4; (e) 1 $\mu$g YP2/YP4; (f) 0.5 $\mu$g YP2/YP4; (g) 5 $\mu$g S-300 YPs; (h) 1 $\mu$g S-300 YPs; (i) 0.5 $\mu$g S-300 YPs.

seen in ultracentrifugation as compared to the other methods was probably the result of a greater bouyancy due to the presence of lipid moieties. Based on the molecular mass of the subunits and the presence of lipids, the estimated molecular mass of the native vitellin would be consistent with the subunits being present in a ratio of 1:1 with two pairs of subunits per protein complex plus 20% lipid. These characteristics are similar to those of other vitellins from lepidopterans (*cf.* Kunkel and Nordin, 1985).

Also contained in the egg was a major protein produced in the ovarioles that appears to be unique to *P. interpunctella.* The ovariole protein was composed of two polypeptides, YP2 and YP4, which were glycosylated polypeptides. The molecular mass of the native protein was estimated previously to be 264 kDa on the basis of gel permeation chromatography (Shirk *et al.*, 1984). In this report, YP2/YP4 was sized at 237 kDa by gel permeation, and at 235 kDa in pore limiting electrophoresis. However, the 237 kDa form was not observed in ultracentrifugation but may have been obscured by the 500 kDa co-purifying protein. The smallest species of YP2/YP4 had a molecular mass of 93 kDa by electrophoresis and 98 kDa with a sedimentation coefficient of 6.5 S by ultracentrifugation. The size of the smallest species suggests that the YP2 and YP4 subunits of YP2/YP4 are present in a 1:1 ratio, and that the larger species are multiples of this dimer. The variability in molecular mass under these conditions can be attributed to one of two factors or a combination of both: (1) proteolytic clipping of YP2, or (2) a weak association between the subunits that can be disturbed when the protein is purified. Proteolytic clipping of YP2 by an endogenous protease(s), as shown in Fig. 3, could produce heterogeneous subpopulations of YP2/YP4 that would result in the multiple bands seen under native electrophoresis conditions. The presence of a highly specific protease for ESP in *B. mori* eggs and embryos has been shown to be responsible for specific utilization of ESP during embryogenesis (Yamashita, 1986). Similarly, a protease has been observed in *P. interpunctella* that degrades YP2 during embryogenesis (DeVera, Shirk and Brookes, unpublished) and may contribute to proteolytic clipping of YP2 observed during the isolation procedure. On the other hand, weak bonding between the two subunits could produce hetero-

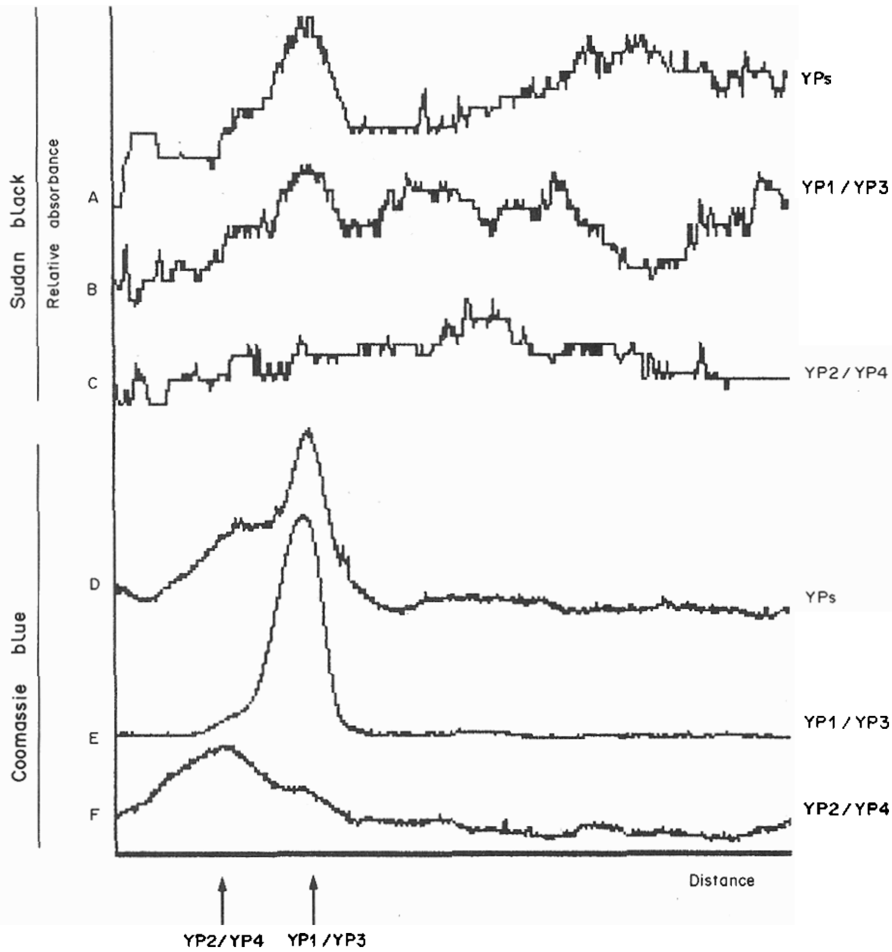



Fig. 5. Yolk proteins stained for lipid moieties with Sudan Black. The lanes are scans of purified yolk proteins stained with Sudan Black (lanes A–C) and then restained with Coomassie Blue (lanes D–F). The axes represent the relative migration distance ($X$) vs relative absorbance ($Y$). Lane designations: (A) S-300 YPs + Sudan Black; (B) YP1/YP3 + Sudan Black; (C) YP2/YP4 + Sudan Black; (D) S-300 YPs + Coomassie Blue; (E) YP1/YP3 + Coomassie Blue; (F) YP2/YP4 + Coomassie Blue.

geneous populations of YP2/YP4 that would result in the multiple bands observed. Similar difficulties with the heterogeneity of native vitellogenin during gel electrophoresis has been reported also for purified vitellogenin from *L. maderaea*, which migrates as several bands during native gel electrophoresis (Engelmann, 1979).

The size and subunit composition of YP2/YP4 appears to be unlike any of the yolk proteins reported for other lepidopterans. Although eggs of *H. cecropia* contain four major yolk polypeptides, only vitellin appears to have a composition similar to that of vitellin (YP1/YP3) in *P. interpunctella*. Paravitellin (70 kDa), produced in the follicle cells of *H. cecropia*, exists as a singular 70 kDa monomer in the yolk as does microvitellin (reluctin), a 30 kDa polypeptide produced in the fat body that is similar in size to YP4 (Telfer *et al.*, 1981). However, neither paravitellin nor microvitellin appears to form any higher subunit associations as do YP2 and YP4. Microvitellin also has been found in eggs and haemolymph of *M. sexta* but again does not form any higher subunit associations (Kawooya and Law, 1983). Egg-specific protein, a 72 kDa polypeptide produced by the follicle cells from *B. mori* was found to form a trimer (Zhu *et al.*, 1986), but a polypeptide similar to YP4 or microvitellin has not been described in this insect. From these observations, we find that YP2/YP4 in *P. interpunctella* is unlike yolk proteins found in other lepidopterans in that YP2 and YP4 form a large major yolk protein that is produced by the ovarian tissues.

The function of YP2/YP4 during embryogenesis is currently under investigation. The findings in *B. mori* that normal embryonic and larval development can occur without the presence of vitellin (Yamashita and Irie, 1980), and that egg-specific protein is utilized preferentially during early embryogenesis (Irie and Yamashita, 1983) through cleavage by a specific protease (Yamashita, 1986) focuses our attention on the specific utilization of the ovarian produced proteins that we have observed

## REFERENCES


Cleveland D. W., Fisher S. G., Kirschner M. W. and Laemmli U. K. (1977) Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. *J. biol.Chem.* **252,** 1102–1106.

Engelmann F. (1979) Insect vitellogenin: identification, biosynthesis, and role in vitellogenesis. *Adv. Insect Physiol.* **14,** 49–108.

Irie K. and Yamashita O. (1980) Changes in vitellin and other yolk proteins during embryonic development in the silkworm, *Bombyx mori. J. Insect Physiol.* **26,** 811–817.

Irie K. and Yamashita O. (1983) Egg-specific protein in the silkworm *Bombyx mori*: Purification, properties, localization and titre changes during oogenesis and embryogenesis. *Insect Biochem.* **13,** 71–80.

Izumi S., Tomino S. and Chino H. (1980) Purification and molecular properties of vitellin from the silkworm, *Bombyx mori. Insect Biochem.* **13,** 81–85.

Kawooya J. K. and Law J. H. (1983) Purification and properties of microvitellogenin of *Manduca sexta*. Role of juvenile hormone in appearance and uptake. *Biochem. biophys. Res. Commun.* **117,** 643–650.

Kunkel J. G. and Nordin J. H. (1985) Yolk proteins. In *Insect Physiology, Biochemistry and Pharmacology*, Vol. 1 (Edited by Kerkut G. A. and Gilbert L. I.), pp. 84–111. Pergamon Press, Oxford.

Martin R. G. and Ames B. N. (1961) A method for determining the sedimentation behavior of enzymes: application to protein mixtures. *J. biol. Chem.* **236,** 1372–1379.

Natio H. K., Wada M., Erhart L. A. and Lewis L. A. (1973) Polyacrylamide-gel disc-electrophoresis as a screening procedure for serum lipoprotein abnormalities. *Clin. Chem.* **19,** 228–234.

O'Farrell P. Z., Goodman H. M. and O'Farrell P. H. (1977) High resolution two-dimensional electrophoresis of basic as well as acidic proteins. *Cell* **12,** 1133–1142.

Ono S., Nagayama H. and Shimura K. (1975) The occurrence and synthesis of female and egg-specific protein in the silkworm, *Bombyx mori. Insect Biochem.* **5,** 313–329.

Osir E. O., Wells M. A. and Law J. H. (1986) Studies on vitellogenin from the tobacco hornworm, *Manduca sexta. Archs Insect Biochem. Physiol.* **3,** 217–233.

Ouchterlony O. and Nilsson L. A. (1973) Immunodiffusion and immunoelectrophoresis. In *Handbook of Immunology* (Edited by Weir D. M.), Vol. 19, pp. 1–39. Blackwell, Oxford.

Shirk P. D., Bean D., Millemann A. M. and Brookes V. J. (1984) Identification, synthesis and characterization of the yolk polypeptides of *Plodia interpunctella. J. exp. Zool.* **232,** 87–98.

Slater G. C. (1969) Stable pattern formation and determination of molecular size by pore-limit electrophoresis. *Analyt. Chem.* **41,** 1039–1041.

Takahashi S. Y. (1987) Studies on the phosphorylation of ovarian proteins from the silkworm, *Bombyx mori. Insect Biochem.* **17,** 141–152.

Telfer W. H., Rubenstein E. and Pan M. (1981) How the ovary makes yolk in *Hyalophora*. In *Regulation of Insect Development and Behavior* (Edited by Sehnal F., Zabza A., Menn J. J. and Cymborowski B.), pp. 637–654. Technical University of Warsaw, Warsaw.

Wray W., Boulikas T., Wray V. P. and Hanock R. (1981) Silver staining of proteins in polyacrylamide gels. *Analyt. Biochem.* **118,** 197–203.

Yamashita O. (1986) Yolk protein system in *Bombyx* eggs: synthesis and degradation of egg-specific protein. In *Invertebrate Reproduction* (Edited by Porchet M. and Andries J.-C.), Vol. 4, pp. 79–84. Elsevier, Amsterdam.

Yamashita O. and Irie K. (1980) Larval hatching from vitellogenin-deficient eggs developed in male hosts of the silkworm. *Nature, Lond.* **283,** 385–386.

Zhu J., Indrasith L. S. and Yamashita O. (1986) Characterization of vitellin, egg-specific protein and 30 kDa protein from *Bombyx* eggs, and their fates during oogenesis and embryogenesis. *Biochem. biophys. Acta* **882,** 427–436.