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Isolation and Partial Chemical Characterization of the Yolk Proteins from *Drosophila* sp. (*robusta* species group)

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The three yolk polypeptides have been isolated and partially characterized. Their molecular weights of YP1, YP2, and YP3 were 48,000, 47,000, and 46,000, respectively, as judged by SDS-polyacrylamide gel electrophoresis. They have different digestion products upon *in situ* peptide mapping by limited proteolysis. Two-dimensional gel electrophoresis showed that their isoelectric points were heterogeneous from 5.92 to 6.54. And they showed three different antigen-antibody reactions when each polypeptides is reacted with antisera made to a mixture of all of three. These data reported here indicate that the yolk proteins are consisted of distinctive polypeptides in *Drosophila* sp. (*robusta* species group).

KEY WORDS: ***Drosophila* yolk proteins, Purification, Partial chemical characterization**

The process of vitellogenesis is a major event in oogenesis in a number of different insects. In *Drosophila,* the three yolk proteins (YP1, YP2, and YP3) are synthesized as vitellogenin precursors in two different tissues of the female fly. Throughout the adult life, the female fat body cells continuously synthesize the three yolk proteins (Bownes and Hames, 1978; Kambysellis *et al.*, 1986). The other synthetic site for yolk protein is the follicle cells surrounding the oocyte (Gutzeit, 1980; Brennan *et al.*, 1982).

The synthesized yolk proteins are then secreted into the hemolymph and then selectively transported into the eggs. Secretion is preceded by at least two steps of protein modification: One step involves the terminal cleavage of about ten amino acids from each of the precusors to the three YPs. A second step is modification which gives the proteins charge heterogeneity (Warren *et al.*, 1979).

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The synthesis of the three yolk proteins is stimulated by both juvenile hormone and 20-hydroxyecdysone (Handler and Postlethwait, 1978; Postlethwait and Handler, 1979). In *D. melanogaster* and *D. grimshawi*, the three yolk proteins are encoded by single copy genes (*yp1, yp2, yp3*) on the X chromosome (Barnet *et al.*, 1980; Hoveman *et al.*, 1981; Riddel *et al.*, 1981; Hotzopolous and Kambysellis, 1987a).

Although the yolk proteins of *D. melanogaster* have been well characterized (Warren and Mahowald, 1979; Mintzas and Kambysellis, 1982), little is known about the other species of *Drosophila*. In this paper, we have isolated and partially characterized the three yolk proteins in more distantly related species, *D.* sp. (*robusta* species group).

Materials and Methods

Isolation of yolk proteins

Laboratory stocks of *Drosophila* sp. (*robusta* species group; Cheju strain) were grown in mass

culture according to the procedure of Travaglini and Tartof (1972). Zero- to 3-hr embryos were collected, and the chorion were removed as previously described (Allis *et al.*, 1977).

The yolk proteins were isolated by the methods of Minitzas and Kambysellis (1982) with a minor modification. The isolated embryos were homogenized in 10 vol. of 50 mM Tris-HCl, pH 8.2, 0.25 M NaCl and then centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was dialyzed against 50% saturated ammonium sulfate solution for overnight at 4°C, and the precipitated fraction was removed by centrifugation. The soluble fraction was dialyzed against 80% saturated ammonium sulfate solution as the above, and the precipitated faction, containing the three yolk proteins, was dissolved in 50 mM Tris-HCl, pH 8.2, 0.25 M NaCl. This fraction was dialyzed against deionized water for 2 days at 4°C The resulting water-insoluble fraction was collected by centrifugation and stored at −20°C.

Analytical techniques

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was done by the method of Laemmli (1970). Two-dimensional gel electrophoresis was performed according to the methods of O'Farrell (1975). Peptide mapping was carried out by the limited proteolysis method of Cleveland *et al.* (1977). Protein concentration was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard reference.

Immunological techniques

The antiserum of the yolk proteins was prepared in New Zealand White rabbit (Bailey, 1984). Double immunodiffusion was done by the method of Ouchterlony (1958), and Western blotting was carried out essentially according to the procedure of Towbin *et al.* (1979).

Results

Isolation scheme of yolk proteins

The isolation scheme for yolk proteins utilized the insoluble nature of these proteins in neutral low-salt buffer (Warren and Mahowald, 1979). Electrophoretic analysis of curde embryo extracts on 10% SDS-polyacrylamide gel reveals three major yolk proteins (YP1, YP2, and YP3), with a similar electrophoretic mobility (Fig. 1, lane 2). Crude embryo extracts were subjected to ammonium sulfate fractionation, as described in Materials and Methods. Approximately one fourth of the proteins present in crude embryo extracts were precipitated between 50% and 80% ammonium sulfate (Fig. 1, lane 3 and Table 1). Dialysis of the 50-80% ammonium sulfate fraction against H_2O resulted in precipitation of a pure three yolk proteins preparation (Fig. 1, lane 4). The purification scheme and the yield obtained in each fraction were summarized in Table 1. The molecular weights of the three yolk proteins of *Drosophila* sp. were 48,000 for YP1, 47,000 for YP2, and 46,000 for YP3 as determined by SDS-polyacrylamide gel electrophoresis.

Verification that the yolk proteins are three separate polypeptides

Separation of the yolk proteins using two-dim-

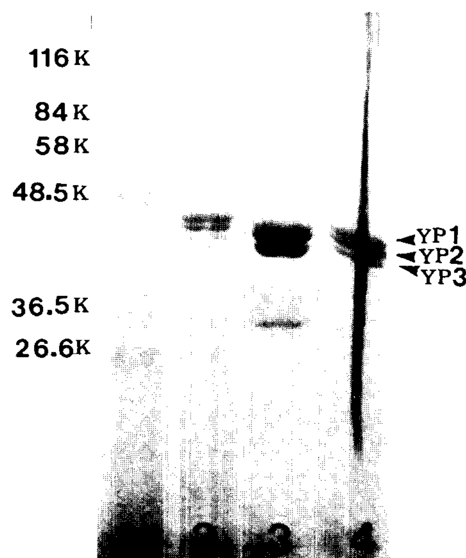

Fig. 1. SDS-polyacrylamide gel electrophoresis of the yolk proteins during purification. Protein fractions were denatured by heat, 2% SDS, and 3% 2-mercaptoethanol and subjected to electrophoresis on 10% slab gel. Lane 1, molecular weight markers; lane 2, crude embryo extract; lane 3, 50-80% ammonium sulfate yolk protein fraction; lane 4, final yolk protein preparation. The arrows indicated the three yolk proteins.

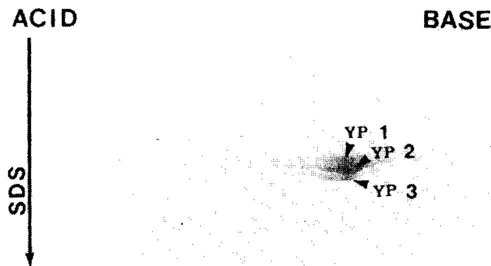

Fig. 2. Two dimensional gel electrophoresis of the partially purified yolk proteins, The proteins was separated by thin layer horizontal isoelectric focusing on a pH gradient about 3 to 10, and then by SDS-polyacrylamide gel electrophoresis.

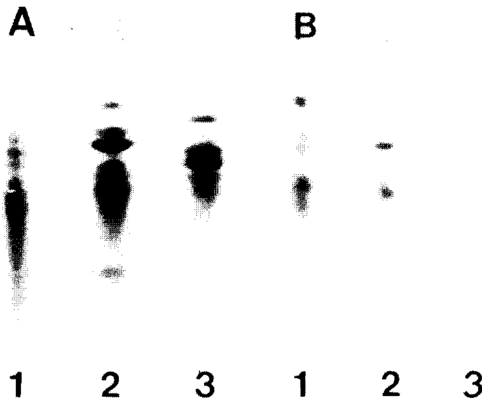

Fig. 3. *In situ* peptide maps of the tree yolk proteins. Twenty μg of the purified yolk proteins were initially run on exponential gradient (8-15%) polyacrylamide gel. After staining with Coomassie brilliant blue, the individual protein band was cut out and loaded on peptide mapping gel and subjected to *in situ* peptide mapping with (A) 0.1 μg and (B) 0.05 μg of chymotrypsin. The resulting peptides were then visualized by silver staining. Lane 1, 2, and 3 are YP3, YP2, and YP1 polypeptide, respectively.

ensional gel electrophoresis revealed distinctive patterns (Fig. 2). The three yolk polypeptides focused over a broad pH range of approximately 5.95 to 6.54 with each number of the triplet to a

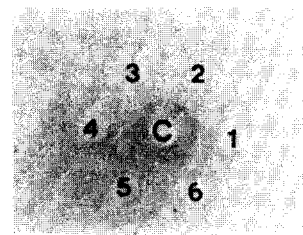

Fig. 4. Double immunodiffusion of the yolk proteins. C, purified yolk proteins; 1, full strength antisera; 2, 3, 4, 5, and 6 contain the following dilutions of the antiserum in sequence: 1/2, 1/4, 1/8, 1/16, and 1/32.

Table 1. Purification of yolk proteins in *Drosphila* sp. (*robusta* species group).

Fraction	Total proteins (mg)	Yield (%)
Crude embryo extract	390	–
50-80% ammonium sulfate fraction	88.4	22.6
H_2O-insoluble fraction	64.3	16.4
Final yolk protein fraction	30	9.2

slightly different pH.

Peptide mapping by limited proteolysis gave different digestion patterns of each of the three yolk polypeptides. Following chymotrypsin digestion, YP1 and YP2 yielded some fragment of similar size and also some unique fragment (Fig. 3), but the YP3 produce a unique pattern of fragments (Fig. 3).

Double immunodiffusion of the yolk polypeptdes with antisera made to the triplet resulted in the formaion of the three precipitin bands (Fig. 4). These data were verified by Western blotting using the same antisera (Fig. 5). The triplet bands did not detected in hemolymph samples derived from 3 days-old male and 1 day-old female (Fig. 5, lanes 6 and 7), but they were detected in hemolymph samples from 3 days-old female flies after eclosion (Fig. 5, lanes 2-5).

Discussion

The isolation and characterization of the yolk proteins have been accomplished in several spe-

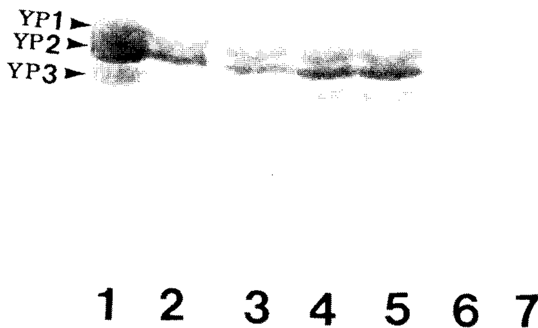

Fig. 5. Western blot of male and female hemolymph proteins separated by the exponential gradient (8-15%) SDS-polyacrylamide gel electrophoresis and reacted with anti-yolk protein antiserum. Lane 1, purified yolk proteins; lane 2, 10 days-old female; lane 3, 7 days-old female; lane 4, 5 days-old female; lane 5, 3 days-old female; lane 6, 3 days-old male; lane 7, 1 day-old female hemolymph.

cies of *Drosophila* (Warren and Mahowald, 1979; Mintzas and Kambysellis, 1982; Kambysellis *et al.*, 1986). Since the yolk proteins are of importance for studying the sex-limited and tissue-specific regulation of vitellogenesis, *D. melanogaster* were extensively used as a model system (Bownes and Hames, 1977; Bownes and Hames, 1978; Postlethwait *et al.*, 1980; Gutzeit, 1980; Bownes and Nothiger, 1981; Garabedian *et al.*, 1985; Hotzopolous and Kambysellis, 1987b; Bownes and Reid, 1990). However, no report on the yolk proteins using *D.* sp. (*robusta* species group) is yet available.

Our results indicate that the yolk proteins of *D.* sp. consist of three unique polypeptides with similar molecular weight as shown in those of *D. melanogaster* and *D. grimshawi*. The three yolk proteins (YP1, YP2, and YP3) in three species of *Drosophila* are similar in their molecular weights; *D. melanogaster*: 47, 46, 44 KD/*D. graimshawi*: 46.7, 45.5, 43.7 KD/*D.* sp.: 48, 46, 45 KD (Warren and Machowald, 1979; Minitzas and Kambysellis, 1982; Kambysellis *et al.*, 1986).

When examined by isoelectric focusing, each of the three polypeptides has a slightly different *pI*. Since the proteins were denatured prior to isoelectric focusing in the present study, the differences in *pI* may be not due to variation in exposed charges of common polypeptides, but due to differences in the primary structure between the three yolk proteins. The heterogeous *pI* values upon isoelectric focusing may then be due to posttranslational modification (Minitzas and Kambysellis, 1982; Bownes, 1986). A more difinitive examination of the relation between the three yolk polypeptides comes from the peptide mapping by limited proteolysis. Digestion with chymotrypsin indicates that the three yolk polypeptides are different. Double immunodiffusion of the three yolk polypeptides and the antisera produces three precipitin reaction. Western blotting using this antisera shows that the three polypeptides may be identical with the hemolymph vitellogenic proteins of female fly.

The precusor-product relationship between the hemolymph vitellogenic proteins and the yolk proteins in *Drosophila* also appears unique among the organisms investigated. In other organisms, there appears to be major specific cleavages of the vitellogenic proteins with widely varing sizes (for review see Byrne *et al.*, 1989). This does not appear to be the case in *Drosophila*. The vitellogenic and yolk proteins are very similar in size even though they are clearly different polypeptdes (Warren and Mahowald, 1979; Mintzas and Kambysellis, 1982).

While the three yolk polypeptides in *D.* sp. appear to be unique polypeptides on the basis of two-dimensional gel electrophoresis, peptide mapping, and immunological techniques, the exact extents of differences need to be determined. The chymotrypsin digestion patterns suggest a similarity between YP1 and YP2. This finding is of interest, since the *yp1* is more homologous, in both sequence and structure, with *yp2* than with *yp3* in *D. melanogaster* and *D. grimshawi* (Barnett *et al.*, 1980; Hung *et al.*, 1982; Hotzopoulos and Kambysellis, 1987a). The work presented here can facilitate further biochemical and developmental genetic studies in *D.* sp.

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Drosophila sp.(_robusta_ species group)의 난황 단백질의 분리 및 부분적 화학적 특성

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제주먹초파리(*Drosophila* sp.; *robusta* species group)에서 3종류의 난황 단백질을 분리하여 부분적인 특성을 조사하였다. SDS-polyacrylamide gel 전기영동으로 결정된 분자량은 YP1이 48,000, YP2가 47,000, 그리고 YP3가 46,000이었다. 3종류의 난황 단백질들은 limited proteolysis로 *in situ* peptide mapping 했을 때 생성된 peptides 양상이 서로 달랐다. 2차원 전기영동으로 분석한 이들의 등전점은 pH 5.92에서 6.54에 이르는 이질적인 분포를 보였다. 본 연구 결과는 제주먹초파리의 난황 단백질은 3종류의 polypeptide로 구성되었음을 시사하였다.