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Processing of Pro-vitellogenin in Insect Fat Body: A Role for High-Mannose Oligosaccharide

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Several discrete events were resolved in the processing of vitellogenin in *Blattella germanica*. Using tunicamycin to inhibit the synthesis of high-mannose oligosaccharide, a high molecular weight pro-vitellogenin peptide (apo-proVG, M_r 215,000) was identified in fat body. Dosages of tunicamycin which inhibited glycosylation of vitellogenin by 98% inhibited its synthesis by as much as 59%, yet led to an intracellular accumulation of apo-proVG. Reversibility and dose dependency of these effects on vitellogenin synthesis, glycosylation, proteolytic processing, and secretion were demonstrated. In control insects, glycosylation of apo-proVG yielded a M_r 240,000 pro-vitellogenin peptide (proVG). FITC-Concanavalin A bound to purified proVG but not to apo-proVG, thus confirming an absence of high-mannose oligosaccharide in the apo-protein. Following its glycosylation, proVG was processed rapidly in fat body to M_r 160,000 (VG160) and M_r 102,000 (VG102) peptides which subsequently were secreted into hemolymph. After uptake into developing oocytes, the VG160 peptide was processed further prior to chorionation, yielding subunits of M_r 95,000 and 50,000. Uniqueness of the peptides of mature vitellin (M_r 102,000, 95,000, and 50,000) was indicated by comparison of the CNBr fragments of each purified subunit. Staining of CNBr fragments with FITC-Concanavalin A also indicated that high-mannose oligosaccharides are attached at one or more sites within each vitellin subunit. Resolution of the substructure of this insect vitellin and identification of events involved in the processing and secretion of its fat body apo-protein provide a basis for further study of the assembly and transport of vitellogenin, its packaging in eggs, and utilization during embryogenesis.

INTRODUCTION

Oogenesis in most egg-laying animals depends upon the rapid synthesis, secretion, and subsequent ovarian sequestration of vitellogenins, the extra-ovarian precursors of yolk (Kunkel and Nordin, 1985; Wahli *et al.*, 1981; Tata and Smith, 1979; Engelmann, 1979). Typically occurring as oligomeric phosphoglycolipoproteins, vitellogenins thus provide a useful model for studying mechanisms involved in the post-translational processing, secretion, and selective pinocytosis of protein (Wahli *et al.*, 1981; Kunkel and Nordin, 1985). Stored in oocytes as vitellins, these yolk proteins comprise a primary nutrient for developing embryos and have been described in numerous species. In contrast, the synthesis of vitellogenin and its transport to oocytes is less well understood. This is due to the transitory stay of vitellogenin in extra-ovarian tissues, to its complex processing, and to the difficulty in distinguishing among multiple discrete vitellogenins found in certain vertebrate (Wahli *et al.*, 1981; Wang and Williams, 1980) and insect species (Kunkel and Nordin, 1985). Among vertebrates, vitellogenesis has been best studied in the chicken and in *Xenopus laevis*. In these species, vitellogenins are synthesized in the liver as pro-peptides which sequentially are glycosylated, processed to complex glyco-conjugates (Gottlieb and Wallace, 1982), phosphorylated (Wang and Williams, 1982; Gottlieb and Wallace, 1981), lipidated, and secreted. Following endocytosis by developing oocytes (Woods and Roth, 1984) but prior to deposition in yolk platelets, vitellogenin then is processed further into multiple subunits (lipovitellins, phosvitins, and phosvettes) containing discrete nutrients (Woods and Roth, 1984; Christmann *et al.*, 1977).

Among insects, the simple processing of monomeric vitellogenins in higher Dipterans (Warren *et al.*, 1979; Mintzas and Kambyselis, 1982) is contrasted to the complex processing of the high molecular weight, oligomeric vitellogenins of most species (Harnish and White, 1982). Although biosynthetic processing of high molecular weight vitellogenins has been examined previously in *Locusta migratoria* (Chen, 1980; Chen *et al.*, 1978) and in the cockroach, *Leucophaea maderae* (Koeppe and Offengand, 1976), the precise relationships between putative pro-vitellogenin peptides and the various subunits of vitellogenin and vitellin in these species were not established. We have examined processing of a representative high molecular weight insect vitellogenin, that of the cockroach, *Blattella germanica* (Nordin *et al.*, 1984; Kun-

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kel *et al.*, 1981). Unlike the vitellogenins of vertebrates, the oligosaccharides of insect vitellins and vitellogenins (Nordin *et al.*, 1984) and of other glycoproteins of insects (Ng and Dain, 1976) are exclusively of the simple, high-mannose type (Kornfeld and Kornfeld, 1985). Using tunicamycin to inhibit the synthesis of N-linked oligosaccharides, we have identified a M_r 215,000 apo-provitellogenin peptide in *B. germanica*, as well as three distinct steps in its processing; its glycosylation and proteolysis in fat body and additional limited cleavage within oocytes. Also addressed is the nature of the role of attached oligosaccharide in the processing and secretion of this insect vitellogenin. A preliminary account of this report has been published (Kunkel *et al.*, 1983).

MATERIALS AND METHODS

Materials. The following chemicals and reagents were obtained from the designated sources: [^{35}S]Met[6] (1000 Ci/mmole), [^{3}H]GlcN (20 Ci/mmole), Enhance fluorographic and Liquifluor scintillation fluid, New England Nuclear; XOMAT-A1 film, Kodak; agarose and acrylamide, BioRad; phenyl methyl sulfonyl fluoride (PMSF), *N*-[α]-*p*-tosyl lysine chloromethyl ketone hydrochloride (TLCK), fluorescein isothiocyanate Concanavalin A (FITC-Concanavalin A), and dimethyl sulfoxide (Me_2SO), Sigma; tunicamycin was a gift from Dr. Robert Hammill, Eli Lilly Co.

Animals. Cultures of *B. germanica* were maintained at 30°C on Purina Rat Chow and distilled water. Developmental synchrony was maintained by regulating temperature and food availability at each molt (Kunkel, 1966). During the last larval instar, a 70% starch, 30% Rat Chow diet was administered to diminish fat body urate stores (Parsons *et al.*, 1981). Synchronous, newly emerged adult females were fed, mated with adult males, and incubated at 30°C. In all isotopic and drug studies, adult females at peak vitellogenesis (Day 5 following emergence) were used.

Antisera production. Vitellin used as immunogen was purified from eggs by salt fractionation, TEAE-Cellulose chromatography, and sedimentation in glycerol gradients (5% to 30%) (Kunkel and Pan, 1976). Antiserum was produced in rabbits (Vaitukaitis, 1981) and absorbed with hemolymph (10% in phosphate-buffered saline (PBS); 2.7 m*M* KCl, 0.9 m*M* $CaCl_2$, 0.5 m*M* $MgCl_2\,6H_2O$, 137 m*M* NaCl, 1.4 m*M* KH_2PO_4, 8.1 m*M* $Na_2HPO_4 \cdot 7H_2O$, pH 7.4) collected from avitellogenic last-larval insects in order to precipitate a minor population of antibodies directed against contaminating serum proteins. The IgG fraction then was prepared by precipitation using Na_2SO_4 and by DEAE-Cellulose chromatography. Antiserum to unfractionated hemolymph of larval insects was prepared similarly.

Immunoprecipitation of vitellogenin. Vitellogenin was isolated from fat body and from hemolymph samples by immunoprecipitation with vitellin antiserum. Tissues were prepared and processed at 4°C. Hemolymph samples (4 μl) were expressed from limbs autotomized at the trochanter, collected in microcapillary tubes, and dispersed into 9 vol of PBS, 10^{-4} *M* PMSF. Samples were cleared of hemocytes by centrifugation (10 min at 500*g*) and were adjusted to 0.4 ml with 0.3 *M* NaCl, 1% Triton X-100, 1% sodium deoxycholate, 50 m*M* Tris-HCl, pH 8.1, 5×10^{-4} *M* PMSF, 5×10^{-4} *M* TLCK (NTDT buffer). Fat bodies were dissected and rinsed in cockroach Ringer's solution (Prosser and Kirschner, 1973), homogenized in 0.4 ml of NTDT, and their high-speed supernatants (20 min at 15,000*g*) recovered. Vitellogenin was immunoprecipitated by the incubation of samples with 0.1 ml of antiserum to vitellin and 5 μg of purified vitellin (as carrier) for 30 min at 30°C, and for 1 hr at 4°C. Immunoprecipitates were collected by centrifugation, washed in NTDT, and solubilized in sample buffer for SDS PAGE (Laemmli, 1970). Freezing of samples prior to immunoprecipitation reduced the recovery of vitellogenin and was avoided.

Drug and isotope treatment. Based on preliminary labeling experiments using [2-^{3}H]Man, the secretion of glycosylated vitellogenin into hemolymph was inhibited efficiently in insects injected with 0.25 μg, or more, of tunicamycin and incubated for 4 hr at 30°C. Unless indicated otherwise, this dosage was administered in each experiment. Tunicamycin was dissolved in Me_2SO and diluted with an equal volume of Ringer's solution prior to injection (Uni-Metric repeating syringe). Radioisotopes were prepared and injected in Ringer's solution. Total injected volumes did not exceed 5 μl per insect.

Electrophoresis. Fused quantitative immunoelectrophoresis (Laurell, 1966) was performed in 1% Agarose gels which were 50 m*M* in Tris-citrate buffer, pH 8.6. SDS PAGE (Laemmli, 1970) of vitellogenin immunoprecipitates and vitellin was performed in 3 to 15% linear gradient gels. Electroelution of peptides from gel slices was performed at constant current (20 mA) in SDS PAGE electrode buffer using a multisample device constructed essentially according to Green *et al.* (1982).

CNBr cleavage of purified vitellin subunits. Following SDS PAGE of purified vitellin (see above), the M_r 102,000, 95,000, and 50,000 subunits were isolated by electroelu-

[6] The abbreviations used are: BSA, bovine serum albumin; CNBr, cyanogen bromide; GlcNAc, *N*-acetyl glucosamine; GlcN, glucosamine; Man, mannose; Met, methionine; PMSF, phenyl methyl sulfonyl fluoride; TLCK, *N*-[α]-*p*-tosyl lysine chloromethyl ketone hydrochloride; FITC-Concanavalin A, fluorescein isothiocyanate-Concanavalin A; Me_2SO, dimethyl sulfoxide; PBS, phosphate-buffered saline; SDS PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TEAE-Cellulose, *O*-(triethylaminoethyl)-cellulose; DEAE-Cellulose, *O*-(diethylaminoethyl)-cellulose.

tion from gel slices. SDS and Coomassie blue were extracted by the method of Henderson *et al.* (1979). Each isolated peptide then was lyophilized, dissolved in 70% formic acid, and incubated with CNBr (Croft, 1980). The resulting peptide fragments were separated by SDS PAGE in 15% gels and were stained using FITC-Concanavalin A.

FITC-Concanavalin A binding analyses. In analyses of FITC-Concanavalin A binding to purified vitellogenin peptides and to CNBr peptide fragments, polyacrylamide gels were incubated sequentially with glutaraldehyde, borohydride, and lectin (Tanner and Anstee, 1975) and photographed using UV illumination and a Wratten type 65 filter.

Fluorography and scintillation spectrophotometry. In fluorography, gels were treated with EnHance, dried, and exposed at −70°C for 24 hr. Counting of radiolabeled, immunoprecipitated vitellogenin and purified vitellin was accomplished using Protosol and Liquifluor. Spillover between ^{3}H and ^{35}S windows was determined using channel ratios (Bush, 1964), an internal standard, and standards quenched with dinitrophenol.

RESULTS

Specificity of Vitellin Antiserum for Vitellogenin

Isolation of vitellogenin from fat body and hemolymph samples required the use of antiserum prepared against purified oocyte vitellin. Following absorption with hemolymph from avitellogenic larval insects, the specificity of this antiserum for vitellogenin was assessed by fused quantitative immunoelectrophoresis (Fig. 1). Hemolymph collected from vitellogenic insects at Day 5 following adult emergence was mixed by diffusion from adjacent wells in agarose gels with purified vitellin as a standard, and with hemolymph sampled either from adult males, larval females, or females in their last-larval instar. Following diffusion, samples were electrophoresed through a lower gel region containing vitellin antiserum. Only the vitellin standard and a single hemolymph protein specific to adult females, i.e., vitellogenin, were immunoprecipitated. As indicated by the pattern of fused immunoprecipitates, vitellogenin possessed all antigenic determinants contained in vitellin. The minor region of partial immunological nonidentity detected within vitellogenin (Fig. 1, arrows) is believed to represent determinants lost from vitellin during its processing in oocytes (see below). Each sample also was electrophoresed further into an upper gel region containing antiserum to unfractionated larval hemolymph. Despite the demonstrated presence in all hemolymph samples of serum proteins at high concentrations (upper gel), none of these proteins were precipitated by absorbed vitellin antiserum (lower gel). Similarly, as assayed by SDS PAGE and fluorography (see below), no ^{35}S-labeled proteins prepared from the fat body or hemolymph of adult male or larval insects were precipitated by vitellin antiserum further demonstrating its specificity for vitellogenin.

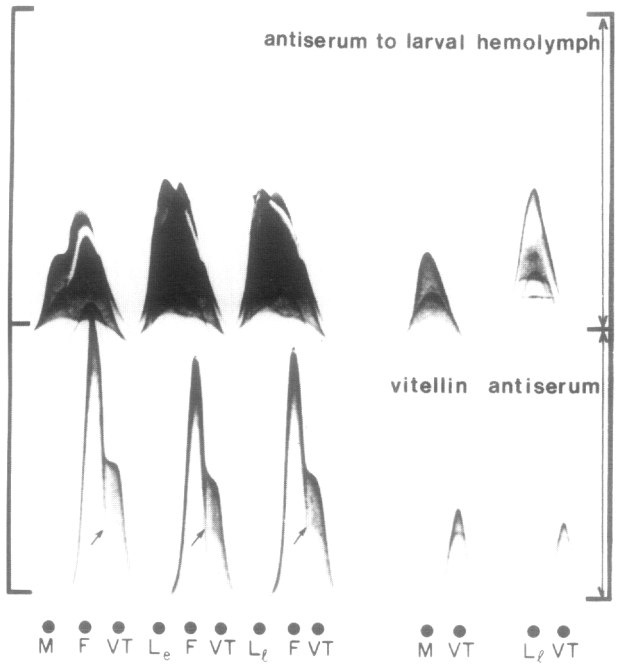

FIG. 1. Specificity of vitellin antiserum for vitellogenin demonstrated by fused quantitative immunoelectrophoresis. Hemolymph sampled from vitellogenic adult female insects (F) was mixed by diffusion from adjacent wells in an agarose gel with (i) a purified vitellin standard (VT), and (ii) with hemolymph sampled from insects at various developmental stages (adult males, M; early larval females, L_e; females in their last-larval instar, L_1). Samples then were electrophoresed through two gels; a lower gel containing antiserum to vitellin, and a second gel containing antiserum to unfractionated larval hemolymph. Antigens bound, and precipitated by each antiserum were stained with Coomassie Blue R-250.

Tunicamycin Inhibition of the Processing of Vitellogenin in Fat Body

Biosynthetic processing of vitellogenin in fat body was analyzed initially by injecting insects with [^{35}S]Met, isolating vitellogenin by immunoprecipitation, and determining its subunit composition by SDS PAGE and fluorography. Vitellogenin immunoprecipitated from fat bodies of control insects (Fig. 2, center lanes) contained major peptides of M_r 160,000 (VG160) and 102,000 (VG102), as well as a M_r 240,000 component (proVG). In contrast, vitellogenin secreted from fat body and immunoprecipitated from hemolymph samples (Fig. 2, left lanes) was composed almost exclusively of VG160 and VG102 and contained no appreciable amounts of high M_r peptides, including proVG. Scanning of fluorographs

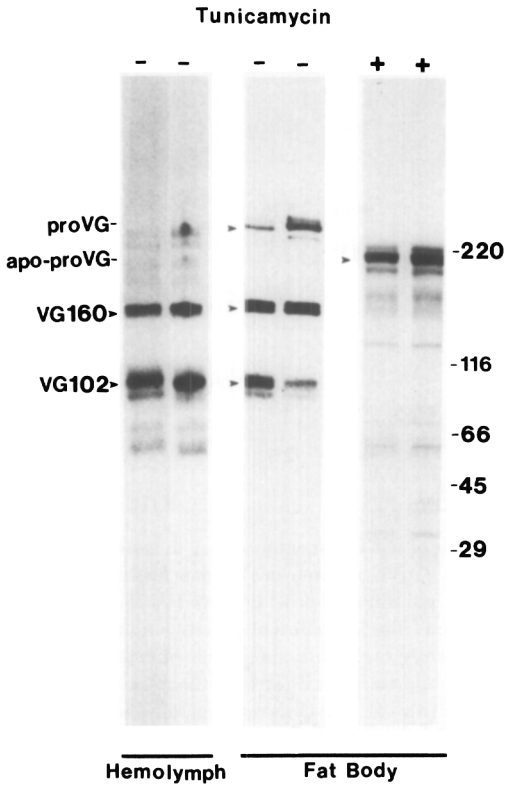

FIG. 2. Inhibition by tunicamycin of vitellogenin processing in fat body. Vitellogenic insects were injected with tunicamycin (0.25 μg) (+) or with 50% Me_2SO in Ringer's solution as a control (−). Vitellogenin, labeled for 1 hr at 30°C by injecting [^{35}S]Met (20 μCi per insect), was immunoprecipitated from fat body or hemolymph samples using antiserum to vitellin. The substructure of ^{35}S-labeled vitellogenin then was assessed by SDS PAGE and fluorography. Each lane contains the vitellogenin isolated from the fat body or hemolymph sampled from a single insect. Positions of M_r standards are indexed in the right margin (myosin, 220,000; β-galactosidase, 116,000; BSA, 66,000; ovalbumin, 45,000; carbonic anhydrase, 29,000). The estimated M_r of designated vitellogenin peptides are proVG, 240,000; apo-proVG, 215,000; VG160, 160,000; VG102, 102,000.

by soft-laser densitometry served to illustrate these results quantitatively (Figs. 3A, B). Based on the absence in hemolymph of detectable amounts of vitellogenin peptides of high M_r, the possibility was examined that VG160 and VG102 derive from the M_r 240,000 proVG peptide within fat body prior to secretion.

Additional information concerning processing in fat body was provided by use of tunicamycin to inhibit the synthesis of high-mannose oligosaccharides normally attached to vitellogenin of *B. germanica* (Nordin *et al.*, 1984). Preliminary experiments served to establish minimal doses of tunicamycin which efficiently inhibited the incorporation of [2-^{3}H]Man into secreted hemolymph vitellogenin (0.25 μg tunicamycin per insect, see Methods). Analyses of the substructure of ^{35}S-labeled vitellogenin immunoprecipitated from fat bodies of insects administered tunicamycin at this dosage revealed an apparent accumulation of a major M_r 215,000 vitellogenin peptide designated apo-proVG (Fig. 2, right lanes and Fig. 3C). The M_r 240,000 proVG peptide of control insects was absent, and only minor amounts of the VG160 and VG102

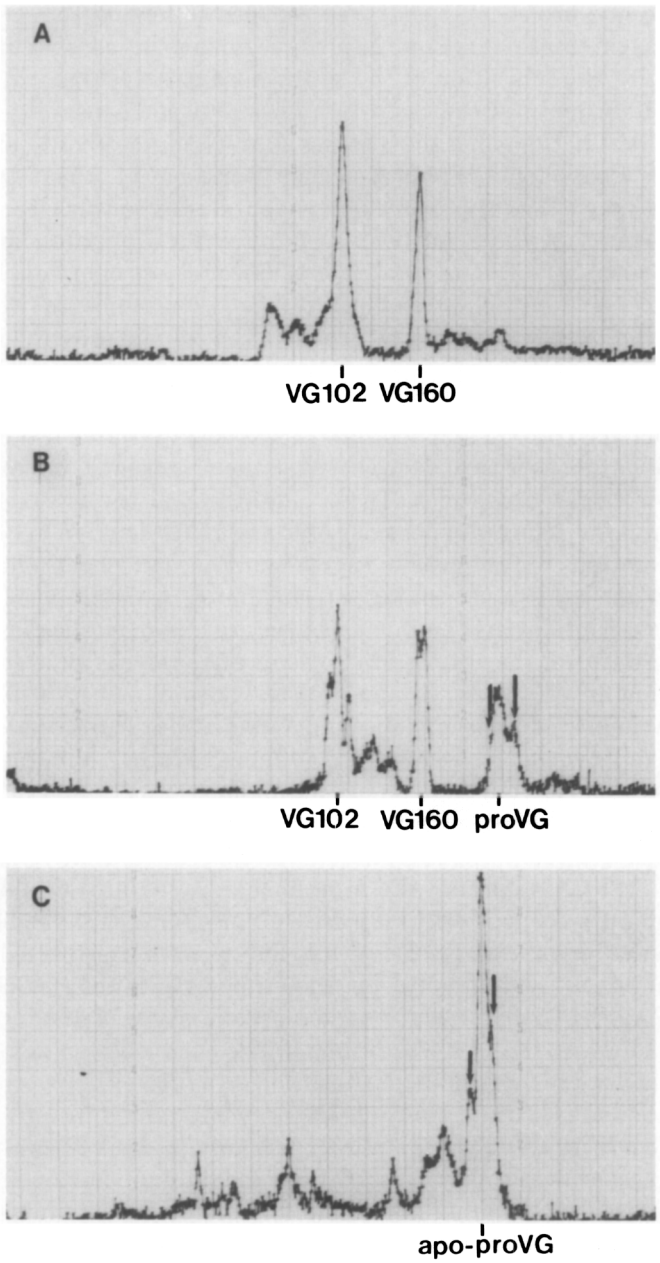

FIG. 3. Inhibition by tunicamycin of vitellogenin processing in fat body illustrated by scanning densitometry of fluorographs. Vitellogenin labeled with [^{35}S]Met was immunoprecipitated from hemolymph (A), from fat body (B), and from the fat body of insects injected with tunicamycin (C). Following SDS PAGE, fluorographs (see Fig. 2) were analyzed by scanning densitometry. Designation of vitellogenin peptides is as in the legend of Fig. 2. The positions of two minor high M_r vitellogenin peptides of fat body (see text) are indexed by arrows (B and C). The left margin corresponds to the bottom of SDS polyacrylamide gels.

peptides were detected. The simplest interpretation of these data is that the M_r 215,000 vitellogenin peptide (apo-proVG) represents an uncleaved, nonglycosylated precursor of proVG. This interpretation is supported by the following observations.

First, the M_r of proVG in SDS PAGE (240,000) relative to apo-proVG (215,000) was reduced following tunicamycin treatment (Figs. 2 and 3) by an amount consistent with the inhibition of the synthesis and attachment to vitellogenin of the 14 to 16 high-mannose oligosaccharides of the general structure $Man_9GlcNAc_2$ known to be components of this molecule (Nordin *et al.*, 1984). It is noted that the molecular weights assigned to vitellogenin peptides do not account for the lipidation or phosphorylation of vitellogenin. Second, a proportional decrease in the apparent M_r of the two minor satellite vitellogenin peptides of proVG likewise was observed following tunicamycin treatment (Fig. 2, center and right lanes; Figs. 3B, C, arrows). Thus, correspondence between proVG and apo-proVG was substantiated by an apparent correspondence between their associated minor vitellogenin peptides. These peptides, and the various minor vitellogenin peptides of lower M_r detected in fluorographs are believed to represent either heterogeneous or alternative processing intermediates, or possibly the pro-peptides and products of discrete minor vitellogenins. Third, the results of two separate analyses showed that proVG, but not apo-proVG, contains substantial amounts of high-mannose oligosaccharide. These analyses involved (i) assay of FITC-Concanavalin A binding to each isolated peptide, proVG and apo-proVG, and (ii) direct measurements of the glycosylation of vitellogenin synthesized in the presence, or absence, of tunicamycin.

In FITC-Concanavalin A binding analyses, proVG and apo-proVG peptides first were isolated from the fat bodies of approximately 20 control, and tunicamycin-treated animals, respectively, by immunoprecipitation, SDS PAGE, and electroelution from gel slices (see Methods). Following SDS PAGE to immobilize each isolated peptide and to confirm its purification, gels then either were incubated with lectin (Fig. 4B) or were stained for protein (Fig. 4A). ProVG (lane 2), but not apo-proVG (lane 1), specifically bound FITC-Concanavalin A. Levels of nonspecific binding were low: co-incubations with D-mannose inhibited the binding of FITC-Concanavalin A to proVG and to a selected standard, ovalbumin, and no binding of lectin to nonglycosylated standards was observed.

Direct measurements of glycosylation were accomplished by co-injecting [^{35}S]Met and [^{3}H]GlcN and monitoring the incorporation of each isotope into immunoprecipitated vitellogenin (Fig. 5). In control insects, fat body vitellogenin was labeled readily with each isotope and was secreted into hemolymph (Figs. 5A, B). Within

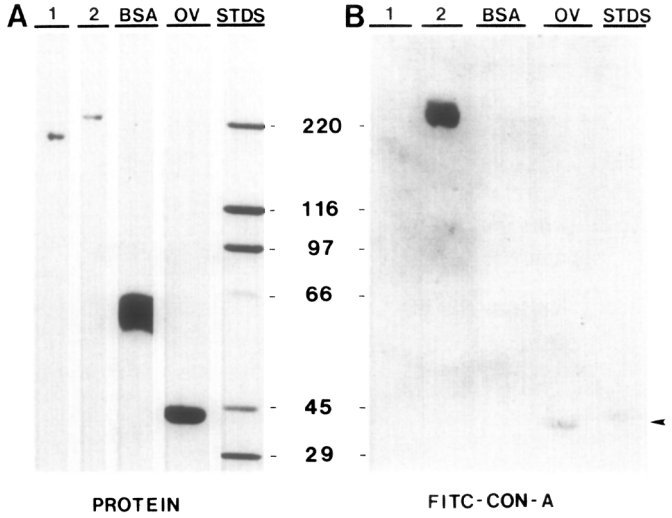

FIG. 4. Binding of FITC-Concanavalin A to isolated proVG and apo-proVG peptides. ProVG and apo-proVG peptides were purified by electroelution from gel slices following SDS PAGE of vitellogenin immunoprecipitated from fat bodies of control, and tunicamycin-treated insects, respectively (see Methods). Each purified peptide then was electrophoresed and stained either with Coomassie blue to confirm its purity (A), or with FITC-Concanavalin A (B). The fluorescence image was reversed to provide contrast. Controls for the specific and nonspecific binding of FITC-Concanavalin A included ovalbumin (OV, 10 μg; arrow, right margin) and BSA (10 μg), respectively. Lane 1, apo-proVG; lane 2, proVG. M_r standards (STDS) are designated as in the legend to Fig. 2.

4 hr following isotope injections, approximately 40% of this secreted vitellogenin had been sequestered by oocytes (Fig. 5C). By comparison, in insects injected with tunicamycin the average rate of incorporation of [^{3}H]GlcN into fat body vitellogenin was inhibited by 98%. Since the majority of this vitellogenin was shown to occur as apo-proVG (Figs. 2 and 3), these results establish directly the inhibition by tunicamycin of the glycosylation of this peptide. Measurements of the incorporation of [^{35}S]Met also showed that tunicamycin reduced the rate of synthesis of vitellogenin by as much as 59% of control values.

Tunicamycin Inhibition of Vitellogenin Secretion

Aglycosyl vitellogenin synthesized in fat body following tunicamycin treatment was not secreted. This result was established by the above double-labeling experiments (Figs. 5A, B). Further, as shown by treating insects with varying amounts of tunicamycin (0 to 2 μg per insect) for 4 hr at 30°C, the minimum dosage of tunicamycin which effectively inhibited the glycosylation of vitellogenin (0.25 μg per insect) also completely inhibited its secretion. This effect was reversible and within 24 hr after tunicamycin injection, rapid secretion of normally processed vitellogenin resumed. Thus, these

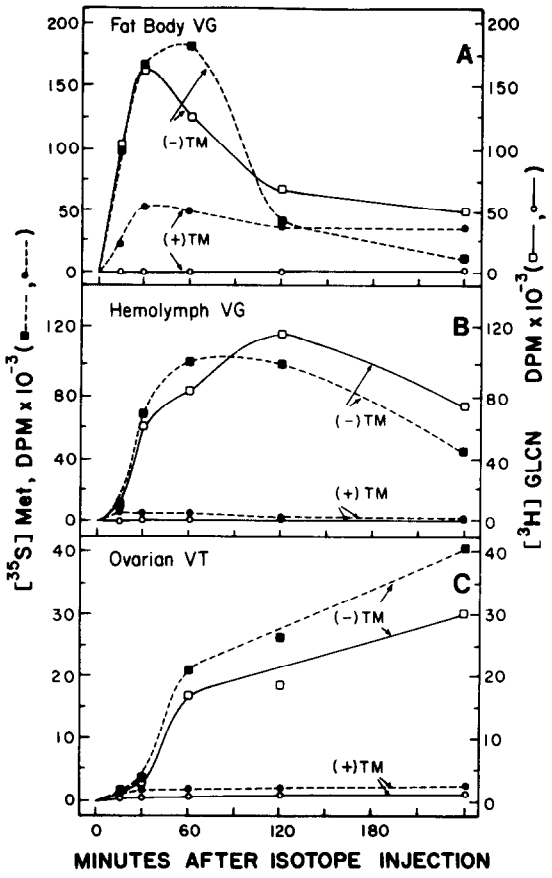

FIG. 5. Tunicamycin inhibition of the glycosylation and secretion of vitellogenin. Vitellogenic insects were injected with tunicamycin (0.25 μg) (●, ○), or with 50% Me_2SO in Ringer's solution as a control (■, □), and incubated for 4 hr at 30°C. Vitellogenin then was labeled by injection of [^{35}S]Met (3 μCi) (●, ■) and [^{3}H]GlcN (3 μCi) (○, □). At the indicated intervals following isotope injections, the amount of each isotope contained in vitellogenin (VG) immunoprecipitated from fat body (A) and hemolymph (B) and in vitellin (VT) purified from ovaries by TEAE-Cellulose chromatography (C) was determined.

results are consistent with an essential role for glycosylation not only in the proteolytic processing of vitellogenin in fat body, but also in its intracellular transport.

Proteolytic Processing of VG160 in Oocytes

In control insects, secreted vitellogenin was sequestered rapidly by developing oocytes (Fig. 5C). Analyses of the peptide substructure of this vitellin revealed that an additional step in processing occurs during oogenesis. During Day 3 to Day 5 of the vitellogenic cycle, the vitellin peptide corresponding to the VG160 peptide of hemolymph vitellogenin was lost concomitantly with the appearance of two vitellin peptides of M_r 95,000 and 50,000 (Fig. 6). This apparent processing of VG160 occurred prior to chorionation, and thus is distinct from the subsequent degradative processing of yolk which accompanies embryogenesis (Storella and Kunkel, 1979). In this experiment, the course of the vitellogenic cycle was established using vitellin antiserum and quantitative immunoelectrophoresis to determine the concentrations of vitellogenin in the hemolymph of synchronous adult female insects. Vitellogenesis began on Day 1 following adult emergence. Maximal concentrations of hemolymph vitellogenin were detected on Day 5, and eggs were ovulated on Day 7. Additional evidence that the M_r 50,000, 95,000, and 102,000 subunits of vitellin in mature oocytes represent discrete peptides was given by analyses of the binding of FITC-Concanavalin A to CNBr fragments prepared from each isolated subunit. At least one unique glycosylated fragment in each subunit was resolved following SDS PAGE (Fig. 7). Thus, these data also indicate that each subunit contains one, or more, sites of glycosylation.

DISCUSSION

Several discrete tissue-specific events in the processing of vitellogenin of *B. germanica* were identified and are summarized in Fig. 8. Processing proceeds from the glycosylation of apo-proVG in fat body yielding a M_r 240,000 proVG peptide. Prior to secretion, proVG then is cleaved (limited cleavage No. 1) to VG160 and VG102. Subsequent uptake of secreted vitellogenin from hemolymph by developing oocytes leads to the further processing of VG160 (limited cleavage No. 2) to M_r 50,000 and 95,000

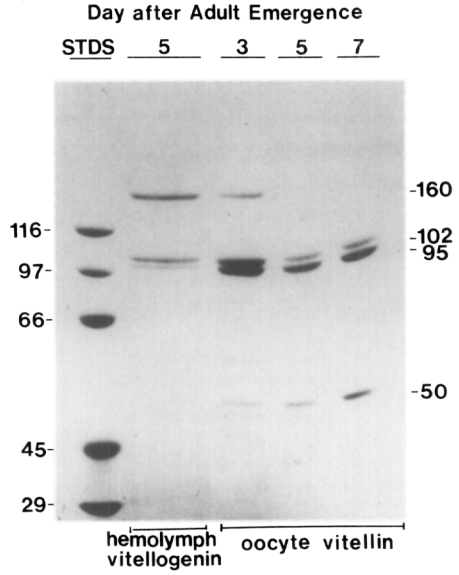

FIG. 6. Proteolytic processing of VG160 in developing oocytes. The substructure of vitellogenin purified from hemolymph at Day 5 following adult emergence, and of vitellin purified from oocytes at Days 3, 5, and 7 was assessed by SDS PAGE and Coomassie blue staining. The estimated M_r of vitellin peptides ($M_r \times 10^{-3}$) is indexed in the right margin, and the M_r of standards (STDS) ($M_r \times 10^{-3}$) in the left margin.

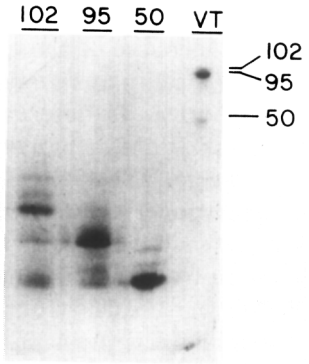

FIG. 7. Binding of FITC-Concanavalin A to CNBr fragments of the purified subunits of vitellin. The M_r 102,000, 95,000, and 50,000 subunits of vitellin were isolated by electroelution from gel slices following SDS PAGE of purified vitellin. Purity of the isolated subunits was confirmed prior to further analysis. Each subunit then was cleaved using CNBr and the resulting fragments were separated by SDS PAGE. The gel then was stained using FITC-Concanavalin A (see Methods). Lanes containing the products of each cleaved subunit are designated by their initial M_r (102, 102,000; 95, 95,000; 50, 50,000). Purified vitellin (VT) also was electrophoresed in order to establish the positions of each intact subunit (right margin).

peptides. Together with VG102, these peptides comprise the subunits of mature vitellin. Previously, the substructure of vitellogenin of *B. germanica* has been studied using hemolymph of ovariectomized insects as a source of protein (Kunkel *et al.*, 1981). Minor amounts of VG160 were detected, but neither its processing products nor the normal developmental stage or tissue involved in its proteolysis were identified. Also, the vitellin subunits of M_r 95,000 and 102,000 were not resolved. With regard to oocyte-directed processing, a pattern of cleavage of vitellin subunits similar to that proposed for *B. germanica* has been reported in the cockroach *L. maderae* (Koeppe and Ofengand, 1976) and was shown to be associated with trimerization of vitellin during its storage in eggs (Masler and Ofengand, 1982). In *B. germanica* stage-specific trimerization of vitellin subunits (18S → 33S; Fig. 8) likewise occurs following ovulation (Storella and Kunkel, 1979; Gochoco *et al.*, 1984). Accordingly, the potential role of a stage-specific protease in the packaging of yolk in these species currently is being investigated, as is a documented loss of lipid (unpublished observation, J.G.K.) which accompanies the ovarian uptake of vitellogenin (17S → 18S; Fig. 8). By comparison, ovarian processing in vertebrates of vitellin to lipovitellin, phosvitin, and phosvette subunits is recognized as a prerequisite to the packaging of yolk in platelets (Tata and Smith, 1979; Christmann *et al.*, 1977).

With regard to the processing of vitellogenin of *B. germanica* in fat body, glycosylation was indicated to be required for normal cleavage of a major pro-vitellogenin peptide. This was shown by a lack of the normal cleavage of apo-protein synthesized following tunicamycin injection (Figs. 2 and 3). Thus, attachment of oligosaccharide is suggested to be required for apo-proVG's recognition by, or transit to, compartmentalized fat body proteases. Additional evidence for lack of efficient transport of apo-proVG in fat body was given by the essential lack of its secretion (Fig. 5). However, whether secretion of vitellogenin depends only upon glycosylation or also involves subsequent cleavage presently is unresolved. In control insects, a small amount of unprocessed ^{35}S-labeled proVG peptide was detected in hemolymph samples pooled from several insects (unpublished results). Thus, unlike glycosylation, proteolysis may not necessarily be required for vitellogenin secretion. Alternatively, since vitellogenin in *B. germanica* occurs as a dimer in its native form (M_r 560,000; 2 × 240,000 plus 12% lipid) it is possible that this proVG is secreted in association with processed VG160 and VG102.

Based on a similar lack of the normal proteolytic processing and secretion of apo-proteins, the pro-peptides of several vertebrate (Roos *et al.*, 1980) and viral glycoproteins (Ng *et al.*, 1982) have been identified using tunicamycin. Also, during the preparation of this manuscript an apo-pro-vitellogenin in fat body of *L. migratoria* tentatively has been identified using tunicamycin to inhibit its processing (Wyatt *et al.*, 1984). Alterna-

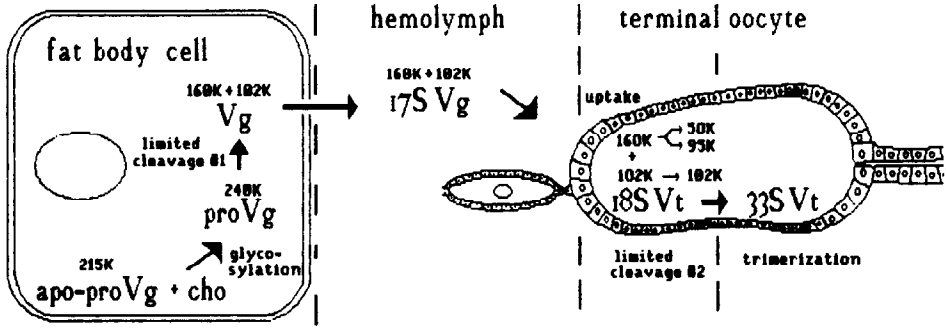

FIG. 8. Proposed processing of vitellogenin in *B. germanica*.

tively, for certain other glycoproteins (Budarf and Herbert, 1982; Mous *et al.*, 1982; Hanley *et al.*, 1982) including vitellogenin of *Xenopus laevis* (Gottlieb and Wallace, 1982), normal processing and transport occurs in the absence of glycosylation indicating that the precise role of attached saccharide varies among glycoproteins (Sidman, 1981). For insect vitellogenin, whatever the range of functions performed by oligosaccharide moieties it must be accomplished within the relatively narrow range of variability observed in the oligosaccharide structure of insect glyco-conjugates (Ng and Dain, 1976; Hughes and Butters, 1981; Nordin *et al.*, 1984). In this context it is noteworthy that integrity of the high-mannose oligosaccharide of vitellogenin in *B. germanica* also is required for its recognition and uptake by developing oocytes (Gochoco *et al.*, 1984).

The present study represents the first example among insects in which discrete events accounting for derivation of a multimeric vitellin from its fat body precursor have been resolved. Using *in vitro* systems, Chen (1980) and Koeppe and Ofengand (1976) identified fat body vitellogenin peptides of approximately M_r 250,000 as provitellogenins in *L. migratoria* and in *L. maderae*, respectively. However, due to the complex structure of these vitellogenins and to limitations associated with the *in vitro* systems employed, the relationship between these putative precursor peptides and the subunits of each vitellogenin and vitellin was not defined. In *B. germanica*, establishment of a role for high-mannose oligosaccharide in the proteolytic processing and transport of pro-vitellogenin also provides a basis for investigations of the subcellular location of certain glycosidases and proteases involved in vitellogenin assembly and secretion. Identification of additional stage-specific processing of vitellin after entry into oocytes, analogous to that occurring among vertebrate vitellins, offers insight into its native structure, its processing and storage, and its patterned utilization as nutrient during embryogenesis.

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