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MOSQUITO VITELLOGENIN SUBUNITS ORIGINATE FROM A COMMON PRECURSOR

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Using a cell-free translation system, we demostrated that the two subunits of mosquito vitellogenin (VG), 200 kDa and 65 kDa, originate from a common precursor. The precursor polypeptide of 220 kDa is a translation product specific to mRNA from vitellogenic mosquitoes. In immunoprecipitation analysis, the 220-kDa polypeptide was recognized by monoclonal antibodies directed either to the large or the small VG subunit. Peptide mapping showed homology between the 220-kDa polypeptide and both subunits, thus providing further proof that the 220-kDa product of translation is the precursor for both VG subunits. In the presence of microsomal membranes, the molecular size of the VG precursor increased to 235 kDa suggesting this as a first step in co-translational modifications of VG.

The major insect yolk protein, vitellogenin (VG), is synthesized by the fat body, secreted into hemolymph, and selectively taken up by developing oocytes where it is stored as vitellin (1). Vitellogenin(s) are synthesized in large quantities in response to hormonal stimuli, and the expression of their genes is sex-, stage-, and tissue-specific. Due to these features, insect vitellogenesis has been widely used for studying gene expression (2). Understanding of the mechanisms governing expression of VG genes is important because vitellogenesis is a key process in egg maturation in insects.

A knowledge of primary gene products and their co- and post-translational modifications is critical for the study of VG gene expression. Detail information is available for *Drosophila* yolk proteins which are only about 45 kDa in size (2). In contrast, vitellogenins of most insects are composed of large (100-180 kDa) and small (47-84 kDa) polypeptides (1). For several insects, these VG subunits appear to be coded by a single mRNA, while for others, independent precursors have been suggested for each VG subunit (3-8). A cell-free translation system was used in only a few of these studies (3,4). In most cases, conclusions about the primary precursor(s) of VG were based on translation of mRNAs in the *Xenopus* oocyte system (which has the capability of protein processing) or from pulse-chase and inhibition studies (3,5-8).

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Previously, we demonstrated that mosquito VG consists of two subunits: a large subunit of 200 kDa and a small subunit of 65 kDa (9). Here, using a cell-free translation system, we report that both the subunits of mosquito VG are first synthesized as a single large precursor of 220 kDa. We have also identified a first step in modification of this VG precursor that occurs co-translationally.

MATERIALS AND METHODS

Animals. *Aedes aegypti* larvae reared at 27°C were fed on the standardized larval diet. Adult females were fed on rats to initiate vitellogenesis. Nonvitellogenic females and males were maintained by feeding 10% sucrose solution.

Preparation of Polyadenylated RNA. Total RNA from fat bodies of female and male mosquitoes was extracted using the guanidine isothiocyanate method (10) as modified by Chomczynski and Sacchi (11). Polyadenylated mRNA [poly(A+) RNA] was obtained by chromatography on an oligo(dT)-cellulose column following the conditions previously described (12,13).

In Vitro Translation of RNA and Processing of Proteins. The translation assay (50 ul) contained 40 ug total RNA or 4 ug poly(A+) RNA, 1 mM amino acid mixture (-methionine), 35 ul rabbit reticulocyte lysate (Promega Biotech) and 5uCi [^{35}S] methionine (NEN 1078 Ci/m mole). In some experiments, 1.8 ul of canine pancreatic microsomal membranes (Promega) was added to 25 ul of total translation mixture. Incubations were carried out at 30°C for 1 h and the reaction was stopped by chilling on ice. Efficiency of translation was estimated by counting the total [^{35}S] radioactivity incorporated into the in vitro synthesized proteins.

Immunoprecipitation of In Vitro Translated Products. To 20 ul of translation medium, 200 ul of TNTE buffer [50 mM TRIS, pH 7.2, 150 mM NaCl, 1% Triton X-100, 5 mM EDTA, 1 mM phenylmethylsulfonylfluoride, 5 mM aminocaproic acid, 1 mM benzoamidine] was added. The translation mixture was first pretreated by incubating for 2 h with 30 ul of 10% solution of rabbit anti-mouse IgG coated Pansorbin (Calbiochem). The supernatant was then incubated for 1 h with VG subunit-specific monoclonal antibodies (mAB); 30 ul of rabbit anti-mouse IgG coated Pansorbin were then added and incubated for 2 h at 4°C. The immune complex was recovered by centrifugation and processed for electrophoresis and fluorography.

Production of Monoclonal Antibodies and Immunoblotting. Production and characterization of mABs specific to large and small VG subunits were as described previously (14). Protein immunoblotting was performed according to Towbin et al. (15) with modifications for mABs described earlier (14).

Peptide Mapping. Vitellogenin was labelled metabolically with [^{35}S]-methionine and the [^{35}S]-labelled precursor polypeptide was obtained by in vitro translation of poly(A+) RNA from vitellogenic fat bodies. Vitellogenin subunits and the 220-kDa precursor were separated by SDS PAGE and subjected to peptide mapping by limited proteolysis with *Staphylococcus aureus* V8 protease (16).

RESULTS

In this study, monoclonal antibodies directed against either one of the two subunits of mosquito VG have been utilized to identify the primary gene product(s). On immunoblots, these mABs specifically recognize either a large VG subunit of 200 kDa or a small one of 65 kDa (Fig. 1). In immunoprecipitation analysis, mABs directed against either a large or small subunit were equally efficient in precipitating the intact VG molecule (Fig. 1).

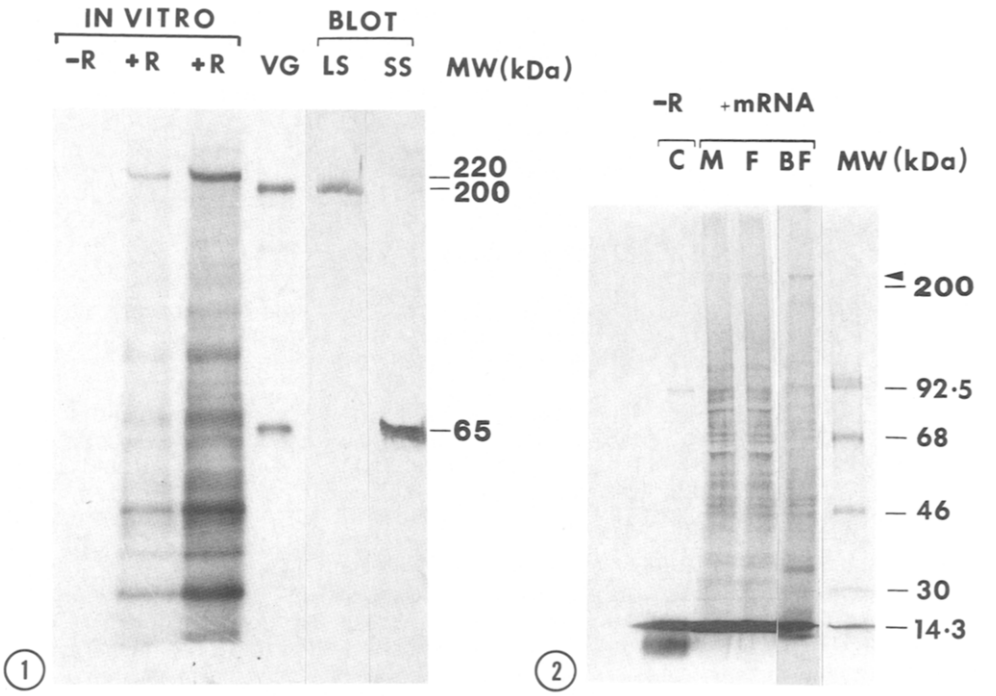

Fig. 1. An in vitro translation of mRNA and immunological analysis of VG from fat bodies of vitellogenic mosquitoes.

IN VITRO: Poly(A+) RNA isolated from fat bodies of vitellogenic mosquitoes was translated in vitro and products analyzed using 5-10% SDS-PAGE followed by fluorography. -R, minus RNA control; +R, total translation products of poly(A+) RNA from the fat body.

VG: vitellogenin subunits after immunoprecipitation of the fat body homogenate by a mAB (B11D12) against the small VG subunit.

BLOT: Specificity of mABs, A1D12 and B11D12, to the large (LS), 200-kDa, and small (SS), 65-kDa, subunits respectively on immunoblotting of proteins from vitellogenic fat bodies separated by SDS-PAGE (5-10% gradient).

Fig. 2. Electrophoretic analysis of in vitro translation products of mRNA isolated from male (M), non-vitellogenic (F) and vitellogenic females (BF). The translation products were analyzed using 5-10% SDS-PAGE followed by fluorography. -R, minus RNA control (C); +mRNA, poly(A+) RNA. MW (kDa), molecular weight standards in kilodaltons. An arrow indicates the 220-kDa polypeptide in translation products from vitellogenic females.

To identify the primary translation products of VG gene(s), poly(A+) RNA isolated from the fat bodies of vitellogenic mosquitoes was translated in a rabbit reticulocyte lysate system. Analyses of translation products by SDS-polyacrylamide gel electrophoresis followed by fluorography revealed several polypeptides of which one of major polypeptides had a molecular mass of 220 kDa (Fig. 1).

Since VG is not synthesized by male or previtellogenic female mosquitoes, the specificity of translation products of fat body RNA from vitellogenic females could be identified by comparing with fat body RNA from male and previtellogenic female

mosquitoes. A polypeptide of 220 kDa was synthesized only with poly(A+) RNA from vitellogenic females, even though the electrophoretic profiles of some other translation products generated with fat body RNA from male, previtellogenic and vitellogenic female mosquitoes were similar (Fig. 2).

We analyzed the primary products of translation by immunoprecipitation with mABs specific to the large (mAB A1D12) or small (mAB B11D12) VG subunits. Poly(A+) RNA, isolated from whole vitellogenic female mosquitoes or their fat bodies as well as whole previtellogenic females and males, were translated in cell-free system. Immunoprecipitation of translation products from the vitellogenic fat bodies or whole vitellogenic mosquitoes showed the presence of only one polypeptide of 220 kDa (Fig. 3). Both mABs, specific to either the large or the small VG subunit, used in this analysis recognized the same 220-kDa polypeptide. This suggests that the 220-kDa polypeptide is indeed a common precursor for both the subunits of VG molecule. SDS-PAGE analysis of translation products of poly(A+) RNA from male and previtellogenic female mosquitoes immunoprecipitated using the same mABs did not reveal any polypeptides (not shown).

As an additional evidence on precursor-product relationship between the 220-kDa polypeptide and the two VG subunits, these polypeptides were subjected to

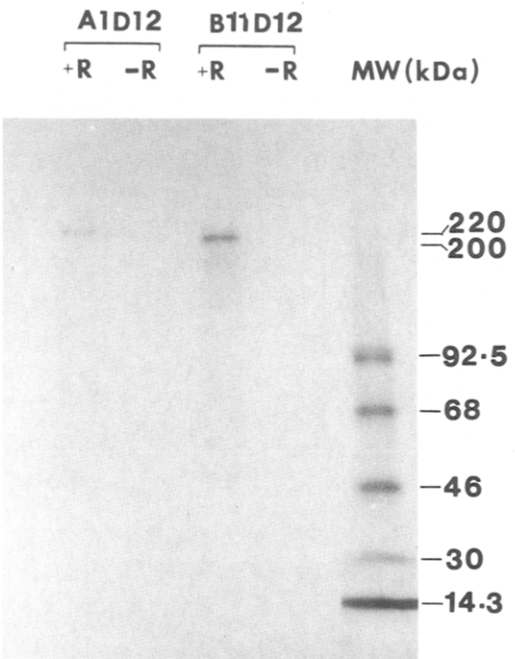

Fig. 3. Analysis of in vitro translation products by immunoprecipitation with monoclonal antibodies.

Translation products of poly(A+) RNA from fat bodies of vitellogenic mosquitoes were immunoprecipitated with mABs specific to the large (A1D12) and small (B11D12) VG subunits. 5-10% gradient SDS-PAGE and fluorography. -R, minus RNA control; +R, in vitro translation of poly(A+) RNA.

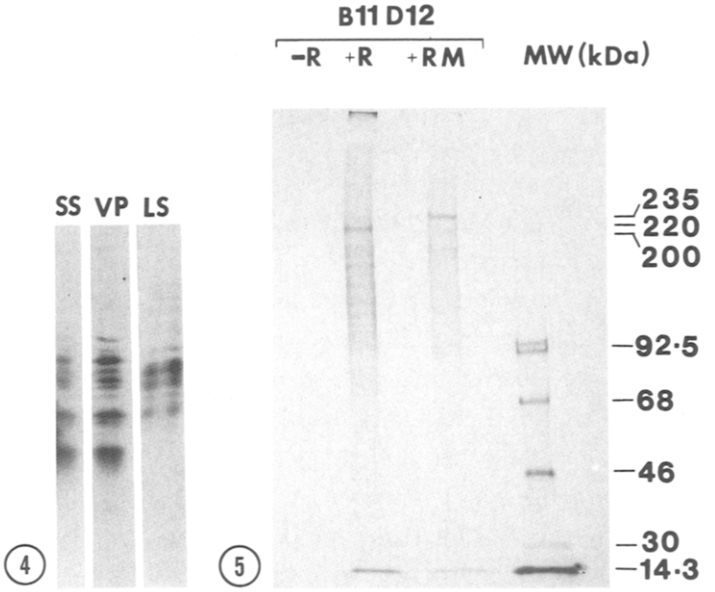

Fig. 4. Peptide mapping with *Staphylococcus aureus* V8 protease of the 220-kDa precursor obtained after in vitro translation and mature VG subunits. The peptides were separated in 15% SDS-PAGE and subjected to fluorography. SS, VP, LS - peptide maps of small VG subunit, VG precursor and large VG subunit respectively.

Fig. 5. In vitro processing of VG precursor by microsomal membranes.

Poly(A+) RNA from vitellogenic fat bodies was translated in the presence of microsomal membranes (+RM). The translation products were immunoprecipitated with a mAB to the small VG subunit (B11D12) and analyzed after electrophoresis followed by fluorography as described above. -R, minus RNA control; +R, plus RNA control, in vitro translation of poly(A+) RNA without microsomal membranes.

peptide mapping using limited proteolysis with *Staphylococcus aureus* V8 protease. The peptide map of the 220-kDa polypeptide had similarities with peptides profiles of both VG subunits generated by limited proteolysis (Fig. 4).

In order to identify the initial step in the processing of the 220-kDa VG precursor, poly(A+) RNA from vitellogenic mosquitoes was translated in vitro in the presence of microsomal membranes. Immunoprecipitation of the translated products with mABs specific to VG subunits revealed that the size of the VG precursor increased to 235 kDa (Fig. 5).

DISCUSSION

We demonstrated that the two subunits composing the mosquito VG originated from a common precursor of 220 kDa. This was established by (1) the speficity of the 220-kDa translation product to poly(A+) RNA from only vitellogenic females, (2) the immunorecognition of the 220-kDa translation polypeptide by mABs directed against either the large or the small VG subunit, and (3) the similarity in the peptide maps of the 220-kDa polypeptide and both VG subunits.

Recently, two VG genes from the mosquito have been isolated (17). Both genes were found to hybridize to mRNA of 6.5 kb which is present only in vitellogenic female mosquitoes (17). The size of the VG precursor detected in our study closely correlates with the size of the product that can be derived from a 6.5 kb mRNA. In another insect, *Locusta migratoria*, which also has VG of large size, VG mRNA was reported to be 6.4 kb in length and its primary product of translation to have a molecular mass of about 200 kDa (6).

The precursors of vitellogenins have been studied in only several insect species. Each of the three *Drosophila* VG polypeptides are coded by separate mRNA (18). In *Bombyx mori*, two independent precursors were suggested for the two VG subunits based on in vitro translation experiments (4). In contrast, a single large VG precursor was identified in several insects with large vitellogenins composed of subunits (3,5-8). In *Locusta*, VG mRNA was first translated into large precursors which were then cleaved to several subunits (5,6). In two species of cockroaches, the VG subunits were also shown to originate from a single precursor as a result of post-translational processing (7,8). This is similar to our findings in the mosquito.

Translation of poly(A+) RNA from vitellogenic female mosquitoes in a cell-free translation system in the presence of microsomal fraction results in an increase in size of the mosquito VG precursor to 235 kDa. An increase in the size of translated products under these conditions is known to be due to co-translational glycosylation (19). This may also be the case for the mosquito VG precursor. Treatment of the primary translation products with endoglycosidase H (Endo H) results in degradation of the 235-kDa precursor processed by microsomal fraction, but not of the non-processed 220-kDa precursor (unpublished data). Since glycosylation has been reported to be required for protein stability (20), the selective degradation of the 235-kDa precursor by Endo H suggests that an increase in molecular mass of the primary VG precursor in the presence of microsomal membranes is most likely due to co-translational glycosylation. The exsistence of similar glycosylation step has previously been reported for the VG precursor of *Blattella germanica by* inhibition of glycosylation with tunicamycin in vivo (7).

Results reported here provide the basis for further expreriments to identify the co- and post-translational processing steps of the mosquito VG into its mature secreted form.

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REFERENCES

1. Kunkel, J.G. and Nordin, J.H. (1985) In: Comprehensive Insect Physiology, Biochemistry and Pharmacology (eds. G.A. Kerkut and L.I. Gilbert), Vol 1, pp. 83-111. Pergamon Press, Oxford.

2. Bownes, M. (1986) Ann. Rev. Ent. 31, 507-531.
3. Harnish, D.G., Wyatt, G.R. and White, B.N. (1982) J. Exp. Zool. 220, 11-19.
4. Izumi, S. and Tomino, S. (1983) Insect Biochem. 13, 81-85.
5. Chen, T.T. (1980) Arch. Biochem. Biophys. 210, 266-276.
6. Wyatt, G.R., Locke, J. and Bradfield, J.Y. (1984) In: Advances in Invertebrate Reproduction (W. Engels ed.) 3, 73-80.
7. Wojchowski, D.M., Parsons, P., Nordin, J.H. and Kunkel, J.G. (1986) Develop. Biol. 116, 422-430.
8. Della-Cioppa, G. and Engelmann, F. (1987) Insect Biochem. 17, 401-415.
9. Raikhel, A.S. and Bose, S.G. (1988) Insect Biochem. (In Press).
10. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294-5299.
11. Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156-159.
12. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, a Laboratory Manual, Cold Spring Harbor Laboratory, New York.
13. Aviv, H. and Leder, P. (1982) Proc. Natl. Acad. Sci. USA 69, 1408-1412.
14. Raikhel, A.S., Pratt, L.H. and Lea, A.O. (1986) J. Insect Physiol. 32, 879-890.
15. Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4350-4354.
16. Cleveland, D.W., Fischer, S.G., Kirschner, M.W. and Laemmli, U.K. (1977) J. Biol. Chem. 252, 1102-1106.
17. Gemmill, R.M., Hamblin, M., Glaser, R.L., Racioppi, J.V., Marx, J.L., White, B.N., Calvo, J.M., Wolfner, M. and Hagedorn, H.H. (1986) Insect Biochem. 16, 761-774.
18. Warren, T.G., Brennan, M.D. and Mahowald, A.P. (1979) Proc. Natl. Acad. U.S.A. 76, 2848-2852.
19. Walter, P. and Blobel, G. (1983) Meth. Enzymol. 96, 84-93.
20. Hohmann, H.P., Bozzaro, S., Merkl, R., Wallraff, E., Yoshida, M., Weinhart, U. and Gerish, G. (1987) EMBO J. 6, 3663-3671.