SEQUESTERED AND INJECTED VITELLOGENIN

Alternative Routes of Protein Processing In *Xenopus* Oocytes

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INTRODUCTION

Vitellogenin (1, 2) is a sex-limited phosphoprotein secreted by the liver in *Xenopus* females (3, 4) and selectively transferred via the circulatory system to growing oocytes, within which it is converted into the yolk proteins lipovitellin and phosvitin (3, 5, 6). Selective uptake (7) and conversion (8) of vitellogenin can also take place in isolated oocytes. The available evidence indicates that vitellogenin is incorporated by a micropinocytotic mechanism and that the derived pinosomes subsequently fuse within the cortex of the oocyte and give rise to yolk platelet primordia (5, 9, 10). The conversion to lipovitellin and phosvitin involves a macromolecular restructuring within yolk platelet primordia rather than breakdown and resynthesis (8, 11), but the conversion mechanism remains unknown.

Protein synthesized by the developing oocyte appears to undergo some degree of turnover whereas sequestered vitellogenin does not (8). This would imply that vitellogenin injected into the cytoplasm of the oocyte might eventually undergo breakdown rather than conversion unless it could traverse the membranes of the pinosomes and primordial yolk platelets. The present report documents a test of this assumption and indicates that indeed vitellogenin injected into, rather than sequestered by, oocytes is not converted to lipovitellin and phosvitin, but instead appears to be simply catabolized.

METHODS

For the preparation of [^{3}H, ^{32}P]vitellogenin, approximately 400 pieces (1 × 2 × 2 mm) obtained from the liver of a female that had been treated with human chorionic gonadotropin (HCG) were incubated in 20 ml of 50% Leibovitz L15 medium containing 10% *Xenopus* male serum, antibiotics, 1 mCi [^{3}H]leucine, and 5 mCi carrier-free $^{32}P_i$ as described elsewhere (11). After 42 h at 20°C, the culture medium was carefully decanted, dialyzed against 0.01 M citric acid-0.06 M 2-amino-2-methyl-1-propanol(monol), centrifuged to remove any debris, and placed on a TEAE-cellulose column (29 × 1.9 cm). [^{3}H, ^{32}P]vitellogenin was eluted by the usual (1,000 ml) gradient procedure (12), concentrated by ultrafiltration (12), and dialyzed against solution O (13), all at 4°C. Samples of the final preparation were placed on either Whatman no. 42 or 3 MM filter paper disks and processed for protein determination using Lab-trol (Dade Div., American Hospital Supply Corp., Miami, Fla.) as a standard (14) or processed for scintillation counting (8), respectively. The final labeled vitellogenin preparation contained 26 cpm as ^{3}H and 117 cpm as ^{32}P per ng protein as measured on disks containing less than 25 μg protein.

Oocytes (0.9–1.0 mm diam) were manually dissected from the ovary of an HCG-treated female (13). 60 oocytes were then either individually injected with 21 nl of the labeled vitellogenin solution (5 ng/nl), using micropipettes attached to a Zeiss micromanipulator (Carl Zeiss, Inc., New York) (15), or were incubated for 30 min in 0.5 ml labeled vitellogenin solution (0.15 mg/ml) and then injected with 21 nl solution O. The injection of each group of oocytes took 60–90 min. All oocytes were then washed 3 times with solution O and, in order to allow metabolism of [^{3}H, ^{32}P]vitellogenin to take place, incubated for an additional 11 h in 50% female *Xenopus* serum dialyzed against solution O.

Upon termination of the incubation period, oocytes were briefly washed several times in solution O, transferred to 0.5 ml 1.5 M NaCl in a small, tight-fitting hand homogenizer, and thoroughly homogenized. The homogenates were then centrifuged (2,000 *g*) in the homogenizer for 30 min to remove a small amount of insoluble material (mostly pigment), and each supernatant was subsequently dialyzed first against 0.01 M citric acid-0.06 M monol-1.0 M NaCl and then against 0.01 M citric acid-0.06 M monol. The dialyzed extracts were applied to a small column (23 × 0.9 cm) of TEAE-cellulose (8) and eluted with a 180-ml 3-bottle gradient (7), using a flow rate of 0.6 ml/min. Samples (up to 0.3 ml) of the effluent fractions were applied with intermittent drying to disks and processed with cold and hot trichloroacetic acid and alcohol-ether for scintillation counting (8). Each disk was counted 10 times for 10 min. All data for ^{32}P were corrected for radioactive decay.

RESULTS AND DISCUSSION

[^{3}H, ^{32}P]vitellogenin with a high specific activity was required for the experiment. This was obtained by culturing liver slices from HCG-stimulated females in the presence of isotopic precursors and male serum (which lacks vitellogenin [3]). A small amount of [^{3}H, ^{32}P]vitellogenin was secreted into the culture medium by the liver slices over 42 h, but it was highly labeled by [^{3}H]leucine and specifically labeled by $^{32}P_i$ (Fig. 1). Its elution position was around 0.64, which corresponds to our previous findings (3).

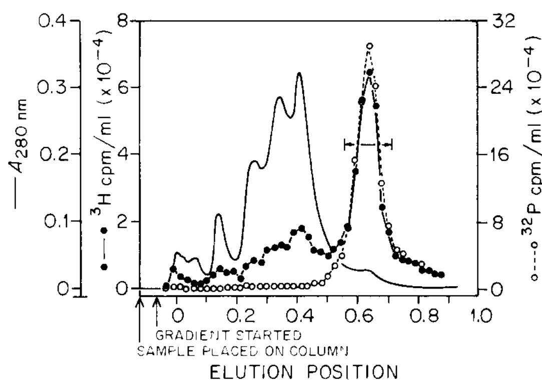

FIGURE 1 Chromatography on TEAE-cellulose of culture medium after incubation with female liver slices. Almost the entire absorbance trace is contributed by unlabeled male serum originally added to the culture medium. Labeled serum proteins were synthesized and secreted into the medium by the liver slices and are indicated by the incorporation of [^{3}H]leucine into protein. The most prominent component, vitellogenin, elutes at position 0.64 and is represented by only a very small amount of protein contributed by the liver slices; it is the only component labeled by $^{32}P_i$ (1, 3). The arrows indicate the fractions collected for concentration.

The fractions containing [^{3}H, ^{32}P]vitellogenin were concentrated, dialyzed against solution O, and adjusted to a concentration of 0.15 mg/ml. Oocytes were then incubated in this medium for 30 min, after which they were washed and, as a control procedure, injected with 21 nl solution O. Under our usual incubation conditions, each oocyte takes up about 0.70 μg vitellogenin/h (8). At the more dilute concentration of labeled vitellogenin used in this experiment, we have determined that the rate of vitellogenin incorporation is about 25% as great, i.e., approximately 88 ng labeled vitellogenin was taken up by each oocyte during the 30-min incubation period.

After an additional 11-h incubation in unlabeled medium, the labeled oocytes were extracted in strong salt and the dialyzed extract was subsequently chromatographed on TEAE-cellulose. The chromatograph (Fig. 2 *a*) indicated that the principal components present were the yolk proteins lipovitellin and phosvitin which eluted at about positions 0.42 and 0.72, respectively (compare with Fig. 7 in reference 3). Labeling in the eluent fractions (Fig. 2 *b*) indicated that [^{3}H, ^{32}P]vitellogenin was absent at position 0.64 and that the ^{3}H label and ^{32}P label were primarily associated with lipovitellin and phosvitin, respectively, as would be expected from the relative abundance of leucine and protein phosphate between the two yolk proteins (2, 3, 8). Approximately 90% of the incorporated labeled vitellogenin can be accounted for in the elution profile.

[^{3}H, ^{32}P]vitellogenin was also prepared at a concentration of 5.0 ng/nl, and 21 nl of this solution was injected into individual oocytes from the same female. Approximately 20% of the injected material immediately leaked out of the oocyte or was somehow lost during the subsequent washings in solution O. In effect about 84 ng labeled vitellogenin was injected into each oocyte or about the same amount that was incorporated by each oocyte during incubation in solution O containing labeled vitellogenin. The injected oocytes were then incubated for 11 h, extracted, and the dialyzed extracts were

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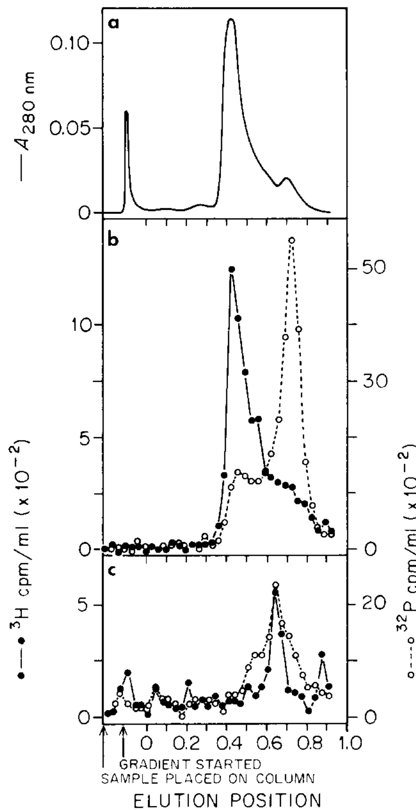

Figure 2 TEAE-cellulose chromatography of oocyte extracts containing approximately 5 mg protein derived from 60 oocytes. (*a*) Absorbance trace (*b*). Labeling in extract from oocytes cultured in the presence of [^{3}H, ^{32}P]vitellogenin. (*c*) Labeling in extract from oocytes injected with [^{3}H, ^{32}P]vitellogenin.

chromatographed and analyzed as was done previously. Although the absorbance profile appeared the same (Fig. 2 *a*), labeling in the eluent fractions (Fig. 2 *c*) indicated that no conversion to lipovitellin or phosvitin had taken place. Rather, a residual amount of labeled vitellogenin was found at elution position 0.64 and small amounts of heterogeneously labeled components appeared throughout the chromatograph. The amount of labeled vitellogenin found was less than half of what would be expected if no breakdown had occurred, so it appears that most of the injected vitellogenin was hydrolyzed into small molecular weight products, some of which were then incorporated into other macromolecules.

These results indicate that vitellogenin is taken up by oocytes and converted into the yolk proteins lipovitellin and phosvitin within a compartment different from and inaccessible to vitellogenin injected into the oocyte cytoplasm. Presumably this compartment is represented by the membrane-bound pinosomes and the forming yolk platelets.

Extracellular protein sequestered by other types of cells has been found to undergo hydrolysis within membrane-bound structures, apparently through the agency of lysosomes (16–18). There does not appear to be a similar process in vitellogenic oocytes, so either typical lysosomes are absent or their activity is suppressed within growing oocytes. Intracellular protein turnover is less clearly understood; most likely it may be mediated either by autophagic vacuoles which subsequently fuse with lysosomes (19, 20) or by neutral proteases within the cytoplasm (21, 22). Acid phosphatase-containing structures and autophagic vacuoles have been observed irregularly in amphibian oocytes, but such oocytes may have been atretic (23). It is apparent from previous results (8) and our present findings, however, that healthy, vitellogenic oocytes do engage in intracellular protein turnover. Since protein can be injected directly into an oocyte and such protein clearly has a different fate from that of sequestered protein, the vitellogenic oocyte would appear to be an excellent model for the study of both the compartmentalization and fate of individual proteins administered to a living cell by a variety of methods.

SUMMARY

Vitellogenin sequestered by vitellogenic oocytes is not degraded but rather undergoes conversion into the yolk proteins lipovitellin and phosvitin, apparently within a membrane-bound compartment. Vitellogenin injected into oocytes is not available to this compartment and is simply degraded rather than converted. Although a mechanism for protein catabolism thus exists within the cytoplasm of growing oocytes, it may not involve the agency of lysosomes.

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