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THREE DIFFERENT MOLECULAR WEIGHT FORMS OF THE VITELLOGENIN PEPTIDE FROM XENOPUS LAEVIS[1]

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SUMMARY: Vitellogenin derived from the blood of estrogen-treated Xenopus laevis is comprised of at least 3 different polypeptides, designated α-, β-, and γ-vitellogenin. The molecular weights of the three polypeptides are 197,000, 188,000 and 182,000, respectively, and the ratio of their relative amounts is approximately 2α: 2β: 1γ.

Vitellogenin is the yolk precursor protein found in the blood of oviparous vertebrates (1). Estrogens can readily induce a pronounced synthesis and secretion of vitellogenin by the liver of Xenopus laevis either in vivo (2) or in vitro (3), so that transcriptional, translational and post-translational events associated with vitellogenin synthesis are now being studied extensively in this amphibian as a model system for steroid-induced protein synthesis (4). Vitellogenin can be readily isolated from the serum of females or estrogen-treated males (5) and has been found to migrate on sodium dodecyl sulfate (SDS) polyacrylamide gels as a single band having a molecular weight of 200,000 (6). Thus, the product of the vitellogenin gene is thought to consist of a single polypeptide (4). In the ovary, vitellogenin is incorporated by growing oocytes within which it is proteolytically cleaved into phosvitin (35,000 daltons) and the heavy (L_1, 120,000 daltons) and light (L_2, 31,000 daltons) subunits of lipovitellin (6). Recently, Ohlendorf et al. (7)

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Abbreviations: C,N,N'-methylene-bis-acrylamide; SDS, sodium dodecylsulfate.

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have provided evidence that L_2 consists of at least 3 peptides having molecular weights of 35,500, 32,000, and 31,600. The origin of this heterogeneity in the vitellogenin cleavage-product remains uncertain.

We have recently begun a study on the structure of vitellogenin using a high resolution SDS-gel electrophoresis system (8). As an initial result, we have found that vitellogenin is comprised of at least 3 polypeptides having different molecular weights. Since this result has important implications for studies on both vitellogenin synthesis and processing, we here provide documentation of our finding.

MATERIALS AND METHODS

All chemicals were obtained from the Sigma Chemical Company. Acrylamide and N,N'-methylene-bis-acrylamide (C) were recrystallized three times from acetone prior to use (9). 2-Amino-2-methyl-1,3-propanediol was recrystallized from ethanol and rinsed with acetone; SDS was reprecipitated from ethanol. All isotopes were obtained from Amersham.

The care of animals together with the injection of hormones and isotopes (10) and the bleeding and collection of plasma (6) were performed as previously described. Vitellogenin was labeled by injecting an animal with 1 mCi each of [^{3}H]leucine (1.0 Ci/mmol, 1.0 mCi/ml) and [^{32}P]orthophosphate (carrier-free, 8 mCi/ml) 8 days after estrogen treatment and 24 hr prior to bleeding (11). Vitellogenin was isolated from plasma either by Mg^{++}-precipitation or by DEAE-cellulose chromatography (12).

SDS gel electrophoresis was performed as described by Wyckoff et al. (8) with the substitution of potassium persulfate (100 mg/100 ml) as the catalyst solution. Protein was either reduced or reduced and carboxyaminomethylated (13) prior to electrophoresis. Carboxyaminomethylation did not change the electrophoretic pattern, but did sharpen the bands (13). All gels (0.6 x 13 cm) contained 2% C (w/v) and were run at 1.5 mA/gel until the tracking dye reached the bottom of the gels (approximately 3 hr) In order to improve the resolution of the vitellogenin bands, some gels were run for an additional 3 hr after the tracking dye had reached the bottom of tubes. The gels were fixed and stained with Coomassie blue as described by Wyckoff et al. (8).

Bands containing labeled vitellogenin were cut out and dissolved in 0.4 ml 30% H_2O_2 at 50°C. Aquasol (10 ml) was then added and the vials were each counted three times for 20 min in a Beckman scintillation counter.

RESULTS

When preparations of vitellogenin were electrophoresed on 10% (w/v) SDS-polyacrylamide gels, only a single, sharp band was seen (Fig. 1A). However, when we

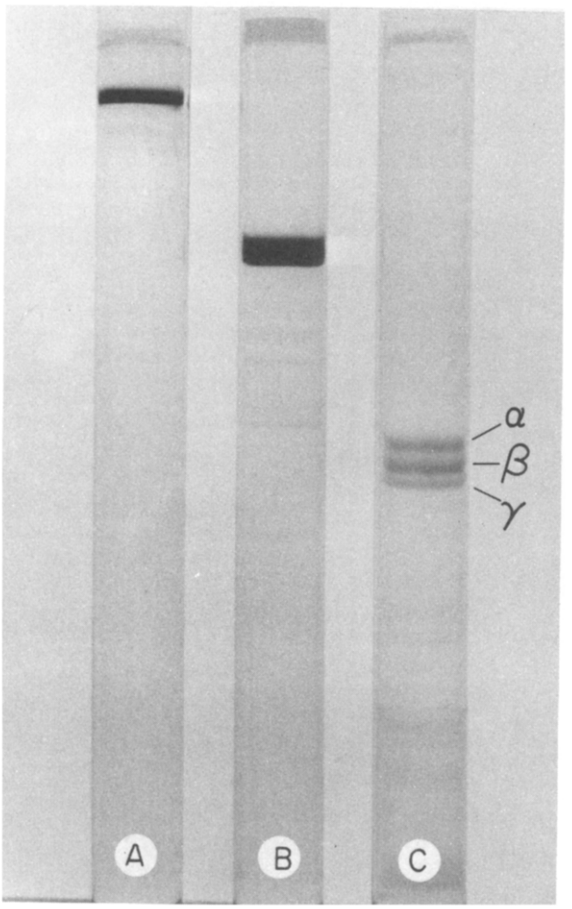

Fig. 1. Electrophoresis of 5 μg reduced and carboxyaminomethylated vitellogenin on SDS-polyacrylamide gels. (A) 10% gel. (B) 5% gel. (C) Same as (B) but run twice as long.

electrophoresed small amounts of vitellogenin (5 μg/gel) on 5% (w/v) gels, we noticed a lamellar structure in the vitellogenin band (Fig. 1B). The lamellar structure of the band was resolved further by running the gels for twice the length of time necessary for the tracking dye to reach the bottom of the gel. We preferred this approach over running gels with an even lower percentage acrylamide because of their fragility. When vitellogenin was run this extra distance on the 5% gels, the lamellar structure resolved into 3 distinct bands, designated as α-, β-, and γ-vitellogenin in the order of their increasing mobility (Fig. 1C).

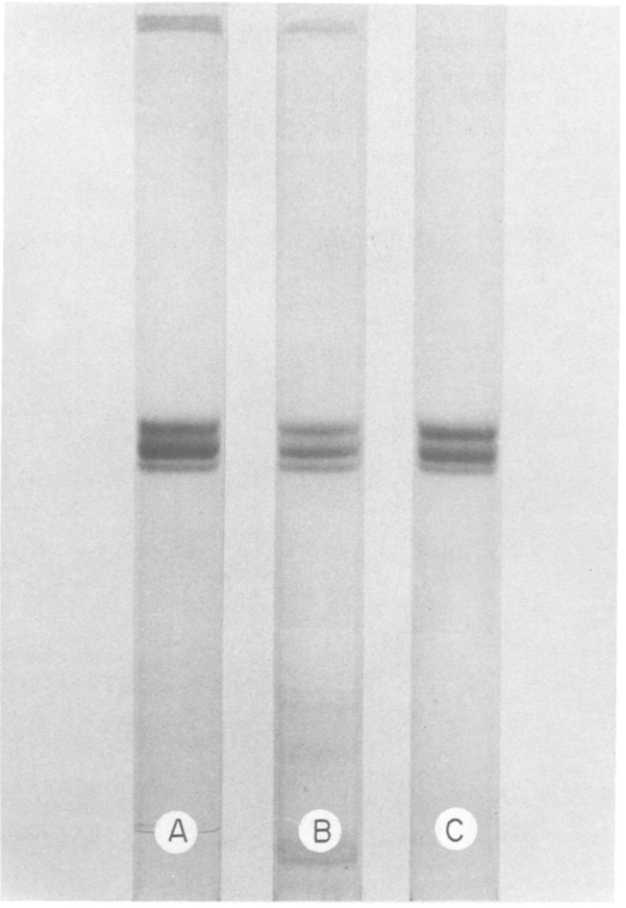

Fig. 2. Electrophoresis of reduced and carboxyaminomethylated vitellogenin on 5% SDS-polyacrylamide gels. The vitellogenin samples were derived from 3 different animals and were prepared by either (A) DEAE-cellulose chromatography or (B) Mg^{++}-precipitation. Alternatively, (C) plasma containing vitellogenin was applied directly to the gel.

In order to ascertain whether the 3 bands of vitellogenin are a preparation artifact, we ran and compared SDS-polyacrylamide gels of plasma samples obtained from estrogen-treated animals as well as vitellogenin isolated by either Mg^{++}-precipitation or DEAE-cellulose chromatography (12). As can be seen in Fig. 2, the same pattern of 3 bands was obtained in all cases. We have also run the vitellogenins obtained from 4 additional animals and have obtained in all cases the identical banding pattern. As a

further check on the origin of the 3 vitellogenin bands, we have established that reduced vitellogenin, carboxymethylated vitellogenin (6), and carboxyaminomethylated vitellogenin all show the identical pattern. Preparation of vitellogenin in the presence or absence of the protease inhibitor, phenylmethyl sulfonyl fluoride (6) likewise had no effect on the 3 bands. Finally, electrophoresis of small amounts of vitellogenin (5 μg/gel) in the SDS-polyacrylamide gel system of Laemmli (14) also resulted in a similar banding pattern. We thus conclude that the 3 bands on SDS-polyacrylamide gels reflect the presence of at least 3 different chain lengths of vitellogenin present in the blood of the animals.

When the migration distances of the 3 vitellogenin bands were compared with molecular weight markers according to procedures previously described (6), sizes of 197,000, 188,000 and 182,000 daltons were obtained for α-, β-, and γ-vitellogenin, respectively. This agrees well with the molecular weight of approximately 200,000 reported previously for unresolved vitellogenin polypeptide (6).

From a visual inspection of the 3 vitellogenin bands, α- and β-vitellogenin seem to be present in equal amounts while γ-vitellogenin appears to be present in a lesser amount. In order to quantitate the ratios of the 3 polypeptides, we doubly labeled vitellogenin with [^{3}H]leucine and [^{32}P]orthophosphate. The latter is a specific marker for delipidated vitellogenin in the blood (5). The labeled vitellogenin was electrophoresed on gels, the bands containing each of the 3 vitellogenin polypeptides excised, and the radioactivity measured. The results (Table 1) indicated that α-, β-, and γ-vitellogenin were present in an approximately 2:2:1 ratio. This was the case for both the ^{3}H and ^{32}P labels, the relative ratios of which were about the same for the 3 bands. The presence of ^{32}P in all 3 delipidated bands further confirms their identity as vitellogenin polypeptides.

TABLE 1

RELATIVE LABELING IN THE 3 VITELLOGENIN BANDS

Band	^{3}H cpm	^{32}P cpm	$^{3}H/^{32}P$
α	182 ± 17	75 ± 7	2.4 ± 0.4
β	180 ± 6	85 ± 4	2.1 ± 0.2
γ	98 ± 13	44 ± 6	2.2 ± 0.5

The results are expressed as the average ± S.D.

DISCUSSION

Native vitellogenin has previously been thought to yield a single component when reduced under denaturing conditions (6). We have further resolved this component into 3 high molecular weight polypeptides. In the blood, native vitellogenin is a lipoprotein with an approximate molecular weight of 460,000, of which 12% (55,000 daltons) is lipid. Thus, native vitellogenin can only contain 2 polypeptide chains. How the 3 chain described here associate to form the native dimer molecule is not known, but the implication is that anywhere from 3 to 6 different native vitellogenin molecules may exist, depending on whether the 3 different vitellogenin polypeptides form homologous or heterologous pairs. Electrophoresis of native vitellogenin has not been able to resolve this issue thus far because of the poorer resolution obtained by this procedure (data not shown).

Of primary interest is the cause of the heterogeneity in the vitellogenin polypeptide. The origin may result either from multiple gene copies for vitellogenin or from heterogeneous post-translational processing of a common gene product. If the latter is the case, then such processing probably occurs in the liver since α-, β-, and γ-vitellogenin are always found in the plasma, even when collected in the presence of phenylmethyl

sulfonyl fluoride. The observations of Ohlendorf et al. (7) regarding at least 3 L_2 peptides can be interpreted as showing that the heterogeneity is partially maintained even after vitellogenin processing within the oocyte. The L_2 and phosvitin peptides appear to be contiguous in the vitellogenin polypeptide (6), but it is not known which peptide product is derived from the end of the vitellogenin polypeptide. If L_2 is at the end, then the observations made to date can be explained by differential post-translational clipping of the end of the vitellogenin polypeptide in the liver. On the other hand, if phosvitin resides at the end of the vitellogenin polypeptide, the known results can only be explained by invoking multiple gene copies for vitellogenin.

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