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The Coelomic Envelope to Vitelline Envelope Conversion in Eggs of *Xenopus laevis*

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An amphibian egg recovered from the body cavity is enclosed by a coelomic egg envelope. Upon transport down the oviduct, the envelope is converted to the vitelline envelope. The coelomic and vitelline envelopes are distinct in terms of sperm penetrability, ultrastructural morphology, and radioiodination profiles. In this study, the macromolecular compositions of these two envelopes were determined. Isolated envelopes were compared by one- and two-dimensional gel electrophoresis, peptide mapping, and radiolabeling. A protein with a molecular weight of 57,000 (57K) was present in the vitelline envelope but was absent in the coelomic envelope. A glycoprotein with a molecular weight of 43K in the coelomic envelope was converted to a component with a molecular weight of 41K in the vitelline envelope. The 43K-molecular weight component of the coelomic envelopes could be radioiodinated by lactoperoxidase but no labeling of the 41K-molecular weight component occurred in the vitelline envelope. Peptide mapping using limited proteolysis established that the 43K-molecular weight component of the coelomic envelope was a precursor to the 41K-molecular weight component of the vitelline envelope. These molecular alterations may underlie the ultrastructural and physiological changes occurring in these envelopes.

Key words: egg, vitelline envelope, glycoprotein, processing, proteolysis, sperm, *Xenopus*

The oviposited egg of *Xenopus laevis* is surrounded by two types of integuments, an envelope that is produced during oogenesis and jelly coats that are secreted around the egg as it travels down the oviduct. Prior to the passage of the egg from the body cavity to the oviduct, the envelope of a *Xenopus* egg is termed the coelomic egg envelope (CE). After passage of the egg through the first region of the oviduct, the CE is converted by oviductal factors to the vitelline envelope (VE).

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Grey et al [1,2] determined the ultrastructure of the CE and VE from *Xenopus* eggs. The envelopes average approximately 1 μm in thickness. The CE is composed of filaments arranged in randomly oriented bundles or fascicles 50–120 nm in diameter and the exterior surface of this envelope has a netlike appearance, being permeated by large pores or channels. The filamentous fascicles are dispersed in the VE, giving this envelope a relatively smooth exterior; there are no large pores or channels in the VE as there are in the CE.

The envelopes also differ biologically in terms of their penetrability by sperm. The isolated or in situ CE cannot be penetrated by sperm [2], consistent with observations that, in general, body cavity eggs of anurans cannot be fertilized without prior chemical or enzymatic modification of the envelope [3,4]. Sperm penetrate the VE in situ and can penetrate an isolated VE from either the inside or outside surfaces.

The macromolecular compositions of the VE and its derivative, the fertilization envelope (FE), have been determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [5–7]. The VE is composed of six glycoprotein components and one protein component with molecular weights in the range of 37,000 to 120,000. From radioiodination studies of the intact isolated CE and VE, a major glycoprotein component of the CE could be radioiodinated but the corresponding component of the VE could not be radioiodinated [7]. In this paper, we report further investigations of the macromolecular properties of the CE and VE and provide evidence that these two envelopes are chemically distinct. These macromolecular differences may underlie the morphological and functional differences of the CE and VE.

MATERIALS AND METHODS

Eggs

Xenopus laevis females were purchased (NASCO, Ft. Atkinson, WI) or collected (Orange County, CA). Oviposited eggs were procured as described by Wolf and Hedrick [8] except that the frogs were injected with pregnant mare serum gonadotropin (35 IU/frog) 3–5 days prior to stimulation with human chorionic gonadotropin (500–1,000 IU/frog). Starting 5–6 hr after the injection of human chorionic gonadotropin, eggs were collected three times at 2 hr intervals. The surgical procedures of Grey et al [2] were used to obtain coelomic eggs. Oviducts were ligated at the anterior ends and the frogs were injected with pregnant mare serum gonadotropin. Three to five days later, the frogs were injected with human chorionic gonadotropin (1,000 IU/frog) and, 10 hr later, coelomic eggs were surgically recovered from the abdominal cavity.

Chemicals

Unless otherwise stated, all chemicals were reagent grade from commercial sources. Electrophoresis grade SDS, acrylamide, 2,2′-methylene-bis-acrylamide, ampholines, and SDS-PAGE molecular weight standards were purchased from Bio-Rad Laboratories (Richmond, CA). Ultrapure urea was from Schwartz-Mann (Cleveland, OH) and Nonidet P-40 came from Particle Data Laboratories (Elmhurst, IL). *Staphylococcus aureus* V8 protease was purchased from Miles Laboratories (Naperville, IL). Lactoperoxidase was a gift of Dr. T. Nishihara. Carrier-free $Na^{125}I$ came from Amersham-Searle (Arlington Heights, IL) or New England Nuclear (Boston, MA). Pregnant mare serum gonadotropin and human chorionic gonadotropin were

purchased from Organon (West Orange, NJ) or acquired from the National Hormone and Pituitary Program, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health.

Envelopes

Coelomic egg envelopes were prepared as described by Grey et al [2]. Vitelline envelopes were prepared from dejellied eggs by the procedure of Wolf et al [5]. Jelly was removed by exposure (3–5 min) of the eggs to DB-Tris, pH 8.9 containing 0.045 M 2-mercaptoethanol [9]. DB-Tris consisted of 0.11 M NaCl, 1.34 mM $CaCl_2$, 1.32 mM KCl, 10 mM Tris, adjusted to the indicated pH with HCl. To stop the dejellying process, the solubilized jelly was decanted and the eggs were rinsed in several volumes of DB-Tris, pH 7.8.

Envelopes were routinely dissolved in 2% SDS at 100°C for 90 sec, cooled to room temperature, and centrifuged in a tabletop centrifuge for 5 min to remove any remaining contaminating particulate material of nonenvelope origin. The envelopes are completely solubilized by this procedure [5]. The clear, SDS-dissociated envelope solutions were stored at −20°C.

SDS-PAGE

Samples in a buffer containing final concentrations of 0.0625 M Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% mercaptoethanol, and 0.001% bromophenol blue were heated for 90 sec at 100°C, cooled to room temperature, and analyzed as to their macromolecular composition by the procedure of Laemmli [10] using a slab gel apparatus. After electrophoresis, the gels were stained for protein with Coomassie Brilliant Blue R-250 [11]. Molecular weights of envelope components were estimated using protein standards [12]. A shorthand nomenclature is used to refer to components by their molecular weights (eg, 120K component has a molecular weight of 120,000).

Isoelectric Focusing and Two-Dimensional Gel Electrophoresis

The procedure of Ames and Nikaido [13] was used for the analysis of envelope components by two-dimensional gel electrophoresis (2D-PAGE). This procedure was utilized because the envelopes were not dissociated by the NP-40 and urea lysis buffer of O'Farrell [14]; SDS was required to dissociate the envelopes into their component molecules. Two-dimensional gels were fixed with 10% TCA and stained as for SDS-PAGE.

Iodination

Intact, isolated envelopes were radioiodinated using lactoperoxidase, H_2O_2 and ^{125}I [7,15]. After iodination, the envelopes were rinsed five to six times in ice-cold H_2O and then solubilized in 2% SDS as described above.

Peptide Mapping

The envelope components were analyzed by peptide mapping using modifications of the procedure of Cleveland et al [16]. This procedure involved limited proteolysis of proteins in the presence of SDS by *S aureus* V-8 protease and subsequent separation of the peptide products by SDS-PAGE. A first-dimensional separation of the envelope componets utilized SDS-PAGE. The gel strip containing the separated glycoprotein mixture was cut out and equilibrated for 30 to 60 min with 0.1% SDS, 0.0625 M Tris-HCl, pH 6.8 and was stored at 4°C in a sealed dry tube

for up to 2 days or used immediately. Proteolysis and subsequent peptide mapping was performed in the second dimension by procedures adapted from the Bordier and Crettol-Jarvinen [17] extension of the Cleveland et al technique.

Autoradiography

Slab gels were dried under vacuum and placed against a sheet of Kodak X-Omat R or Kodak XR-5 X-ray film. To lessen the possibility of the peptide mapping gels cracking during the drying process, the gels (15% polyacrylamide) were soaked in 50% ethanol for 1–2 h prior to drying. After a suitable exposure time, the X-ray film was developed according to the manufacturer's instructions.

Protein Concentration Determination

Protein concentrations were estimated by the method of Lowry et al [18] with bovine serum albumin as the standard or by absorbance readings of SDS-dissociated envelopes (VE) using the value of $A^{1\%}_{280nm,1cm} = 8.56$ [5].

RESULTS

The Macromolecular Composition of the CE and VE Using SDS-PAGE

The Coomassie blue-stained gel patterns of the CE and VE were very similar in that five of the glycoprotein components observed were in both CE and VE (Fig. 1). However, two components were distinctly different. A protein with a molecular weight of 57K was present in the VE but not in the CE. The 57K VE component was previously reported as a 60K component and shown to be a protein based upon its staining characteristics in contrast to all other components which were glycoproteins [5,19] as they reacted with a periodic acid-Schiff base stain or a periodic acid-dansyl hydrazine stain. It has also been observed that treating the 57K component with trifluoromethane sulfonic acid, a reagent which deglycosylates glycoproteins, did not reduce its molecular weight on SDS-PAGE [M. Bakos, L. Lindsay, and J. Hedrick unpublished observations]. The other difference was a reduction in the molecular weight of the 43K glycoprotein component in the CE to a 41K component in the VE. The 43K and 41K glycoproteins were major components of the envelopes and gave relatively broad bands on SDS-PAGE. However, slight mobility differences in these envelope components corresponding to 2K mass units were repeatedly observed and were best demonstrated using 7.5% polyacrylamide gels.

The Macromolecular Composition of the CE and VE Using 2D-PAGE

Analysis of the macromolecular components of the CE and VE by 2D-PAGE (Fig. 2) confirmed and extended the differences detected by SDS-PAGE. A11 CE and VE envelope components were polymorphic in terms of isoelectric points. This observation was not unexpected, as this is commonly observed for glycoproteins. However, even the 57K protein component in the VE gave multiple spots in 2D-PAGE (Fig. 2B). The 43K region in gels of the CE gave 12 spots with different isoelectric points (Fig. 2A). However, many of these spots were "doublets" in that they were very closely associated with one another in terms of isoelectric points and appeared to have slightly different molecular weights. By comparison, the 41K region in gels of the VE gave six spots that corresponded to 6 of the 12 spots observed in the

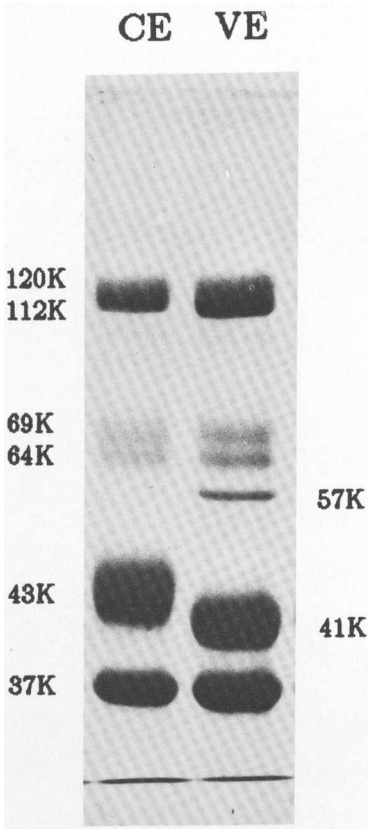

Fig. 1. SDS-PAGE of the CE and VE. Electrophoresis was in a 7.5% polyacrylamide gel with Coomassie blue staining. Each lane contained approximately 25 μg of protein.

43K region of the CE gels. The unique glycoproteins associated with the 43K region of the CE are marked with arrows in Figure 2A.

It seemed likely that the CE preparation was contaminated to some extent by VEs. Grey et al [2] observed that CE preparations were routinely contaminated with envelopes that had VE-like ultrastructures. This contamination was caused by some eggs entering the upper end of the ligated oviduct (where the CE to VE conversion takes place) and then being "regurgitated" into the coelomic cavity. In addition, we have observed that the CE isolation procedure may enrich the VE contamination as the CE tended to adsorb onto glass surfaces whereas the VE did not [19].

Radiolabeling experiments supported the interpretation that the CEs were contaminated with VEs. We previously showed that a component in the isolated particulate CEs could be radiolabeled with ^{125}I, while a similar component in the VE could not be radiolabeled under the same conditions [7]. When isolated particulate CEs were radiolabeled using lactoperoxidase and ^{125}I, and then analyzed by 2D-PAGE, six components in the 43K region were radiolabeled (Fig. 3). The six components were those that were unique to the CE (marked by arrows in Fig. 2A) and were not present in the 41K region of VE gels. Thus, there were six unique components in the

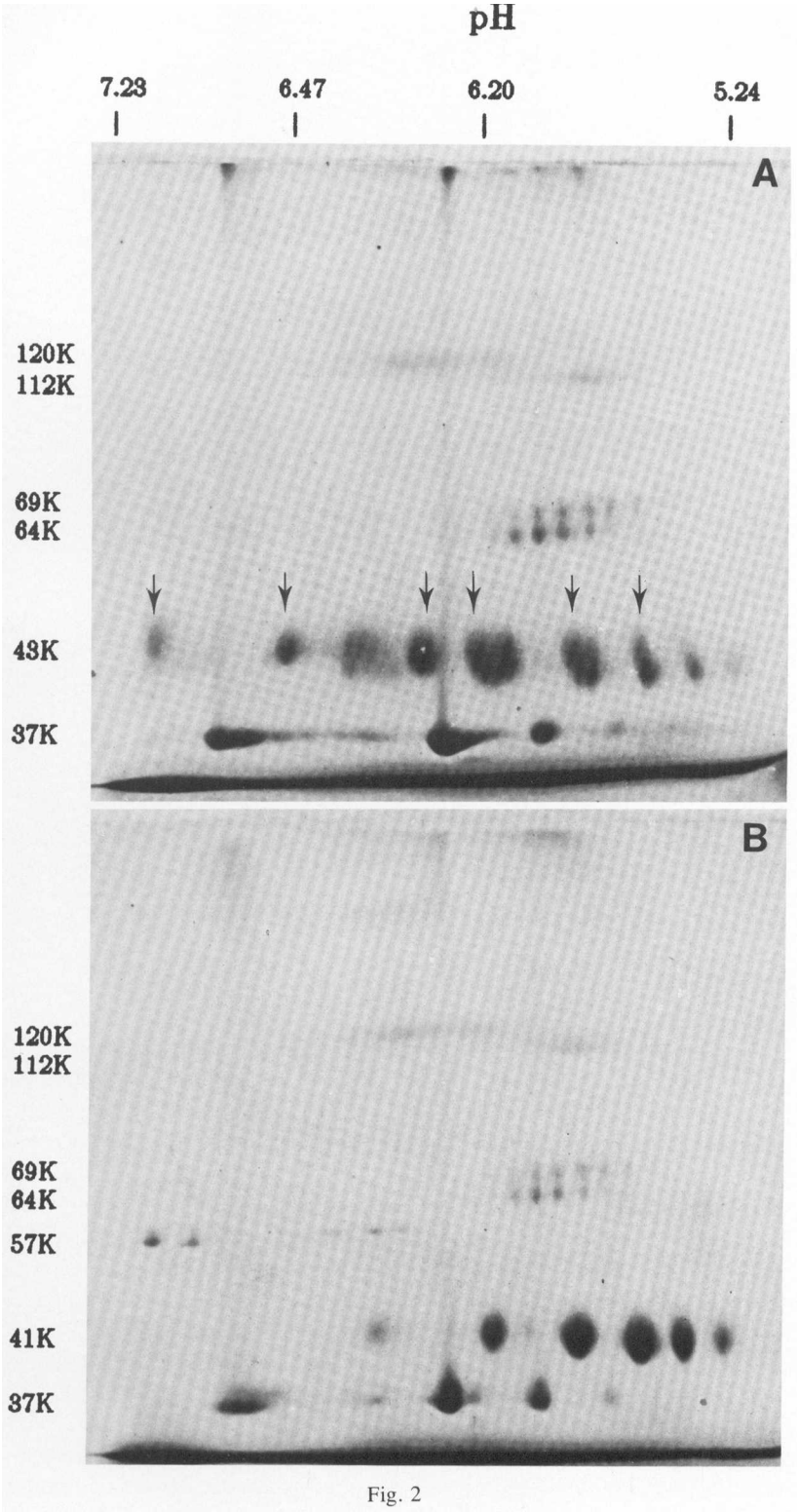
pH
7.23
6.47
6.20
5.24
A
120K
112K
69K
64K
43K
37K
B
120K
112K
69K
64K
57K
41K
37K

Fig. 2

43K region of CE gels and six corresponding components in the 41K region of VE gels; only the 43K components in the CE were radiolabeled in intact envelopes.

Peptide Mapping of the 43K and 41K Envelope Components

The SDS-PAGE separated components of the CE and VE were treated with *S aureus* V8 protease and the peptides (glycopeptides) generated were separated by SDS-PAGE (Fig. 4).The stained peptide maps showed that a unique peptide was present in the digest of the 43K CE component compared to the 41K VE component (marked by an asterisk in Fig. 4; for comparison, the peptides generated by digestion of the 37K component, common to both envelopes, were included). Thus, the 43K and 41K components were highly homologous but a peptide portion was missing from the 41K component. This experiment was repeated using ^{125}I-labeled CEs and VEs (VE radiolabeling was of the dissolved, dissociated envelope; CE radiolabeling was of the particulate envelope). The autoradiograms of 43K and 41K peptide maps

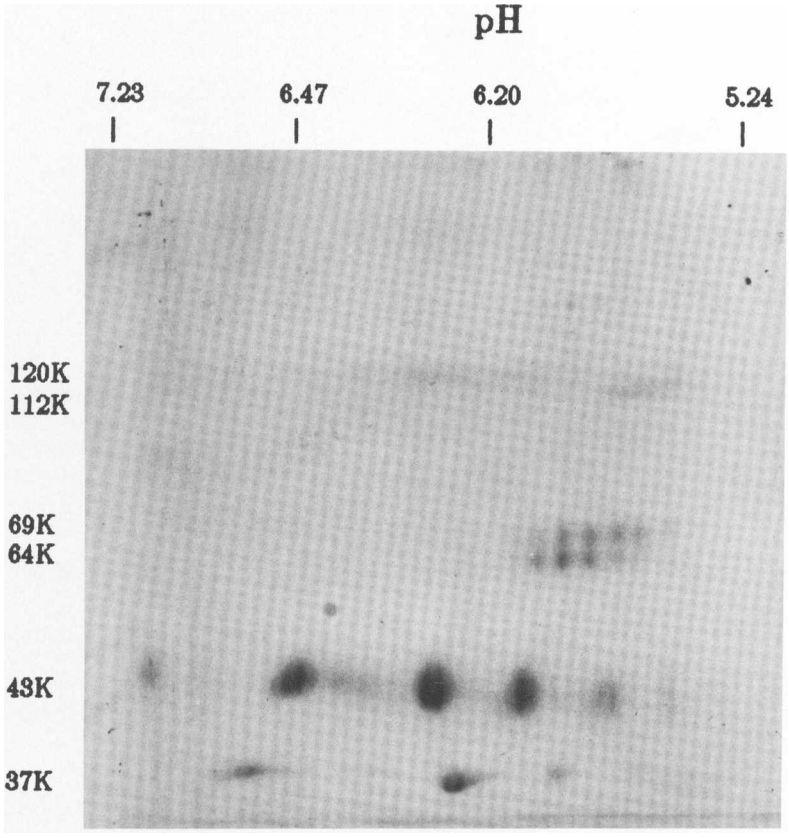

Fig. 3. Autoradiogram of gel from Figure 2A

Fig. 2. Two-dimensional gel electrophoresis of envelope components. First-dimensional isoelectric focusing was performed as in Ames and Nikaido [13]. Second dimension of SDS-PAGE utilized gels containing 7.5% polyacrylamide. Coomassie blue staining of A) ^{125}I-CE (2.2×10^5 cpm) and B) VE. The arrows indicate the 43K CE components that were ^{125}I labeled as shown in Figure 3.

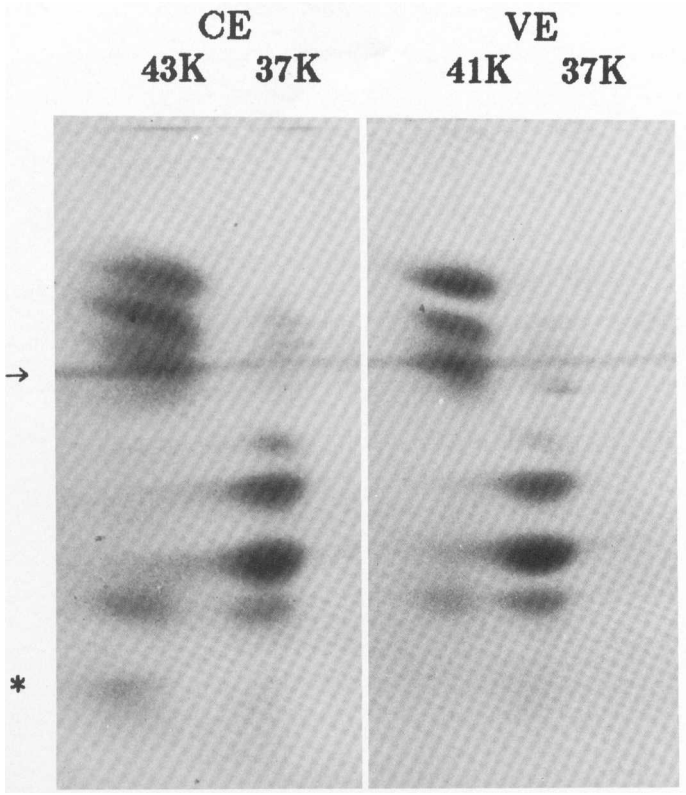

Fig. 4. Limited cleavage peptide maps of the 43K and 37K components of the CE and the 41K and 37K components of the VE; Coomassie blue stained. Components from approximately 60 μg of each envelope were separated by SDS-PAGE using a 7.5% polyacrylamide gel. Proteolysis of the separated components using 10 μg of *S aureus* V8 protease and subsequent separation of the peptides by SDS-PAGE was as described in the test. The arrow denotes the V8 protease band and the asterisk denotes the peptide unique to the 43K CE component.

showed almost identical patterns except that the unique CE peptide shown in Figure 4 was detected only in the CE peptide lane.

From these experiments, we concluded that the 43K CE component was converted or processed to the 41K VE component.

DISCUSSION

The results reported here have established that there are macromolecular differences between the CE and VE. Conversion of the CE to the VE consists of adding a 57K protein component to the envelope and processing of a 43K glycoprotein to a 41K glycoprotein. The 43K component of the CE can be radioiodinated in the intact envelope, whereas the 41K component in the intact VE cannot be radioiodinated. However, the 41K component of the VE when dissolved and denatured, eg, by SDS, can be radioiodinated. Thus, conformational differences in the 43K and 41K components must exist in the intact envelopes. We believe this is the first report of molecular

processing of an egg envelope glycoprotein that correlates with changes in the functional and morphological properties of the envelope.

Table I summarizes the macromolecular composition of the CE and VE as determined here and of the FE as previously reported [5–7]. The chemical difference between the 43K and 41K components has yet to be defined. The apparent molecular weight difference of 2K mass units could be due to limited peptide or oligosaccharide hydrolysis. However, any peptide differences between the two glycoproteins must occur at the C-terminal amino acid end of the molecule, as the N-terminal amino acid is blocked [19]. The apparent shift in isoelectric points to more acidic values when 43K is converted to 41K (Fig. 2) requires that a basic oligomer be removed in the processing event. Since such an oligomer could exist for a protein (a basic peptide) but not for an oligosaccharide (basic oligosaccharides have not been reported in glycoproteins), it seems likely that the processing enzyme is a peptidase rather than a glycosidase.

The processing of the 43K to 41K envelope component likely involves an enzyme secreted by the oviduct. It is possible that the 57K protein component added to the CE may be the enzyme responsible for the 43K to 41K processing event. Based upon inhibitor studies, Miceli et al [20,21] have implicated a "trypsin-like" oviductal protease in the CE to VE conversion of egg envelopes from the toad *Bufo arenarum*. They demonstrated an esterolytic activity toward synthetic substrates but have not yet demonstrated any proteolytic activity associated with the oviductal extracts. Katagiri et al [22] have demonstrated that an extract of the pars recta region of the oviduct from *Bufo Bufo japonicus* can cause the morphological alteration of the CE to VE in *Bufo* eggs. In the eggs of *Xenopus laevis*, a morphological change of the CE to VE can be induced at pH 6 or 9 [2]. However, these nonphysiological conditions would not likely cause the 43K to 41K conversion demonstrated here, but could possibly

TABLE I. A Summary of the Macromolecular Components of *Xenopus laevis* Egg Envelopes

CE	VE	FE[a]
		J[b]
120K	120K	120K
112K	112K	112K
69K	69K	
		66K
64K	64K	
		61K
	57K[c]	57K
43K		42–45K[d]
	41K	41K
37K	37K	37K

[a]Macromolecular components of the fertilization envelope, FE, are taken from [5–7].

[b]A high-molecular-weight jelly component of undetermined molecular weight located at the top of the small pore gel and derived from the F layer of the FE [5].

[c]The only protein component present; all other envelope components are glycoproteins [5–7].

[d]Components contributed by the cortical granule lectin derived from the F layer of the FE [5,23].

cause conformational changes in the envelope glycoproteins. The morphological differences between the envelope forms may be a reflection of conformational differences in the envelope macromolecules. Potential functional changes in the envelope, eg, sperm penetrability, induced by changes in pH have not yet been tested. We have recently detected a 57K component and a peptidase activity associated with a macromolecularly complex secretory fluid obtained from *Xenopus* pars recta tissue cultures. These culture fluids were capable of converting the CE to VE [unpublished observations, M. Bakos and J.L. Hedrick].

Identification of the chemical nature of the processing event involved in the 43K to 41K conversion and isolation of the oviductal hydrolase catalyzing this processing event will be necessary in order to understand the ultrastructural and functional (sperm penetrability) differences in the CE and VE.

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