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The Macromolecular Composition of *Xenopus laevis* Egg Jelly Coat†

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ABSTRACT: The three morphologically and functionally distinct jelly coat layers of *Xenopus laevis* eggs, J_1, J_2, and J_3, were separated by manual dissection, solubilized with dithiothreitol, and physicochemically analyzed. The chemical composition of the three jelly layers varied from 37 to 48% protein and 63 to 52% carbohydrate. The carbohydrate consisted of hexosamines, galactose, and fucose. Some of the carbohydrate in each of the jelly layers was covalently linked to protein through O-glycosidic bonds as β elimination of the carbohydrate moiety in the presence of alkali was observed. In agreement with a previous finding, covalently attached sulfate was localized within the innermost jelly coat layer, J_1. Cellulose acetate electrophoresis at pH 8.0 resolved a total of nine macromolecular components from the three jelly coat layers differentially staining for protein and carbohydrate: J_1 yielded two anodically migrating components; the middle layer J_2 yielded two cathodically migrating macromolecular components; the outermost layer J_3 contained five species, three anodic and two cathodic. Sodium dodecyl sulfate agarose gel electrophoresis analysis yielded nine unique species, six of which stained coincidently for protein and carbohydrate. Immunoelectrophoresis and Ouchterlony double diffusion analyses using antiserum to total jelly components resolved nine different antigenic species with cross-reactivity between one or two macromolecules in layers J_1 and J_3. Analytical sedimentation velocity centrifugation revealed eight distinct species all of which exhibited hypersharp schlieren patterns and whose $s_{20,w}$ values were highly concentration dependent. On the basis of these analyses, *Xenopus laevis* egg jelly layers are composed of at least 8–9 distinct macromolecular species. The majority of these macromolecules are uniquely associated with different jelly coat layers.

A characteristic feature of amphibian eggs is the presence of a water-insoluble gelatinous matrix, termed the jelly coat, surrounding the egg and usually composed of several distinct layers. The egg jelly coat layers are secretory products of the tubular gland cells lining the oviduct and are deposited around the eggs as they traverse the oviduct following their release from the ovary. As determined by light microscopy studies, the jelly coats are morphologically simple or without any peculiar or distinguishing features. Histochemical studies and chemical analyses of several amphibian egg jelly coats have indicated the presence of protein and carbohydrate (for review of work published before 1966, see Monroy, 1965, and Metz, 1967; Lee, 1967; Freeman, 1968; Shivers and James, 1970a; Steinke and Benson, 1970). Immunological analyses of egg jelly coats have indi-

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cated the presence of multiple antigenic components in each of several amphibian species (Shivers, 1965; Katagiri, 1968; Shaver et al., 1970). Thus, although the morphology of jelly coats is relatively simple, their chemistry and macromolecular composition are ill defined and apparently complex. With regard to the biological role of the jelly coat in sperm–egg interactions during fertilization, amphibian egg jelly coats act as a barrier to sperm penetration (Greenslade et al., 1973), are involved in sperm capacitation and/or the acrosomal reaction (Katagiri, 1966, 1973; Metz, 1967; Shivers and James, 1970b; Wolf and Hedrick, 1971b; Elinson, 1973; Shaver et al., 1973), and participate in establishment of a block-to-polyspermy (Wyrick et al., 1974). These functions appear to be associated with particular jelly coat layers and are not properties of the jelly coat as a whole.

Our understanding of the molecular basis of egg jelly coat functions is severely limited by an incomplete knowledge of the number and types of macromolecules which compose the egg jelly coat layers. In this report we describe studies on the macromolecular composition of the egg jelly coat of the South African clawed toad, *Xenopus laevis*. The egg jelly coat of this organism is comprised of three morphologically distinct layers (Freeman, 1968). These studies were greatly assisted because of two fundamental characteristics of *Xenopus laevis* egg jelly coats: (1) maintenance of the egg jelly coat structure is dependent on the integrity of disulfide bonds; their reduction results in solubilization of the jelly coat (Gusseck and Hedrick, 1971); and (2) each of the three morphologically distinct egg jelly coat layers may be directly isolated by manual dissection (Freeman, 1968).

Materials and Methods

Materials. Sexually mature *Xenopus laevis* were imported through Dart and Howes, Ltd., Cape Town, South Africa, or collected in Orange County, Calif. The agarose (pure powder) used for either zone electrophoresis or immunoelectrophoresis was obtained from Aldrich Chemical Co., and the agarose (A grade) used for Ouchterlony double diffusion was obtained from Calbiochem. All other chemicals were of the highest grade obtainable from commercial sources and were used without further purification.

Preparation of Mercaptan-Solubilized, Manually Dissected Jelly Coat Layers. Eggs were obtained from *Xenopus laevis* following injection of human chorionic gonadotropin as previously described (Wolf and Hedrick, 1971a) and stored in a balanced salt solution containing: 110 m*M* NaCl–1.3 m*M* $CaCl_2$–1.3 m*M* KCl–$NaHCO_3$ to yield pH 7.2. The outermost jelly coat layer, J_3, was cleanly removed from eggs by manual dissection with watchmaker's forceps with no contamination by layers J_2 and J_1. This exposed the middle jelly coat layer, J_2, which was invariably dissected with a small amount of adhering J_1. The isolated J_3 and J_2 jelly coat layers were washed several times with 100 m*M* NaCl–50 m*M* Tris-HCl (pH 8.0) and then solubilized by addition of dithiothreitol to a final concentration of 2–5 m*M* (Gusseck and Hedrick, 1971). Complete solubilization occurred in 5–10 min at 22°. The remaining innermost jelly coat layer, J_1, was selectively solubilized by immersing the J_3-, J_2-less eggs in 100 m*M* NaCl–50 m*M* Tris-HCl (pH 8.0) containing 2–5 m*M* dithiothreitol for 5–10 min. The egg and vitelline envelope remained intact during this process. The solubilized jelly coat preparations were centrifuged at 12,000*g* for 10 min and then dialyzed vs. a buffer appropriate to further analyses of the jelly layer.

Chemical Determinations. Protein was determined spectrophotometrically using the equation $\mu g/ml = (A_{215\,nm} - A_{225\,nm})144$ (Waddell, 1956). Fucose was analyzed by the method of Dische and Shettles (1948). Hexose determination was by the phenol–H_2SO_4 test (Dubois et al., 1956) using galactose as a standard and corrected for interference from fucose. Total hexosamine was analyzed following hydrolysis in 4 *N* HCl for 4 hr at 100° under N_2. The liberated hexosamines were first isolated by chromatography on resins of Dowex AG-50W-X2 (Boos, 1953) and then assayed by the procedure of Elson and Morgan (1933). Sialic acid analyses were by the method of Warren (1959) following hydrolysis of samples in 0.10 *N* H_2SO_4 at 80° for 1 hr. *N*-Acetylneuraminic acid was used as a standard and correction was made for fucose inhibition of color development.

Sulfhydryl groups were assayed using Nbs_2[1] (Ellman, 1959). Jelly coat layers were first solubilized at 22° with 5 m*M* dithiothreitol in 100 m*M* NaCl–50 m*M* Tris-HCl (pH 8.0), dialyzed vs. buffer containing 2 m*M* dithiothreitol, and then subjected to gel filtration on a 1 × 18 cm column of Sephadex G-25 equilibrated with 50 m*M* sodium phosphate buffer (pH 8.0). The sulfhydryl content of the proteins eluted in the void volume was measured within 2 hr from the start of the chromatographic run. The sulfhydryl content of intact jelly was determined by incubating 36 fully jellied eggs in 2.5 ml of 100 m*M* NaCl–20 m*M* Tris-HCl–0.10 m*M* Nbs_2 (pH 8.0) and then monitoring the absorbance of the media at 412 nm with time.

Cellulose Acetate Electrophoresis. Electrophoresis was conducted at 50 V for 30 min on 2.5 × 7.6 cm PhoroSlide strips (Millipore Corp.) equilibrated with 100 m*M* Tris-acetate (pH 8.0) at room temperature in a PhoroSlide apparatus. Protein components and sulfated components were visualized by staining with 0.1% nigrosin or 1% Alcian Blue, respectively, in 7% acetic acid. Carbohydrate containing components were also visualized by staining with PAS after fixation in 5% trichloroacetic acid. Distances of migration were measured to within 0.070 mm under a dissecting microscope, and electrophoretic mobility was calculated from the equation:

$$\text{mobility } (cm^2\ V^{-1}\ sec^{-1}) = \frac{(\text{distance migrated})(\text{interelectrode distance})}{(\text{voltage})(\text{time})}$$

A value of 6.08 cm was determined for the interelectrode distance in this system.

Ultracentrifugation. Analytical sedimentation velocity centrifugation was conducted with a Spinco Model E ultracentrifuge equipped with schlieren optics. Sedimentation was at 20° using the AN-D rotor and 12-mm Epon-aluminum double sector cells at 52,000 rpm. Boundary positions were measured with a Nikon microcomparator, and relative peak areas were determined by cutting and weighing procedures from traces of enlarged profiles.

Immunological Techniques. Rabbit antisera to mercaptoethanol solubilized *Xenopus laevis* egg jelly coat was a generous gift of Dr. William A. Frankart. Immuno double-diffusion (Ouchterlony, 1948) was conducted in 1% agarose prepared in physiological saline buffered to pH 7.5 with 5 m*M* Tris-HCl containing 0.1% sodium azide. Approximately 50 μl of sample was added to each 7-mm diameter well. The antigen-to-antigen and antigen-to-antiserum well dis-

[1] Abbreviations used are: Nbs_2, 5,5′-dithiobis(2-nitrobenzoic acid); PAS, periodic acid–Schiff reagent.

Table I: Chemical Composition of Mercaptan-Solubilized Jelly Coat Layers.

Jelly Layer	μg[a]/Egg	Percentage Composition					Sulfate[b] μg/Egg	Moles of SH/ 100,000 g of Protein
		Protein	Fucose	Hexose	Hexosamine	Sialic Acid		
J_1	19.39	36.9	11.6	17.0	30.7	1.0	3.3	7.0
J_2	4.51	47.9	9.3	16.6	24.4	0.4	0.9	15.7
J_3	16.96	38.5	17.6	23.1	18.8	2.0	*c*	12.6

[a] From the sum of the individual components. [b] From Hedrick et al. (1974). The sulfate values are minimal estimates for J_1 and maximal estimates for J_2 due to contamination of J_2 by J_1. [c] No detectable sulfate.

Table II: Electrophoretic Mobilities and Staining Characteristics of Jelly Coat Macromolecules Resolved by Cellulose Acetate Electrophoresis.

Jelly Coat Layer[a]	Electrophoretic Mobilities[b]		
	Alcian Blue	PAS	Nigrosin
J_1	−7.59 ± 0.17	−7.20 ± 0.06	*c*
J_2	–	+1.68 ± 0.09	+2.14 ± 0.08
J_3	–	+2.92 ± 0.07	+2.92 ± 0.07
	–	+1.40 ± 0.03	+1.44 ± 0.04
	–	−2.26 ± 0.04	−2.46 ± 0.05
	–	–	−5.22 ± 0.05

[a] Dithiothreitol-solubilized jelly coat layers were dialyzed vs. 100 m*M* Tris-acetate–5 m*M* dithiothreitol (pH 8.0) prior to electrophoresis on cellulose acetate strips equilibrated with 100 m*M* Tris-acetate (pH 8.0). [b] Values are given as $cm^2\ V^{-1}\ sec^{-1} \times 10^5$ plus and minus the standard deviation of the mean. Each value is the average of six determinations; negative mobilities denote migration toward the anode. [c] Dashes indicate the absence of any components visualized with the indicated stain.

tances were 2.6 and 4.0 mm (edge-to-edge), respectively. Plates were developed at 22° in a moist chamber.

Immunoelectrophoresis was conducted on microscope slides coated with 1% agarose gels equilibrated with 100 m*M* Tris-acetate (pH 8.0). Following electrophoresis of antigen at 25 V/cm for 3–4 hr, antiserum was added to a central trough, and diffused for 24–48 hr in a moist chamber at 22°.

Dodecyl Sulfate Agarose Gel Electrophoresis. Zone electrophoresis of dodecyl sulfate treated samples (10–100 μg of hexose/gel) was conducted in 1% agarose gels containing 0.1% dodecyl sulfate as described by Holden et al. (1971a,b), with omission of sample gels and modification of sample treatment. Samples were first dialyzed overnight at 4° vs. 18 m*M* Tris–0.5 m*M* EDTA–18 m*M* borate (pH 8.2); then dodecyl sulfate (0.1%) and dithiothreitol (17 m*M*) were added and the samples heated at 55° for 30 min. Following addition of Bromophenol Blue and sucrose (4%), the samples (130 μl) were layered on top of the gels (0.5 × 7 cm). Electrophoresis was at room temperature, 2.5 mA/tube, with migration toward the anode until the tracking dye was within 1 cm of the bottom of the gel. The middle of the dye band was marked by the insertion of a needle dipped in India ink. Gels were fixed and stained with Coomassie Blue or PAS as described by Fairbanks et al. (1971). Densitometer tracings were obtained with a Gilford spectrophotometer (Model 2400-S) equipped with a linear transport mechanism and chart recorder. Gels stained with Coomassie Blue or PAS were scanned at 600 or 555 nm, respectively. Relative peak areas were obtained by cutting and weighing procedures.

[^{35}S]Sulfate-Labeled Jelly. Incorporation of $^{35}SO_4^{2-}$ into *Xenopus laevis* egg jelly was as previously described (Hedrick et al., 1975). Following zone electrophoresis of [^{35}S]sulfate-labeled, dithiothreitol solubilized J_1 and J_2 jelly coat layers in 1% agarose containing 0.1% dodecyl sulfate, the gels were cut into 1-mm slices with a Mickle Gel-Slicer. Each slice was solubilized by heating at 100° with 0.5 ml of H_2O in a scintillation vial, after which 10 ml of scintillation fluid was added and the radioactivity quantitated with a liquid scintillation counter.

Results

Chemical Composition. Table I summarizes the results of chemical analyses of *Xenopus laevis* egg jelly coat layers. Summation of the individual components present in the jelly coats yielded a value of 40.9 μg of jelly coat material per egg. This is in excellent agreement with the value of 39.4 μg determined by Freeman (1968) on the basis of nitrogen content and dry weight analyses. Each jelly coat layer consisted of 40–50% protein with the remaining 50–60% being carbohydrate predominantly in the form of fucose, hexosamines, and hexoses with smaller amounts of sialic acid also present. The overall carbohydrate composition of the three jelly coat layers was similar, although quantitative differences did occur. As mentioned in Materials and Methods, it was not possible to prepare jelly layer J_2 completely free from J_1. Thus, interpretation of the values in Table I for J_2 is limited due to the contamination of J_2. The values reported for J_2, therefore, are maximal when $J_1 > J_2$ (e.g., percentage composition of hexosamine) and minimal when $J_1 < J_2$ (e.g., percentage composition of protein). As described previously (Hedrick et al., 1975), jelly from *Xenopus laevis* eggs contained covalently bound sulfate and greater than 90% of the sulfate present in jelly was localized in jelly coat layer J_1.

Consistent with the mercaptan solubilization of the jelly coat layers was the presence of significant amounts (Table I) of free sulfhydryl groups accessible to Nbs_2 in the dithiothreitol-reduced material, whereas intact jelly contained barely detectable levels ($\Delta A_{412\,nm} < 0.008 = <0.2$ mol of -SH per 10^5 g of protein accessible to Nbs_2). Thus, greater than 95% of the Nbs_2-titratable sulfhydryl groups present in the reduced jelly coat layers was "masked" in the intact jelly, presumably as cystine residues. It was noteworthy that the Nbs_2 reaction kinetics with each of the reduced jelly coat layers was complex, indicative of more than one kinetic class of reactive sulfhydryl groups (data not shown) and required 60–120 min to reach completion.

The presence of both protein and carbohydrate suggested the possibility of a glycoprotein nature for at least some of the jelly coat macromolecules. If carbohydrate were attached to protein via O-glycosidic bonds to serine or threonine residues, strong alkali should induce a β elimination reaction with concomitant formation of dehydroalanine or

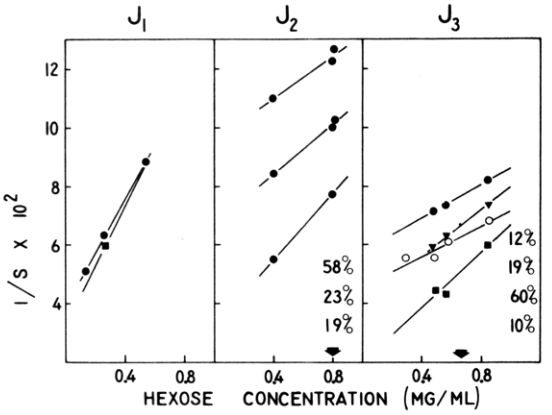

FIGURE 1: Dependence of sedimentation coefficients on concentration wih mercaptan-solubilized jelly coat layers. Centrifugation was in 100 m*M* Tris-acetate–5 m*M* dithiothreitol (pH 8.0). All values of 1/S were derived from the corresponding $s_{20,w}$ values. The arrows denote the concentrations at which relative peak areas as a percentage of the total schlieren pattern were determined. The indicated values are for the respective plots read from top to bottom.

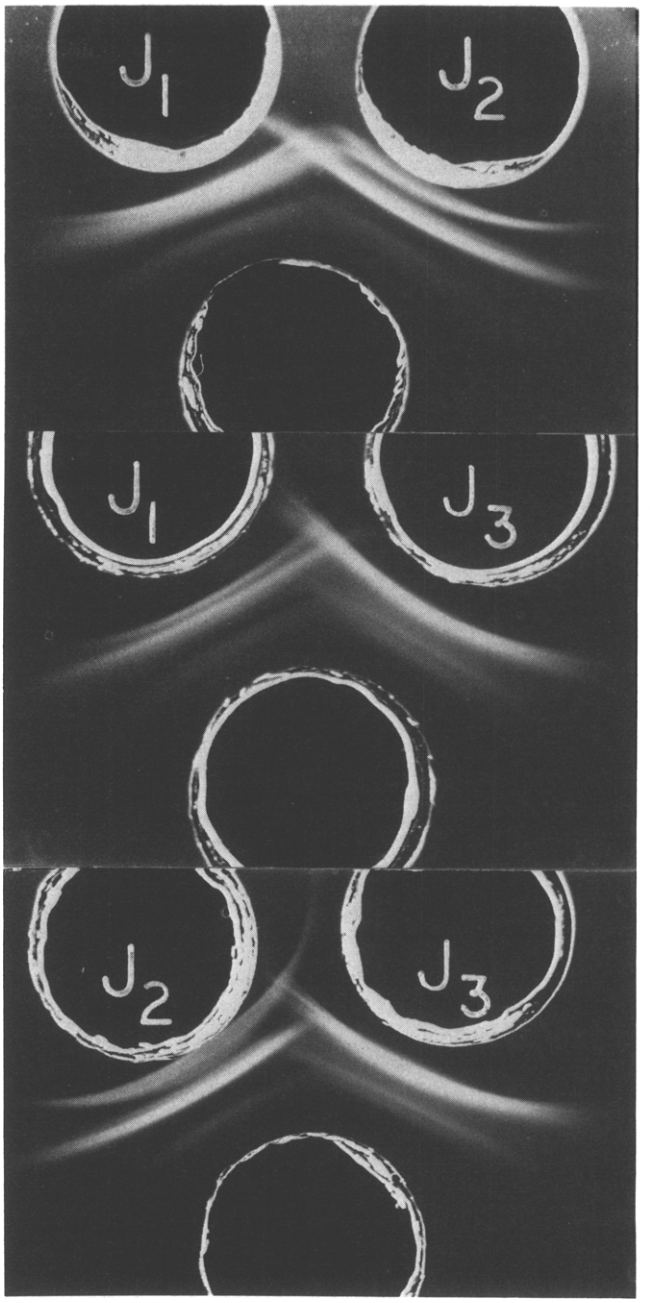

FIGURE 2: Comparative immunodiffusion results of the jelly layers. The bottom well in each section of the figure contained rabbit antiserum against total jelly. Diffusion was for 69 hr; other experimental conditions as described in the text.

α-aminoacrylic acid residues, detectable by their ultraviolet absorption (Carubelli et al., 1965). Incubation of individually solubilized jelly coat layers in 0.4 *N* NaOH at 22° resulted, for all three jelly layers, in a time-dependent increase in the absorbancy at 241 nm (30–40% in 40 min). In addition, amino acid and hexosamine analyses on a purified macromolecule from jelly layer J_3 indicated abnormally high levels of threonine relative to other amino acids and approximately equivalent amounts of threonine, glucosamine, and galactosamine (Oliphant, 1970). Thus, some of the carbohydrate in each of the jelly coat layers is covalently linked to protein through O-glycosidic bonds.

Cellulose Acetate Electrophoresis. Table II summarizes the electrophoretic mobilities and staining characteristics of the jelly coat macromolecules resolved by cellulose acetate electrophoresis in 100 m*M* Tris-acetate (pH 8.0). A total of nine basic and acidic components were observed. J_1 yielded a single broad, rapidly migrating anodic band when stained with either Alcian Blue for strongly acidic substances (e.g., sulfate esters) or with PAS for carbohydrate. The mobilities of this broad band visualized with the two stains were, however, slightly but significantly different, indicating the presence of at least two components. J_2 yielded a single cathodic band when stained for protein with nigrosin or for carbohydrate with PAS. Again, despite overlap of the bands visualized with the two stains, the mobilities were significantly different, indicating the presence of at least two species. J_3 gave the most complex pattern with two cathodic components both of which stained for protein and carbohydrate, and three anodic components, one of which stained with PAS and two of which stained with nigrosine; a total of five macromolecular species were detected. Whereas all three PAS staining components in J_3 stained with approximately equal intensity, the four nigrosine staining components stained disproportionately, in that one band ($\mu = +2.92 \times 10^{-5}$ cm^2 V^{-1} sec^{-1}) bound considerably more stain than the other three. The isoelectric point of the cathodically migrating species in jelly coats J_2 and J_3 must be quite high as they remained cathodic even in 45 m*M* mercaptoethanol buffer (pH 9.5).

Ultracentrifugation. When the mercaptan-solubilized jelly coat preparations were subjected to analytical sedimentation velocity centrifugation a total of eight unique species were observed with sedimentation coefficients ($s_{20,w}$) ranging from 7 S to 23 S. The schlieren peaks were all hypersharp, and as depicted in Figure 1, the $s_{20,w}$ values obtained were all highly concentration dependent. J_1 gave two closely sedimenting peaks, J_2 gave three peaks, the most rapidly sedimenting of which, however, was attributible to J_1 contamination, and J_3 gave four peaks. Area analysis of the sedimentation results was difficult due to the hypersharp nature of the peaks and interpretationally limited by the fact that the relative areas of the peaks varied as a function of concentration. Thus, the area analyses listed in Figure 1 must be considered as only a first approximation. In addition, we have observed that purified macromolecules of jelly layer J_1 exhibit sedimentation characteristics that

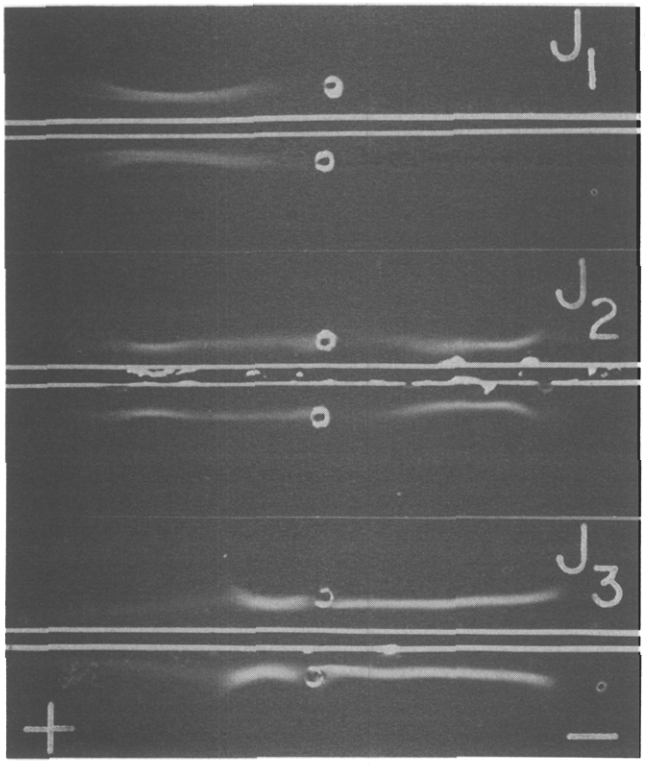

FIGURE 3: Comparative immunoelectrophoresis of jelly layers. Samples were run in duplicate; the center trough in each section contained total jelly antiserum. Other conditions as described in the text.

fit the classic Johnston–Ogston effect (Johnston and Ogston, 1946).

Immunodiffusion. The results of Ouchterlony double diffusion experiments of mercaptan-solubilized jelly coat layers vs. rabbit antiserum prepared against total jelly antigens are depicted in Figure 2. J_1 yielded two major and one minor bands. J_2 contained the three bands of J_1 due to contamination in addition to a poorly resolved doublet. The two precipitin bands unique to J_2 displayed complete antigenic nonidentity with the two major J_1 bands as evidenced by double spur formation. The relation of the minor J_1 band and the J_2 bands could not be ascertained due to the faintness of the bands. J_3 yielded a triplet and a doublet for a total of five precipitin bands; four bands exhibited complete nonidentity with the two major J_1 bands and at least three bands exhibited complete nonidentity with the two J_2-specific bands. Fusion of precipitin arcs closest to the antiserum well, with J_1 and J_3, indicated antigenic identity. There are nine antigenic components present in the jelly coat layers surrounding the egg: one common to J_1 and J_3, two unique to J_1, two unique to J_2, and four unique to J_3.

Immunoelectrophoresis. As seen in Figure 3, immunoelectrophoresis of J_1 yielded a closely resolved doublet of precipitin arcs migrating toward the anode. A diffuse precipitin arc was also observed at the origin when electrophoresis times were reduced. J_2 yielded an anodic doublet due to J_1 contamination, and, in addition, a pair of fused precipitin arcs migrating toward the cathode. The cathodic components, therefore, appear to be antigenically related. J_3 yielded two anodic species which displayed partial antigenic identity as evidenced by single spur formation. In addition, a complex pattern of cathodic components was observed which, apparently due to fusion of adjacent precipitin arcs, yielded only a single irregular precipitin arc probably composed of two or more components.

Table III: Sodium Dodecyl Sulfate Agarose Gel Electrophoresis of Jelly Coat Components.

Jelly Coat Layer[a]	Band[b] (R_m)	Percentage of Stain[c] PAS	CBB	Ratio (PAS/CBB)
J_1	0.43 ± 0.02	84	40	2.1
	0.53 ± 0.02	0	10	0
	0.80 ± 0.02	0	20	0
	0.92 ± 0.02	0	22	0
J_2	0.29 ± 0.01	50	45	1.1
	0.44 ± 0.02	37	19	2.0
	1.03 ± 0.02	6	22	0.3
J_3	0.19 ± 0.03	25	10	2.5
	0.36 ± 0.01	54	36	1.5
	0.46 ± 0.02	19	30	0.6
	0.80 ± 0.04	0	6	0
	0.92 ± 0.04	0	14	0

[a] Jelly coat layers were first solubilized with 5 m*M* dithiothreitol and then treated with dodecyl sulfate and additional dithiothreitol as outlined in Materials and Methods. [b] Mobilities are relative to the Bromophenol Blue tracking dye. Each value is the average of at least three determinations with the indicated standard deviation. [c] Each value is the average of two separate determinations and represents the percentage of stain in the indicated band relative to the total stain on the gel. Coomassie Blue is abbreviated CBB.

Dodecyl Sulfate Agarose Gel Electrophoresis. Table III summarizes the results of zone electrophoresis of dithiothreitol-solubilized, dodecyl sulfate treated jelly coat preparations in 1% agarose gels in the presence of 0.1% dodecyl sulfate. The use of agarose to form a gel matrix was necessitated by failure of the jelly coat macromolecules to effectively penetrate polyacrylamide gels. Under these dissociative conditions a total of 3–5 bands were observed originating from each jelly coat layer. The majority of the bands, especially those of lower electrophoretic mobility, contained both protein and carbohydrate as indicated by staining with Coomassie Blue and PAS, respectively. In general, the more slowly migrating species contained the higher percentage of carbohydrate relative to protein as indicated by their ratio of PAS to Coomassie Blue staining.

J_1 yielded one major component (R_m = 0.43) when stained for carbohydrate but yielded four bands when stained for protein. Electrophoresis of J_1 preparations labeled with [^{35}S]sulfate yielded a single peak of radioactivity coincident with the major carbohydrate-containing component (R_m = 0.43), indicating that this J_1 band contained the sulfated glycoprotein(s) present in the jelly. This band was also characterized by a typically distorted (grape-shot or grape cluster) appearance when very concentrated J_1 solutions were applied to the gel. J_2 yielded two glycoproteins (R_m = 0.29 and 1.03), in addition to a band attributed to J_1 contamination (R_m = 0.44) on the basis that the [^{35}S]sulfate label present in manually dissected J_2 migrated in this position. J_3 yielded three glycoproteins and two rapidly migrating proteins for a total of five species. Consistent with ultracentrifugation experiments, one band (R_m = 0.36) was predominant. The middle band in J_3 (R_m = 0.46) was distinguishable from the major J_1 component (R_m = 0.43) on the basis of its lack of [^{35}S]sulfate incorporation and nondistorted appearance. In contrast, the two most rapidly migrating components in J_1 and J_3 (R_m = 0.80 and 0.92) were indistinguishable in that their mobilities and staining characteristics were identical. Thus there are nine distinctly different species resolved by this technique.

Table IV: Macromolecular Composition of *Xenopus laevis* Egg Jelly.

Jelly Coat	Number Macromolecules Per Jelly Coat Layer[a]				
	CAE[e]	UC	ID	IE	SDS-Agarose
J_1	2	2	3	3	4
J_2	2	2	2	2	2
J_3	5	4	5	4	5
Total different macromolecules[b]	9	8	9[c]	9	9[d]

[a] Number of macromolecules per jelly coat layer. Correction has been made for J_1 contamination of manually dissected J_2 jelly coats. [b] Total number of *different* macromolecules in jelly from sum of the macromolecules unique to each of the three jelly coat layers. [c] Assuming one component common to J_1 and J_3. [d] Assuming two components common to J_1 and J_3. [e] Abbreviations used are: CAE, cellulose acetate electrophoresis; UC, ultracentrifugation; ID, immunodiffusion; IE, immunoelectrophoresis; SDS-agarose, sodium dodecyl sulfate agarose gel electrophoresis.

Discussion

This report is the first comprehensive study on the macromolecular composition of an amphibian egg jelly coat, and greatly extends the initial studies of Freeman (1968) and del Pino (1973) on the morphology and gross chemical composition of *Xenopus laevis* egg jelly coat. The importance of using manually dissected jelly coat layers cannot be overemphasized as was originally noted by Freeman (1968). Use of total jelly with the techniques described in this report resulted in much diminished resolution and sensitivity. The results of these studies clearly demonstrate the high degree of complexity and structural organization, at the macromolecular level, of the jelly layers surrounding *Xenopus laevis* eggs. As summarized in Table IV, the various analyses yielded quite consistent results with respect to both the number of macromolecules present in each of the three jelly coat layers as well as the total number of different macromolecules (8–9) present in the jelly coat. The majority of the macromolecules are uniquely associated with different jelly coat layers.

As indicated by the immunodiffusion and dodecyl sulfate agarose gel electrophoresis experiments, one or two macromolecules appear to be common to jelly layers J_1 and J_3 or to have similar physicochemical properties (e.g., similar charge/mass ratios in the case of dodecyl sulfate gel electrophoresis or similar antigenic determinants in the case of immunodiffusion). Whether or not the macromolecules common to J_1 and J_3 are identical or only share certain physicochemical properties but are otherwise different (e.g., amino acid and carbohydrate content or sequence) cannot be unequivocally determined until the macromolecules are isolated. The observation that most of the jelly coat macromolecules are uniquely associated with different jelly layers undoubtedly reflects the degree of specialization of the jelly secreting cells lining the oviduct.

It has not been possible to correlate relative mobility with molecular weight in the dodecyl sulfate agarose gel system employed in these studies as suitable marker proteins or glycoproteins of well-defined molecular weight large enough to be fractionated in the 1% agarose gels were not available. A further difficulty with the jelly coat macromolecules is that the observed mobilities may not be a function solely of their molecular weights but may also reflect charge differences, i.e., decreasing dodecyl sulfate binding as a function of increasing percentages of carbohydrate present. The most rapidly migrating jelly coat component, a J_2 glycoprotein, observed in a 1% dodecyl sulfate–5.6% acrylamide gel system (Fairbanks et al., 1971) had an apparent molecular weight of 90,000 (experiments not described). Thus, the glycoprotein macromolecules apparently possess large but as yet undetermined molecular weights. Lastly, the existence of jelly coat macromolecules as multimeric structures and, if such exist, the effectiveness of dodecyl sulfate treatment in dissociating such structures are not yet known. The molecular weights determined by this procedure may not be that of the subunits.

It is not to be assumed that macromolecules composing the various jelly coat layers are randomly or homogeneously distributed within a particular jelly coat layer. The nonuniform contamination of jelly layer J_2 by J_1 macromolecules (e.g., Table III) can be interpreted as indicating a structural differentiation within jelly coat layer J_1. In addition, ultrastructural studies employing electron microscopy suggest a nonuniform distribution of macromolecules within layer J_1 (Wyrick et al., 1974; Grey, R. D. and Hedrick, J. L., unpublished observations).

That the majority of the macromolecules present in the jelly coat are each localized within a specific jelly coat layer has obvious implications with respect to a diversity of functions of the three jelly coat layers in the fertilization process. It has been demonstrated that in *Xenopus laevis* eggs a sulfated J_1 glycoprotein participates in establishment of the block-to-polyspermy (Wyrick et al., 1974; Grey et al., 1974; Wolf, 1974), and that jelly coat layer J_1 is required for fertilization of the egg (E. C. Yurewicz and J. L. Hedrick, manuscript in preparation).

As reported here, the jelly coat macromolecules differ with respect to charge, size, and immunological determinants. Whether these differences are due primarily to differences in their protein or carbohydrate moieties or to a combination of both remains to be established. Now that the total macromolecular composition of *Xenopus laevis* egg jelly coat layers is known and adequate assays for each of the macromolecular components is available, isolation of specific jelly coat macromolecules, their physicochemical characterization, and elucidation of their roles in the fertilization process can be undertaken.

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Fucosyl-Glycoprotein and Precursor Pools in HeLa Cells†

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ABSTRACT: An enzymatic-radioactive isotope method has been developed for the direct quantitation of L-fucose in amounts as low at 0.5 ± 0.05 nmol. Fucose kinase is used to transfer [^{32}P]phosphate from ATP to [^{3}H]fucose. The labeled enzymatic products are then separated electrophoretically and the amount and specific activity of the fucose are determined from the known specific activity of the phosphate donor. This assay has been used to measure the GDP-L-fucose and macromolecular fucose in HeLa cells after extraction and purification of the sugar. It has been determined there are 0.5 nmol of GDP-L-fucose in 10^7 cells with a nine- to tenfold dilution of specific activity in converting L-[^{3}H] fucose to GDP-L-[^{3}H]fucose. After 2 to 3 days of labeling, the GDP-L-[^{3}H]fucose pool is essentially at equilibrium with the macromolecular pool, and hence it can be concluded that the dilution of label is due to a nine- to tenfold contribution to GDP-L-fucose from an endogenous source, as compared to exogenously supplied fucose. The fucosyl-glycoprotein pool has been shown to be much larger containing 6 to 8 nmol of fucose in 10^7 cells. It has further been shown that GDP-fucose is the only soluble fucose intermediate present in significant amount.

Radioactive fucose is a useful precursor for studying the biosynthesis of glycoproteins (Bekesi and Winzler, 1967; Jabbal and Schachter, 1971; Trujillo and Gan, 1971) including those in the HeLa cell surface (Shen and Ginsburg, 1968; Atkinson and Summers, 1971; Atkinson, 1973). In HeLa cells, radioactive fucose is found almost entirely in glycoprotein (Shen and Ginsburg, 1968; Atkinson, 1975) and the label is not distributed into other sugars (Kaufman and Ginsburg, 1968). However, the rate at which the radioactive fucose is incorporated into glycoprotein is not necessarily a reflection of the true rate of synthesis because the soluble precursor pool, GDP-fucose, can be derived from two sources: namely, exogenous fucose (Bekesi and Winzler, 1967; Kaufman and Ginsburg, 1968; Ishihara et al., 1968; Ishihara and Heath, 1968) and endogenously synthesized GDP-mannose (Foster and Ginsburg, 1961). Radioactive GDP-fucose will thus be diluted to an extent depending on the endogenous contribution, and thus the specific radioactivity may vary with growth conditions (Nowakowski et al., 1972). Therefore, the determination of glycoprotein synthetic rates from the use of radioactive precursors will depend on measuring GDP-fucose. Furthermore, it may be possible to infer precursor-product relationships from mea-

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