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The Crystalline Yolk-Platelet Proteins and their Soluble Plasma Precursor in an Amphibian, *Xenopus laevis*

By M. R. REDSHAW and B. K. FOLLETT
Department of Zoology, University College of North Wales, Bangor, Caerns., U.K.

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A single lipophosphoprotein complex, vitellogenin, was isolated and purified from the plasma of oestrogen-stimulated female toads by preparative ultracentrifugation and chromatography on TEAE-cellulose (triethylaminoethylcellulose). The protein contains 12% lipid, 1.5% phosphorus, 1.6% calcium and smaller amounts of carbohydrates and biliverdin. In amino acid composition it is identical with total yolk-platelet protein. The platelet protein, however, is fractionated on TEAE-cellulose into two components, a high-molecular-weight lipovitellin and a smaller phosvitin. Analyses of the soluble plasma vitellogenin suggest that it is a complex of two phosvitin molecules covalently bound to one lipovitellin dimer, and that it is the immediate precursor of the yolk proteins, into which it is converted by a molecular rearrangement. Uptake of vitellogenin from the plasma into the growing oocyte, and its subsequent crystallization as a yolk platelet, appear to be enhanced by gonadotrophic hormones.

Hitherto, virtually all investigations into the properties of the yolk proteins and of their precursors have concentrated on the domestic fowl (e.g. McIndoe, 1959; Schjeide & Urist, 1960; Heald & McLachlan, 1963; Schjeide *et al.* 1963; Cook & Martin, 1969). In this species vitellogenesis proceeds first by the synthesis of the yolk proteins in the liver, followed by their transportation in the bloodstream to the ovary and their deposition as granules in the maturing ovum. The insoluble material in the granules is mainly lipoprotein in nature (lipovitellins, 'light' lipoproteins) but does include a lipid-free phosphoprotein, phosvitin. The plasma precursors of the yolk proteins, however, are soluble under physiological conditions. They have recently been referred to collectively as 'vitellogenins' (Pan, Bell & Telfer, 1969). Their synthesis is induced by oestrogens secreted by the ovary.

This pattern of vitellogenesis seems common to all vertebrates and during the past 5 years has been studied extensively in Amphibia by using the South African toad *Xenopus laevis* as experimental material (Follett & Redshaw, 1968, 1971; Wallace, 1967, 1970*b*; Wallace & Dumont, 1968; Wallace & Jared, 1969; Wallace, Jared & Nelson, 1970). The present paper is concerned with the chemical and physical properties of the single plasma vitellogenin and with the two yolk proteins into which it is cleaved in the egg. The cleavage processes and the conversion of the soluble vitellogenin into insoluble platelet proteins are also considered.

MATERIALS AND METHODS

Animals. Mature male and female *Xenopus* were supplied by the South African Snake Farm, Fish Hoek, Cape Province, South Africa. They were maintained in tap water at 20°C and fed twice weekly on diced ox liver.

Experimental treatments. Vitellogenin synthesis was stimulated by the subcutaneous implantation of a 15mg pellet of fused oestadiol-17β (Follett & Redshaw, 1968). Blood was collected by cardiac puncture. Radioactive vitellogenin was produced by injecting chronically oestrogen-treated toads (implanted for 6 weeks) with three doses (30μCi) of high-specific-radioactivity (50Ci/mg of P) $Na_3{}^{32}PO_4$ into the dorsal lymph sac. A few days later the toads were anaesthetized and bled.

Isolation of plasma vitellogenin and total yolk-platelet protein. Plasma from chronically oestrogen-treated toads (50–70 days stimulation) was centrifuged at 80000*g* for 9h at 4°C in a 3×23ml swing-out rotor of an MSE Superspeed 40 preparative ultracentrifuge. The pellet was resuspended in a solution containing 0.14M-NaCl, 20mM-KCl, 20mM-$CaCl_2$ and 60mM-$NaHCO_3$ (solution A), and was recentrifuged. The green protein solution was then exhaustively dialysed against cold water and freeze-dried. The resultant green fluffy lipoprotein was either utilized in this relatively impure form or processed further by chromatography.

Total yolk-platelet protein was prepared by the method of Schjeide, Levi & Flickinger (1956). Ovarian tissue was homogenized in 0.15M-NaCl and the insoluble proteins were collected by centrifugation at 1700*g* for 10min. They were resuspended and centrifuged several times before being finally dissolved in 0.5M-NaCl. This final step allows the removal of pigment granules and cell

debris. The proteins were reprecipitated from solution by dialysis against water, and dried.

Electrophoretic and chromatographic procedures. Acrylamide-gel electrophoresis was used to assess both the increase in vitellogenin in the plasma of oestrogen-treated toads (7.5% gel) and the homogeneity of the various vitellogenin fractions prepared chromatographically (4–5% gel). The discontinuous buffer system of Ornstein & Davis (1964*a*,*b*) was employed, the proteins being stained with a 1% (w/v) solution of Amido Black 10B in aq. 7% (v/v) acetic acid.

Vitellogenin, prepared by preparative ultracentrifugation, was further purified by chromatography on an agarose gel column (2.5cm × 130cm) at 4°C (Sepharose 4B; Pharmacia, Uppsala, Sweden). The flow rate of solution A (kept at pH 7.5 by 0.02M-tris–HCl) was 6ml/h and the 3ml fractions were monitored for protein by measuring their extinction at 280nm. An indication of the molecular weight of the vitellogenin was obtained by calibrating the agarose column with a series of proteins of high molecular weight (Andrews, 1965).

Anion-exchange chromatography on 100–300-mesh triethylaminoethylcellulose (TEAE-cellulose) [Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.] was used to purify both the plasma vitellogenin and the total yolk-platelet protein. The method was essentially that of Wallace (1965). Samples (approx. 200mg) were dialysed to equilibrium against the starting buffer (0.01M-citric acid–0.06M-2-amino-2-methylpropan-1-ol, pH 9.9) and then applied to the column (2.5cm × 45cm). Proteins were eluted with an exponential gradient of decreasing pH value and increasing concentration, the limiting buffer being 0.25M-citric acid–0.75M-2-amino-2-methylpropan-1-ol, pH 7.5). The flow rate was 50ml/h. The eluate was monitored continuously at 280nm by means of a 2mm flow-through cell, and fractions containing protein were pooled, dialysed and freeze-dried.

Amino acid analysis. Protein samples (0.5–1.0mg) were hydrolysed in 0.5ml of nitrogen-flushed 6M-HCl in evacuated glass ampoules for 24h at 110°C. Analyses were performed on a Technicon amino acid AutoAnalyzer operating by the method of Piez & Morris (1960). Tryptophan was not assayed. Losses of serine resulting from acid hydrolysis were estimated by hydrolysing protein samples for 12, 24, 36, 48, 72 and 108h and constructing a curve of serine residues (per 1000 residues) against hydrolysis time. Extrapolation to zero time gave the true serine content of the protein. This procedure was mandatory for phosvitin, since more than 50% of all residues are serine; other proteins having much less serine showed negligible losses when hydrolysed for 24h.

Chemical and radioactivity analyses. Protein P measurement, the simplest means of estimating both vitellogenin and the yolk proteins, was performed after alkaline hydrolysis, by the procedure of Fiske & SubbaRow (1925).

Total plasma Fe, Cu, P, phospholipid, protein-bound P, Ca and total lipid were measured as previously described (Follett & Redshaw, 1968). Protein was determined by either the Folin–Ciocalteu reagent (Lowry, Rosebrough, Farr & Randall, 1951), with bovine serum albumin as the standard, or by direct measurement of E_{280}^{1cm}. Protein-bound Ca was measured by the centrifugal ultrafiltration method of Toribara, Terepka & Dewey (1957). This method was also used to prepare plasma ultrafiltrate. Protein-bound hexose was assessed by the ethanolic-orcinol method (Winzler, 1955), protein N by the micro-Kjeldahl procedure and protein-bound biliverdin as described by Redshaw, Follett & Lawes (1971).

For the determination of labelled material in the eluates from chromatography columns, portions (0.1ml) were applied to glass-fibre filter discs (Whatman GF/B, 2.1cm diam.), which were then mounted in a Millipore filter holder. The discs were washed with an excess of 10% (w/v) trichloroacetic acid to remove non-protein-bound radioactivity, and then dried. They were counted for radioactivity in a Beckman liquid-scintillation system in standard vials containing 5ml of phosphor solution [0.4% 2,5-diphenyloxazole, 0.03% 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene in toluene]. Under these conditions quenching did not occur.

Physical determinations. Sedimentation studies were performed with a Spinco model E analytical ultracentrifuge, normally operated at 20°C and 59780rev./min with the phase plate set at 60–70°. Photographs were taken every 4min. Protein concentration (assessed by an interferometer) was nominally 0.5–1.0%. The solvent differed, depending on the protein preparation used, being at pH 9.5 or high molarity (1M-NaCl) when yolk was analysed since yolk platelets are insoluble at neutral pH and low molarity.

RESULTS

After oestrogen administration to sexually immature *Xenopus* a protein (vitellogenin) appears newly in the plasma (Plate 1*a*). Chemical determinations (Follett & Redshaw, 1968) show that the concentration of this protein, together with its bound constituents (P, Ca, phospholipid), increases steadily in an implanted animal until after 8–10 weeks of treatment it accounts for more than 90% of the total plasma proteins (Plate 1*b*). Its rate of migration in acrylamide gels implied that it had a high molecular weight, suggesting that a convenient step in its isolation and purification would be to submit plasma from a chronically treated toad to preparative ultracentrifugation. This method is also the least harmful that could be applied and should not remove the protein-bound Ca. Such treatment of the deep-green plasma resulted in the formation of a clear, green viscous pellet. The remaining plasma had a straw colour typical of that from untreated toads. The amino acid composition of this 'crude' vitellogenin preparation is shown in Table 1, and its chemical composition in Table 2.

After ultracentrifugation some of the protein was passed through an agarose-gel column. The elution profile (Fig. 1) showed a single protein peak with negligible contamination from other serum proteins. The peak material was isolated and analysed (Tables 1 and 2) and was found to be similar to the 'crude' vitellogenin. Its elution position from the agarose column suggests a molecular weight of about

Table 1. *Amino acid composition of* Xenopus *vitellogenin and ovarian proteins*

The results are expressed as residues of amino acid/1000 residues. Vitellogenin I is 'crude' vitellogenin prepared by preparative ultracentrifugation; vitellogenin II is the protein obtained after agarose-gel chromatography and vitellogenin III is the protein obtained after TEAE-cellulose chromatography. Each result is the mean of five individual analyses.

	Composition (residues/1000 residues)					
	Vitellogenin					
	I	II	III	Total platelet protein	Yolk lipovitellin	Yolk phosvitin
Gly	51.4	51.5	50.1	54.2	53.4	28.4
Ala	85.0	85.0	82.9	94.1	101.8	22.2
Val	45.6	43.6	45.5	47.0	50.8	5.8
Leu	81.0	82.5	77.9	82.0	85.1	14.0
Ile	35.9	35.1	37.0	37.9	40.6	3.3
Ser	117.0	121.4	115.0	107.4	81.3	560.0
Thr	55.3	56.8	54.8	51.1	56.4	7.2
½Cystine	13.0	13.5	8.1	9.0	9.1	0
Met	23.0	18.7	14.8	16.9	20.1	0
Asp	83.8	85.8	92.3	91.3	87.7	49.8
Glu	138.0	136.3	137.0	135.4	138.2	101.4
Lys	78.8	76.0	83.0	75.5	73.0	75.6
Arg	53.0	51.7	58.8	58.2	56.4	59.6
His	28.7	28.0	32.0	28.2	28.7	27.7
Tyr	30.2	29.7	26.6	31.6	29.6	9.4
Phe	35.6	35.9	34.8	41.9	42.3	7.2
Pro	47.5	46.5	48.7	40.5	50.3	29.8

600000, although such estimates may be subject to considerable error for complex lipoproteins. As predicted, however, it was totally excluded from Sephadex G-200.

The most convenient method of further purifying the vitellogenin is to submit it to TEAE-cellulose chromatography, and this was done by using 200mg of the 'crude' preparation after ultracentrifugation. A single trailing peak with R_F 0.66 was obtained (Fig. 2*a*). Amino acid analyses of this peak (Table 1) showed it to be identical with the other vitellogenin preparations. However, its chemical composition was altered slightly (Table 2); most notably, the lipid and Ca contents were lowered after TEAE-cellulose treatment.

A further advantage of TEAE-cellulose is that it may be used to separate the protein components of the yolk platelets (Wallace, 1965), the solubility characteristics of which make them unsuitable for chromatography on agarose gels. Total platelet protein was prepared from whole *Xenopus* ovaries and like the vitellogenin was light-green in colour. Its amino acid composition is shown in Table 1, indicating it to be similar to vitellogenin. On TEAE-cellulose, however, the platelet protein showed two peaks (Fig. 2*b*). The larger had R_F 0.41 and resembled in amino acid and chemical composition (Tables 1 and 2) the avian yolk protein, lipovitellin, and is hence called amphibian lipovitellin (Lv). The smaller peak, comprising about 8% only of the total platelet protein, had R_F 0.92 and is termed amphibian phosvitin (Pv) (Table 1). Like the homologous avian protein, over half of the residues are serine. From the alkali-labile-P content (11%) it was calculated that 72% of these serine residues are phosphorylated.

Further chromatography of *Xenopus* lipovitellin on TEAE-cellulose suggested that some minor heterogeneity was present but discrete subfractions were unobtainable. When phosvitin was chromatographed on DEAE-cellulose by using a linear gradient of 0–0.5M-NaCl in 50mM-tris–HCl–5mM-EDTA, pH 8.0, as eluent, fractions were obtained that had identical amino acid composition but differed in their alkali-labile-P content.

Chronically oestrogen-treated toads readily synthesized labelled vitellogenin if injected with sodium [^{32}P]phosphate (Fig. 2*a*). This radioactive protein was converted into the two yolk-platelet proteins, phosvitin and lipovitellin, after being introduced into the circulation of female toads actively engaged in yolk deposition. Isolation of the labelled yolk proteins by TEAE-cellulose chromatography showed that the label was distributed between the protein peaks according to the ratio of their P contents. Total yolk-platelet protein consists of 91% lipovitellin and 9% phosvitin; thus the overall ratio of P contents should be 0.915, since the P content of phosvitin is 95mg/g but that of lipovitellin only 7.5mg/g. The observed value

Table 2. *Chemical composition of* Xenopus *vitellogenin, lipovitellin and phosvitin*

The values for composition are expressed as g/100g of protein. Vitellogenin I was isolated by preparative ultracentrifugation of plasma from chronically oestrogen-treated toads and vitellogenin III is the protein obtained after TEAE-cellulose chromatography.

	Vitellogenin			
	I	III	Lipovitellin	Phosvitin
Total lipid	12.3	4.5	20.0	Trace
Phospholipid	10.0	—	8.0	—
Neutral lipid	3.0	—	11.0	—
Total P	1.52	0.75	0.75	9.5
Protein-bound P	1.35	1.1	0.5	9.5
Protein Ca	1.60	Trace	0	0
Ca/P ratio	1.05	—	—	—
Protein N	14.6	14.9	15.8	11.5
N/P ratio	10.8	13.5	31.5	1.2
Carbohydrate	0.7	0.3	0.3	—
S	0.85	—	0.4	Trace
Fe		2.2×10^{-3}		
Cu		5.0×10^{-4}		
Biliverdin (molar ratio)	2	2	1	0
Serine residues/1000	117	115	81.3	560.0
Percentage of serine residues phosphorylated				72
Phosvitin/lipovitellin ratio	$\frac{0.090}{0.910}$		$\frac{0.046}{0.954}$	

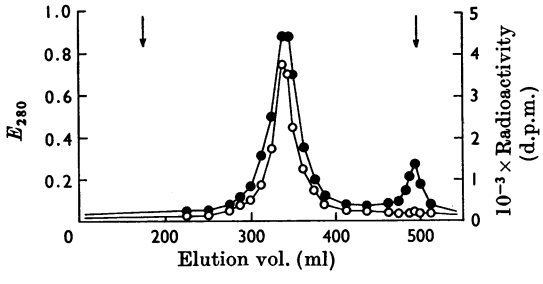
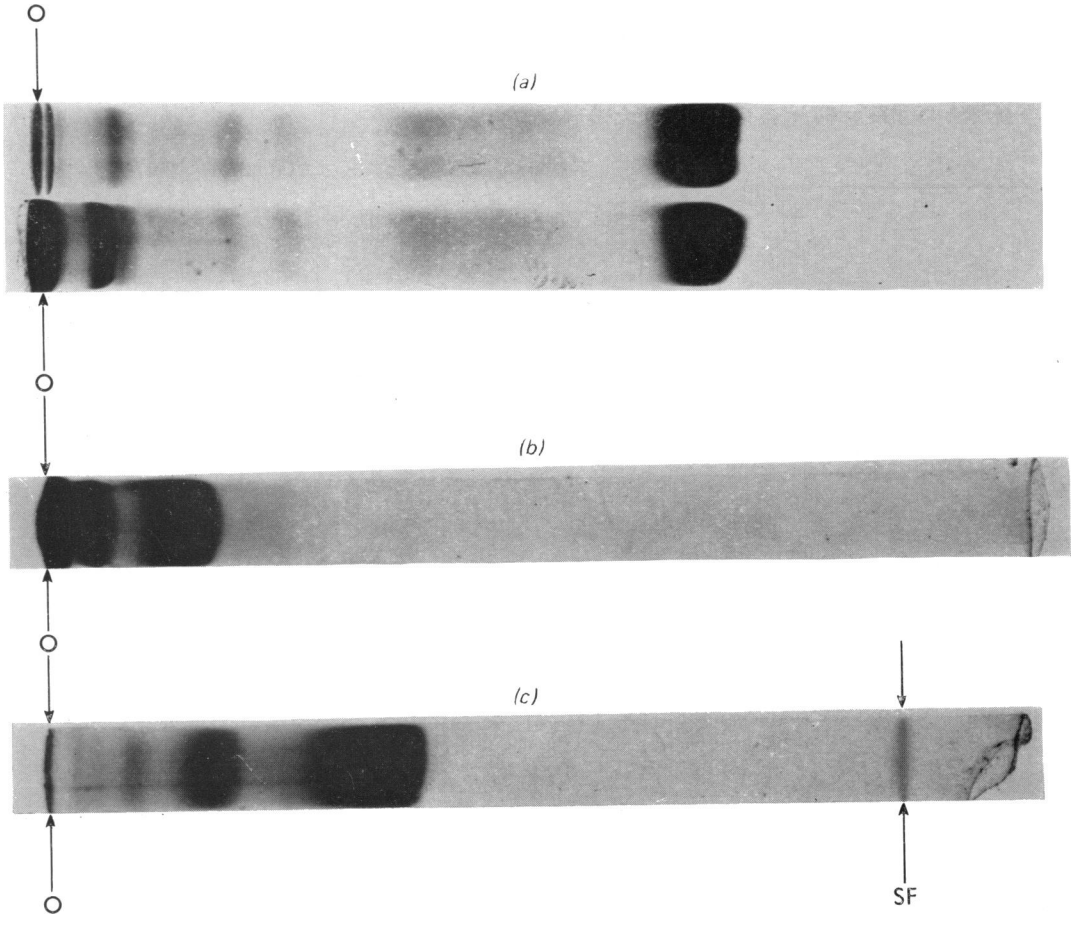


Fig. 1. Agarose-gel chromatography of 'crude' vitellogenin prepared by ultracentrifugation. The column was 2.5cm × 130cm and was eluted with solution A, maintained at pH 7.5 by 0.02M-tris–HCl. The major protein peak (E_{280}, ●) was eluted after 340ml had passed through the column. This peak contained all of the alkali-labile P (d.p.m., O). A smaller peak representing lower-molecular-weight contaminants was eluted with the retention volume. The left-hand arrow indicates the void volume of the column (175ml) and the right-hand arrow the retention volume (487ml).

based on the radioactivity associated with each of the protein peaks in Fig. 2(*b*) gave a Lv/Pv ratio of 0.92. In the lipovitellin the label was associated with both the phospholipid and protein-bound P parts of the molecule. Similar results were obtained when [^{14}C]valine was incorporated into the vitellogenin. The specific radioactivity of the two yolk proteins reflected their relative valine contents, i.e. a (lipovitellin[^{14}C]valine)/(phosvitin[^{14}C]valine ratio of 8.75.

Electrophoresis of the purified plasma vitellogenin in 4.5% acrylamide gel gave the pattern of bands shown in Plate 1(*c*). Although 75% of the protein was present as the fastest migrating component (R_F 0.425), three other bands were detectable with R_F values 0.22, 0.11 and 0.06. These minor components are probably aggregates of the 'basic' molecule. No evidence for such high-molecular-weight species was obtained during agarose-gel chromatography at neutral pH values (Fig. 1).

Analytical ultracentrifugation yielded a confusing series of results. If plasma from a chronically oestrogen-treated toad (containing 12–15g of vitellogenin/100ml of plasma) was diluted with a plasma ultrafiltrate from control toads, then a pattern was obtained (Fig. 3*a*) with predominantly a single component having a sedimentation coefficient ($s^0_{20,w}$) of 17.7S. In other determinations, in potassium chloride–potassium phosphate solutions as the diluent, patterns resembling the result from acrylamide-gel electrophoresis were often recorded (e.g. Fig. 3*b*, cf. Plate 1*c*). Here, the sedimentation rates indicated probable monomers, dimers and trimers with $s^0_{20,w}$ values of 17.5S, 25.0S and 35.0S respectively. After chromatography on TEAE-cellulose slightly different values were obtained. Ultracentrifugation of yolk-platelet proteins in alkaline solution (pH 9.5) gave two peaks, the major



EXPLANATION OF PLATE 1

Acrylamide-gel electrophoresis patterns. (*a*) Plasma from male *Xenopus* before and after treatment with a pellet of fused oestradiol-17β. The 7.5% gel was run in the discontinuous-buffer system of Ornstein & Davies (1964*a*,*b*). Note the appearance of a densely staining band close to the origin, which represents *Xenopus* vitellogenin. (*b*) Plasma obtained after 60 days treatment of the toads with oestradiol. Negligible amounts of contaminating proteins are visible (7.5% gel). (*c*) Aggregates of vitellogenin that appear when 'crude' vitellogenin is subjected to electrophoresis in 4% gel. Some 75% of the protein is present as the monomer with R_F 0.425. In the 7.5% gel, some protein denaturation occurs, accounting for the dense 'origin' band (O). The solvent front (SF) is indicated by the Bromophenol Blue band.

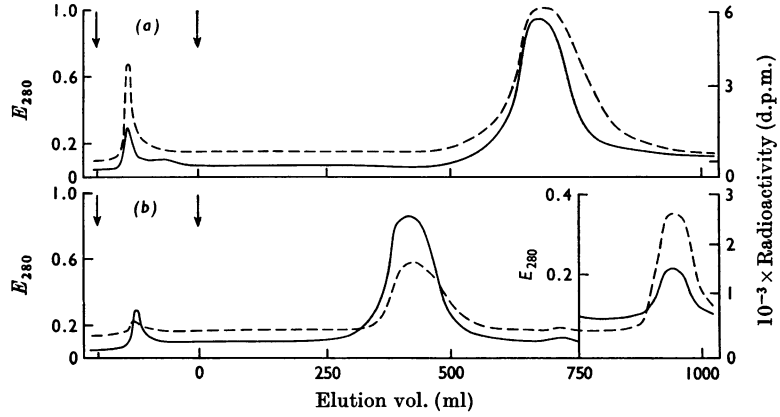


Fig. 2. TEAE-cellulose chromatography of vitellogenin and yolk-platelet proteins. (*a*) Vitellogenin was eluted as a single peak from the column at R_F 0.66. ——, E_{280}; ----, alkali-labile ^{32}P. (*b*) Yolk-platelet protein dissolved in the starting buffer was eluted as two peaks. The peak with R_F 0.41 is amphibian lipovitellin and the small peak eluted at R_F 0.92 is phosvitin. The left-hand arrow indicates the point of application of the sample to the column, and the right-hand arrow indicates the commencement of the gradient.

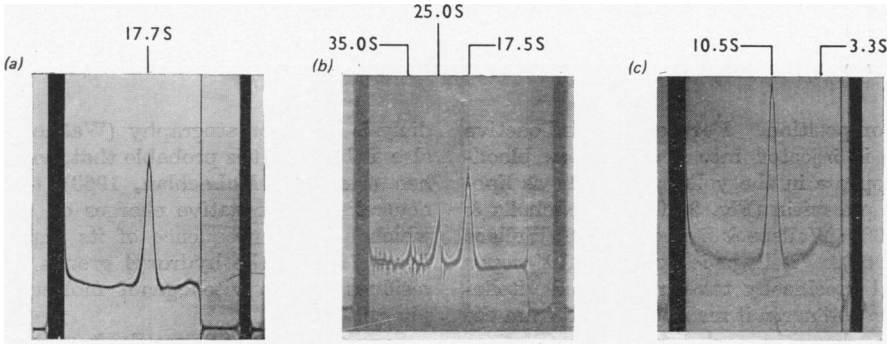


Fig. 3. Ultracentrifuge patterns of *Xenopus* vitellogenin and yolk lipovitellin. Sedimentation diagrams were recorded after centrifugation for 16 min (*a*, *b*) and 28 min (*c*) at 59780 rev./min with the phase plate set at 60°. The $s_{20,w}$ values are indicated. (*a*) *Xenopus* vitellogenin (protein concn. 0.8 g/100 ml) prepared by preparative ultracentrifugation and redissolved in centrifugally ultrafiltered plasma from control female toads. (*b*) Aggregates of *Xenopus* vitellogenin (protein concn. 0.75 g/100 ml), which often appear when 'crude' vitellogenin is redissolved in 50 mM-sodium phosphate buffer, pH 7.5, containing 0.15 M-NaCl. (*c*) *Xenopus* yolk-platelet protein dissolved in the starting buffer (pH 9.9) for TEAE-cellulose chromatography, containing 0.15 M-NaCl (protein concn. 0.65 g/100 ml). The smaller peak probably represents phosvitin and the larger peak represents the lipovitellin dimer.

one representing lipovitellin, with $s^0_{20,w}$ 10.5S (Fig. 3*c*). The minor peak, probably caused by phosvitin, was estimated to have a sedimentation coefficient of about 3.3S.

Some physical properties of vitellogenin, largely derived from the results of Wallace (1970*a*), are shown in Table 3.

DISCUSSION

In *Xenopus*, and probably also in *Rana catesbeiana* (Urist & Schjeide, 1961) a single vitellogenin appears in the plasma in response to oestrogen stimulation. This is in contrast with the domestic fowl where two vitellogenins are synthesized *de novo* and a third is greatly increased in concentration (Schjeide *et al.* 1963). However, the amphibian yolk platelet does contain a lipovitellin and a phosvitin that are similar to the avian yolk-granule proteins, and the evidence that these proteins are derived, in *Xenopus*, by a molecular rearrangement of the single vitellogenin is unequivocal. Total yolk-platelet protein has an amino acid composition essentially indistinguishable from that of vitellogenin and there are other striking similarities in

Table 3. *Some physical properties of* Xenopus *vitellogenin, lipovitellin and phosvitin*

Vitellogenin I is 'crude' material prepared by preparative ultracentrifugation. Vitellogenin III, lipovitellin and phosvitin have been purified by TEAE-cellulose chromatography. The results for vitellogenin III and the yolk-platelet proteins are from Wallace (1963*a*,*b*, 1970*a*). Absorptivity is measured at 280nm on solutions of known concentration as determined by dry-weight analysis.

	Vitellogenin			
	I	III	Lipovitellin	Phosvitin
Absorptivity (l/g·cm)	—	0.77	0.74	0.32
Partial specific volume, $\bar{v}$ (ml/g)	0.700*	0.746	0.787	—
Sedimentation coefficient, $s^0_{20,w}$ (S)	17.7	13.6	10.5	3.3
$10^7 \times$ Diffusion coefficient $D^0_{20,w}$ (cm^2/s)	—	2.75	3.75	5.08
$10^{-5} \times$ Molecular weight (calculated from $D^0_{20,w}$ and $s^0_{20,w}$)	5.4	4.7	2.9	0.51
$10^{-5} \times$ Molecular weight (by sedimentation equilibrium)	—	4.5	—	—
$10^{-5} \times$ Protein molecular weight†	4.4	3.9	2.4	0.38
R_F on TEAE-cellulose column	—	0.66	0.41	0.92
Molecular diameter (monomer)	—	—	80Å	40Å
$10^{21} \times$ Volume of one structural yolk-platelet unit (ml)			602	
Molecular ratio (phosvitin/lipovitellin)	1.6–2.3		1.6–2.3	

* Estimated value; see the text.

† These values indicate the estimated protein molecular weights obtained by ignoring the lipid, P, carbohydrate and gegenions (e.g. Na^+), which constitute 7–10% of the phosvitin molecule.

chemical composition. Further, if radioactive vitellogenin is injected into the *Xenopus* bloodstream it appears in the yolk specifically as lipovitellin and phosvitin (Fig. 2; Follett, Nicholls & Redshaw, 1968; Wallace & Dumont, 1968; Wallace & Jared, 1969). Vitellogenic oocytes of *Xenopus* actively and specifically take up labelled vitellogenin *in vitro* and again it may be isolated from the platelets as phosvitin and lipovitellin (Wallace *et al.* 1970). Other more indirect immunological evidence also supports this viewpoint (antibody prepared against lipovitellin cross-reacts with vitellogenin; Wittliff & Kenney, 1969; Follett & Redshaw, 1971).

The plasma vitellogenin, being the primary storage reserve material for future embryonic development, is of necessity a complex molecule. It has a high P content (1.52%), much of which is lost during TEAE-cellulose chromatography. This loss seems to be mostly of P present in the phospholipid component of the molecule. The total lipid content of plasma vitellogenin is 12.3%, of which 75% is phospholipid, the remainder being triglyceride.

In the plasma the vitellogenin appears to be loosely complexed with Ca, and the Ca/P atomic ratio was calculated to be 1 (Follett & Redshaw, 1968; Wallace, 1970*a*). The Ca remains complexed with the vitellogenin during ultracentrifugation (Table 2) or paper electrophoresis (Munday, Ansari, Oldroyd & Akhtar, 1968), but is rapidly lost during dialysis or chromatography (Wallace, 1970*a*; see also Table 2). It is probable that, as in the laying hen (Heald & McLachlan, 1963), the Ca^{2+} ions neutralize the negative charges on the molecule, which are a consequence of its high P content. Some 72% of the hydroxyl groups of the serine residues in the vitellogenin molecule are phosphorylated.

Small amounts of Fe are also bound to the vitellogenin (Wallace, 1970*a*). The green colour of *Xenopus* vitellogenin is caused by biliverdin, a haem breakdown pigment, which seems to be attached as a prosthetic group (Redshaw *et al.* 1971). Although its function is unknown, biliverdin appears to be an obligatory constituent of the molecule and is also found in equimolar amounts bound to yolk-platelet lipovitellin. Minor quantities of carbohydrate are present in vitellogenin, but these do not appear to be associated with any nucleic acid components. Vitellogenin is therefore unlikely to be the means whereby maternal DNA and RNA are transported to the amphibian oocyte, as was proposed by Baltus, Hanocq-Quertier & Brachet (1968).

Although evidence was found for aggregates of the 'basic' vitellogenin molecule in both the analytical ultracentrifuge and acrylamide-gel electrophoresis, the significance of these components to the physiological system is unclear. Certainly no evidence for such aggregates was found when plasma was submitted to agarose-gel chromato-

graphy, and R. A. Wallace (personal communication) has failed to find aggregates if purified vitellogenin is analysed in the ultracentrifuge. It remains possible, however, that under physiological conditions an equilibrium exists in the plasma between the monomer and higher-molecular-weight aggregates.

The physicochemical results of Wallace (1970*a*) obtained with *Xenopus* vitellogenin have been used to estimate its molecular weight in the plasma. Under these conditions it contains 1.6% Ca, and its density would be some 7% greater than that of the chromatographically purified material. The revised partial specific volume, estimated to be 0.7 (Table 3), gives a molecular weight for 'physiological' vitellogenin of about 540000. This is close to Wallace's (1970*a*) estimate of 470000.

Xenopus vitellogenin has proved to be extremely resistant to chromatographic separation into subunits. Even reagents or conditions that normally cause dissociation of protein aggregates (including chicken lipovitellin), such as alkaline pH, 6M-urea, EDTA or guanidine hydrochloride, fail to dissociate the molecule. However, the amino acid analyses indicate that *Xenopus* vitellogenin consists of 9% phosvitin and 91% lipovitellin, and it would appear likely that in *Xenopus* the two proteins are synthesized separately by the liver cell but are linked covalently just before secretion. This step seems to be absent in the chicken and consequently the two vitellogenins remain independent in the plasma.

Rather more information is available about the amphibian yolk-platelet proteins but the way in which the soluble vitellogenin is converted within the oocyte into the highly ordered yolk platelet is still open to speculation. The platelet proteins are combined together as a crystalline lattice of two polar phosvitin molecules (mol.wt. about 40000) embedded in a lipovitellin dimer (mol.wt. about 400000). A phosvitin dimer forms by the juxtaposition of neighbouring phosvitin–lipovitellin molecules (Wallace & Dumont, 1968). Purified yolk-platelet protein dissolves in alkaline solvents, in which the lipovitellin component dissociates reversibly into two equal units (Cook & Wallace, 1965), and the phosvitin dissociates from the lipovitellin, and can be purified. In chicken phosvitin the predominant serine residues occur in groups of eight (Williams & Sanger, 1959); whether a similar situation occurs in the *Xenopus* protein is uncertain. The yolk lipovitellin has a higher neutral-lipid content than the plasma vitellogenin. The source of this extra lipid is not known, but it may be derived from a non-protein-bound lipid fraction, mainly esters of cholesterol, of which the amount in the plasma is increased by oestrogen treatment (Follett & Redshaw, 1968).

Gonadotrophic hormones are a prerequisite for the uptake of vitellogenin by growing oocytes. The hormones appear to act on the follicle cells, which probably produce an enzyme that initiates the crystallization process by additional phosphorylation of the phosvitin molecule (see Follett & Redshaw, 1971).

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