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Biochemical analysis of vitellogenin from rainbow trout *(Salmo gairdneri)* : fatty acid composition of phospholipids

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Summary. Vitellogenin was obtained from three year-old vitellogenic trout.

Two procedures of isolation were compared : dialysis against distilled water and ultracentrifugation in the density interval 1.21-1.28 g/ml.

Similar patterns were observed by gel filtration and electrophoresis for both preparations of vitellogenin, indicating that electric charge and molecular weight were not modified by either procedure.

The apparent M_r of the native form was 560,000 in gel filtration, whereas that of the monomer was estimated as 170,000 by sodium dodecylsulfate-polyacrylamide gel electrophoresis. Minor proteins were also detected (100,000-88,000-78,000).

The main components were protein (79 %), and lipids (19 %), Carbohydrates accounted for 0.3 % when protein phosphorus and calcium each represented 0.7 % of total weight.

Phospholipids (70 % of total lipids) mainly consisted of phosphatidylcholine in which n-3 docosahexanenoic acid accounted for one-third of total fatty acids.

The results show the high levels of essential fatty acids in structural lipids which are known to be involved in embryo development.

Introduction.

Vitellogenin (VTG), the lipophosphoprotein synthesized during vitellogenesis by oviparous vertebrates, has been characterized in some fish species including rainbow trout (Hara and Hirai, 1978 ; Campbell and Idler, 1980 ; Norberg and Haux, 1985). The complex is generally isolated by precipitation. However VTG can be obtained by ultracentrifugation keeping the complex in a soluble form.

Previously (Fremont *et al.*, 1984) we used sequential ultracentrifugation for comparing chemical composition and fatty acid distribution in VTG and plasma lipoproteins ($d < 1.21$ g/ml) obtained by the same procedure. However, it is quite possible that methods used for isolation change the properties of VTG, specially that related to the insolubility of the complex in aqueous solutions. In the present study we therefore compared some properties of VTG obtained either by ultracentrifugation (VTG_U) or by dialysis against bidistilled water (VTG_D).

In addition, we characterized the phospholipid moiety of VTG which is the main vehicle for the essential fatty acids involved in the embryonic development of trout (Leray *et al.*, 1985).

Material and methods.

Animals.

Three year-old female trout *(Salmo gairdneri)* from an autumn strain were reared at 9-15 °C in a fish farm under the control of the « Institut National de la Recherche Agronomique, France ». Fish were fed a commercial diet in which protein and lipid accounted for 43-45 % and 8-10 %, respectively, of the dry matter. The essential n-3 polyunsaturated fatty acids (PUFA) were provided at a level of 1 % of the diet (in weight). When serum VTG reached a high level (September-October), blood was taken after one-week starvation by puncture in the dorsal caudal artery. Serum was isolated by a low-speed centrifugation and antiproteolytic agents were added : 0.01 % sodium azide (NaN_3) and 0.005 % phenylmethylsulfonylfluoride (PMSF). Samples were stored at -80 °C until analysis.

Analytical procedures.

— *Isolation of vitellogenin.*

Dialysis. — A volume of serum was introduced into a membrane tubing (Spectrapor) and placed for dialysis in bidistilled water (5 l for 30 ml serum) at 4 °C for 2 days. After 3-4 changes of water, the precipitated VTG was collected by a 30-min centrifugation at 15 000 × g and solubilized in 0.5 M NaCl. It was twice purified by reprecipitation in 10 vol. of distilled water. The final material was designed as VTG_D.

Ultracentrifugation. — Serum lipoproteins of $d < 1.21$ g/ml were isolated by flotation in a NaCl-KBr solution according to Havel, Eder and Bragdon (1955) for 40 h at 10 °C. A rotor 50 Ti Beckman was operated at 145 000 × g. After removing lipoproteins, the subnatant fraction was raised to $d = 1.28$ g/ml. The floating VTG was obtained after a 72-h run at 10 °C and collected as previously described (Fremont *et al.*, 1984).

The VTG containing saline solution was exhaustively dialyzed against 0.5 M NaCl to remove KBr and the excess of NaCl. The final material was designed as VTG_U.

Gel filtration. — Several trade marks of beads were used : Sepharose CL 6B, Sephacryl S 300 (Pharmacia fine Chemical, Upsala, Sweden), AcA 22 (IBF, France) and Biogel 1.5 M (Biorad, Richmond, USA). The elution solvent was 0.5 M NaCl containing 0.01 % NaN_3 and 0.005 % PMSF. In some assays, the influence of ionic strength (μ) and Ca^{2+} were tested. The saline solutions were :

0.5 M NaCl (μ = 0.5) ; 0.8 M NaCl, 0.5 M NaCl + 0.1 M $MgCl_2$, 0.5 M NaCl + 0.1 M $CaCl_2$ (μ = 0.8) ; 1.25 NaCl (μ = 1.25) ; 0.5 M $MgCl_2$, 0.5 M $CaCl_2$ (μ = 1.5). Before each fractionation, the column was equilibrated with the elution solvent for at least 2 days. The sample of VTG (20-50 mg protein) was applied to the column (1.5 × 90 cm or 1.6 × 100 cm) and eluted with the tested buffer at a flow rate of 10 (or 12) ml h^{-1} ; fractions of 2.5 ml were collected after monitoring absorbance at 280 nm. The column was calibrated for molecular weights (M_r) with standard proteins : thyroglobulin (669,000), ferritin (440,000), and catalase (232,000). The markers *Escherichia coli* and tryptophane were used to determine the void volume (V_0) and the total volume (V_t) respectively. The apparent M_r of VTG which was eluted at V_e was estimated from the linear relation $K_{AV} = f \log M_r$ with $K_{AV} = V_e - V_0/V_t - V_0$.

Electrophoretic methods. — Agarose gel electrophoresis was carried out according to Noble (1968) as previously described (Fremont *et al.,* 1981). Proteins were visualized by Amidoblack 10 B.

Polyacrylamide gel electrophoresis (PAGE) in non-denaturing conditions was performed by the method of Davis (1964) on 5 % gels using the vertical slab apparatus of Biorad (Protean 32 cell). The molecular weights of subunits were estimated by SDS-PAGE in linear gradient of 3-15 % polyacrylamide according to Laemmli (1970) using the following markers : myosine (200,000), galactosidase (116,250), phosphorylase b (92,500), bovine serum albumin (66,200), ovalbumin (45,000). In some assays, samples were incubated at 100 °C for 3 min in the presence of a reducing agent (one-fifth of the volume of 400 mM dithiothreitol).

The sample of protein (ca 50 µg per well) was deposited in glycerol. Bromophenol blue was used as a tracing dye. The running time was 4-5 h at 50 mA per slab in electrode buffer TRIS-glycine, pH 8.3. Afterwards, migration gels were stained for proteins with Coomassie Brillant blue R 250.

— *Chemical analysis.*

Lipids were extracted with chloroform-methanol (2 : 1) according to Folch *et al.* (1957) Triglycerides and cholesterol were quantified using enzymatic procedures (Biochemica test combination, Boehringer, Mannheim, France). Lipid phosphorus was estimated by a modification of the method of Bartlett (1959). All reactions were performed in the same tube using a heating block to mineralize the lipid extract with 10 NH_2SO_4. The color was developed at 100° after adding the Fiske and Subbarow reagent and the optical density read at 830 nm.

The phosphorus content was estimated from a standard curve obtained with $HKPO_4$ and phospholipids calculated by multiplying the phosphorus value by 25 considering lecithin as the major phospholipid. Phosphoproteins were assayed as acid-insoluble-nonlipid phosphorus according to Bergink *et al.* (1973). After washing the precipitate by solvents, protein phosphorus was estimated in the same way as lipid phosphorus.

For qualitative identification, lipid classes were separated by thin-layer chromatography on Silicagel H (Merck). The developing solvent system was hexane — diethyl ether — formic acid (80-20-2 v/v/v) for total lipids and nonpolar lipids ; the solvent system was methanol/hexane/acetic acid/boric acid (40-20-30-10-1.8 v/v/v/v/w) for phospholipids (Gilfillan *et al.,* 1983).

For quantitative analysis, a silica cartridge (25 mm × 10 mm) (SEP-Pack waters, Framingham, USA) was used to separate non-phosphorus lipids from phospholipids with 30 ml of chloroform followed by 5 ml of chloroform/methanol (Juaneda and Rocquelin, 1985). Phospholipid molecular classes were separated by high-performance liquid chromatography (HPLC) using a Beckman apparatus. The column (10 × 250 mm) was packed with silica (5 µm ultrasphere-Si) ; the chromatographic system was programmed for elution using two solvent systems (Geurts Van Kessel *et al.,* 1977). System A : hexane/2 propanol/water (6-8-0.75 by vol.) and system B : hexane/2 propanol/water (6-8-1.4 by vol.).

A linear gradient elution ranging from 0 to 100 % of solvent B was used for 10 min. The phospholipid extract (3-5 mg) was applied to the column in 500 µl of hexane/2 propanol (6-8 by vol.). Peaks were detected at 206 nm and fractions containing the separated phospholipids were collected for phosphorus estimation and fatty acid composition.

The methyl esters of fatty acids were obtained by transmethylation with 10 % HCl in methanol. The analysis was performed by gas-liquid chromatography (GLC) in a Packard chromatograph (model 427) fitted with a flame ionization detector. A 50 m × 0.2 mm vitreous silica capillary column with CP wax 52 CB (Chrompack, France) phase was used. The column was programmed from 140 °C to 225 °C at 6 °C/min. The injector and detector temperatures were 250 °C and 290 °C, respectively. The carrier gas was hydrogen at a flow rate of 2 ml/min. The peaks were identified with standards by calculating the equivalent chain length using a Delsi (Enica 10) integrator equipped with a microcomputer proceeding to an automatic expression of percentages.

Protein was estimated by the technique of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Amino acids were determined after acid hydrolysis in 6 N HCl with norleucine as the internal standard for 24 h and 48 h at 115 °C in sealed test tubes. Hydrolysates were analyzed using a Liquimat III, Kontron analyzer equipped with a polystyrene sulfonic (DC-6A 10 µm) resin column (0.4 × 30 cm). A computing integrator was used for calculations.

Hexoses were estimated according to Winzler (1955) using the H_2SO_4-orcinol reaction. Hexosamines were obtained by the method of Rondle and Morgan (1955) modified by Kraan and Muir (1957) based on the reaction of Ehrlich, D-galactosamine and D-glucosamine were used as standards. Sialic acids were analyzed according to Svennerholm (1957) using N-acetyl neuraminic acid.

Calcium was determined by flame-emission-spectrophotometry (Eppendorf apparatus). In some assays VTG was mineralized at 550° for 12 h before quantification of the cation.

Results and discussion.

Molecular filtration of VTG_U or VTG_D on Sepharose CL 6B Biogel 1.5 M, Sephacryl S 300 or AcA 22 gave a single peak when a saline solution of NaCl was used as eluant (fig. 1), whatever the ionic strength (μ = 0.5, 0.8, 1.25 or 1.5). When NaCl was partially or totally replaced by a Ca^{2+}-containing saline solution, the same profile was obtained with either gel except with AcA 22. On this gel, VTG eluted as a broad fraction showing a shoulder on the ascending slope. This fraction was incompletely resolved into two closely spaced peaks ; they eluted in the region of the unique peak obtained in the absence of Ca^{2+} but the apparent M_r could not be estimated because of band spreading. When Mg^{2+} replaced Ca^{2+} without changing the ionic strength, no effect was observed. Only Ca^{2+} changed the profile, whatever the ionic strength, and there was no relation between the elution profile and the concentration of the cation in the buffer. This observation was unexpected considering that Ca^{2+} is a normal component of VTG. In the native complex the cation is not masked since similar values were obtained when Ca^{2+} was estimated either in crude VTG or after mineralization of the complex. The calcium binding properties of VTG is well described in amphibians. One atom of calcium would be associated with every protein phosphate group (Wallace, 1970) but any associated calcium can be removed by gel filtration (Ansari *et al.*, 1971 ; Wallace and Bergink, 1974). The binding of Ca^{2+} with the phosphate moiety of VTG was also described in other oviparous vertebrates, fish (Hori, Kodama and Tanahashi, 1979) and birds (Verrinder Gibbins and Robinson, 1982).

According to Wiley, Opresko and Wallace (1979), the complex remained soluble in serum because of the presence of other proteins but the addition of divalent cations to purified VTG resulted in VTG precipitation without degradation except when Ca^{2+} was used. The authors suggest that the extensive degradation of VTG might be caused by a Ca^{2+}-activated protease. Such a degradative effect of Ca^{2+} on our purified VTG might explain the heterogeneous shape of the peak eluted from AcA 22 while the unaltered profile obtained on other gels could be due to a lower capacity of resolution.

Another explanation might be the existence of changes in the hydrophobicity of VTG induced by the added Ca^{2+}. The zone spreading observed on AcA 22 would be related to the ability of this gel to interact with the modified VTG.

The apparent M_r estimated on the gels from the elution volume in the absence of Ca^{2+} was 560,000 ; the value was between that found by Hara and Hirai (1978) and Campbell and Idler (1980) (600,000 and 440,000 respectively).

Both peaks obtained from VTG_U or VTG_D after gel filtration migrated close to the origin at pH 8.6 as a prominent band in agarose electrophoresis (fig. 2). When either VTG was subjected to polyacrylamide gel electrophoresis under non-dissociating conditions a single slow migrating band was also observed.

The SDS electrophoretic patterns of purified VTG were also similar for VTG_U and VTG_D showing several bands after protein staining. The M_r of the main band was 170,000 while those of minor bands were 110,000, 88,000, 78,000. The main band represented most likely the VTG monomer corresponding to the

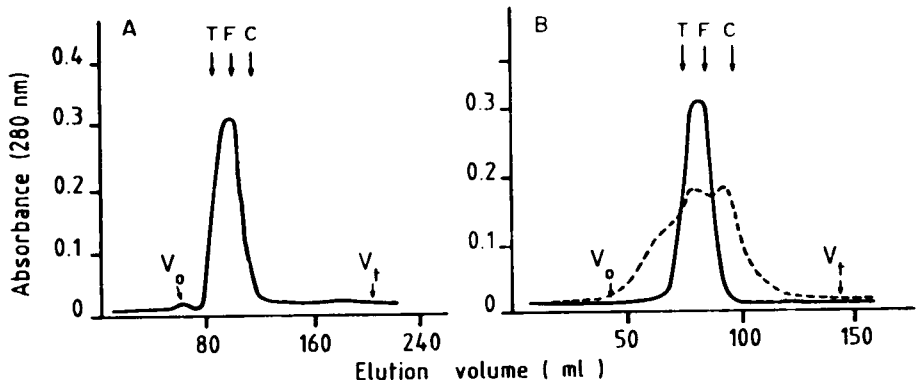

FIG. 1. — *Gel filtration of trout vitellogenin obtained either by ultracentrifugation, or by dialysis;* serum was obtained from female trout during vitellogenesis. 20-50 mg of protein was applied to the column in the appropriate saline solution.

A. Sephacryl S-300 was packed in a 1.6 × 100 cm column. The protein was eluted with a saline solution at the flow rate of 12 ml h^{-1}. The same profile was obtained whatever the elution solvent 0.5 M NaCl ; 0.8 M NaCl ; 0.5 M NaCl + 0.1 M $MgCl_2$; 0.5 M NaCl + 0.1 M $CaCl_2$.

B. AcA 22 was packed in a 1.5 × 90 cm column. The protein was eluted with a saline solution at the flow rate of 10 ml h^{-1}.
The profile (———) was obtained with the following elution solvents : 0.5 M NaCl ; 0.8 M NaCl ; 0.5 M NaCl + 0.1 M $MgCl_2$; 0.5 M $MgCl_2$.
The profile (- - -) was obtained with 0.5 M NaCl + 0.1 M $CaCl_2$ or 0.5 M $CaCl_2$ as elution solvent.

The apparent M_r was determined in 0.5 M NaCl. The standard proteins used as markers were : thyroglobulin (T), ferritin (F) and catalase (C).

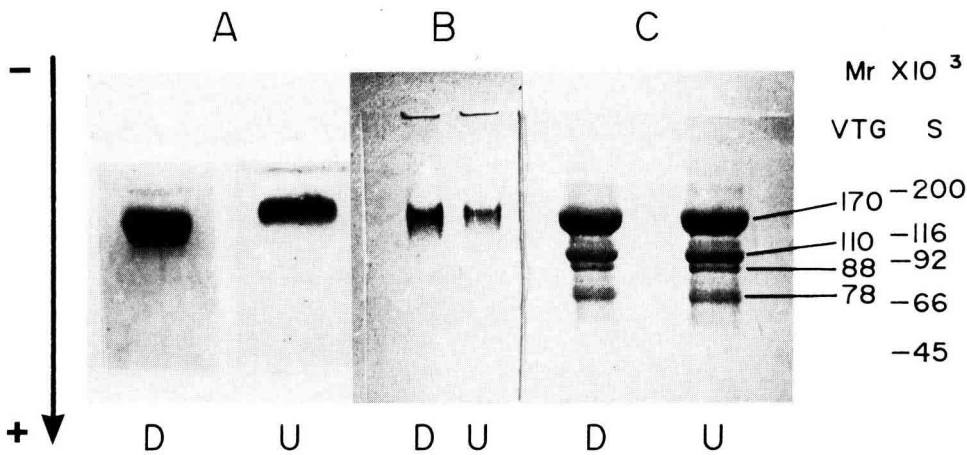

FIG. 2. — *Gel electrophoresis of trout vitellogenin* (VTG) *obtained by ultracentrifugation* (U) *or by dialysis* (D) *and purified by gel filtration on AcA 22 using 0.5 M NaCl as eluting buffer.*
A. 0.8 % agarose plates at pH 8.6. Protein was stained by Amidoblack 10 B. B. PAGE in 5 % polyacrylamide slab under non dissociating conditions. Protein was stained by Coomassie Brillant blue R 250. C. SDS-PAGE in linear gradient of 3-15 % polyacrylamide in the presence of dithiothreitol. Molecular weights were estimated by standards (S) as described in Material and Methods. Protein was stained by Coomassie Brillant blue R 250.

protein described by others [Hara and Hirai (1978), M_r 220,000 ; Chen (1983), M_r 170,000 ; Babin (1987), M_r 175,000].

The comparison of this pattern to that obtained in gel filtration indicated that the dissociation of peptides having lower molecular weights than the monomer only occurred when electrophoresis was performed in the presence of SDS. The similarity between VTG_U and VTG_D in gel filtration and electrophoresis showed that the step of precipitation did not cause major structural modifications in the complex.

Chemical analysis of VTG_U (table 1) revealed the predominance of protein (79 % by weight). Protein phosphorus accounted for 0.7 % of total VTG or 0.88 % reported to the protein moiety (0.028 atom per 100 g).

TABLE 1

Chemical composition of trout vitellogenin obtained by ultracentrifugation in the density range of 1.21-1.28 g/ml.

Component	Weight (%)	
Protein	79	
Lipid	19	
phospholipid	13,3	70*
triglyceride	4,2	22
cholesterol	1,5	8
Carbohydrate	0,3	
sialic acid		22**
hexosamine		25
hexose		53
Protein phosphorus	0,7	
Calcium	0,7	

* % of total lipids ; ** % of total carbohydrates.

The values estimated by Campbell and Idler (1980) and by Norberg and Haux (1985) were slightly lower : 0.6 and 0.63 per 100 g of protein, respectively. The percentages were similar in goldfish since Hori, Kodama and Tanahashi (1979) found 0.7 % and De Vlaming *et al.* (1980) 0.79 %. As reported by the latter authors, the protein phosphorus content of VTG from several species of fish is low compared to that of VTG from *Xenopus laevis* (Wallace, 1970) and birds (Deeley *et al.*, 1975 ; Verrinder Gibbins, Van de Voort and Braham, 1981). The complex contained 0.7 % calcium ; thus the calculated atomic phosphorus ratio was 0.77 assuming that calcium phosphatidyl residues were not all bound to calcium. VTG lipids (19 % of total weight) consisted of phospholipids (70 %), triglycerides (22 %) and cholesterol (8 %). The distribution was similar to that previously reported for VTG obtained by ultracentrifugation (Fremont *et al.*, 1984) and to that found by Norberg and Haux (1985) for VTG isolated by selective precipitation using Mg^{2+}.

As reported by Hara and Hirai (1978), carbohydrates were present but they were minor components, comprising 0.3 % of VTG ; this content was lower than that of *Xenopus* VTG (Tata, 1976).

The amino acid distribution (table 2) resembled that reported by Campbell and Idler (1980), Hara and Hirai (1978) for trout. The major amino acids were alanine, glutamic acid and leucine. The molar ratio serine/protein phosphorus was 0.055/0.028, showing that about half of seryl residues were phosphorylated in trout VTG (it was possible that some threonyl residues were also phosphorylated).

TABLE 2

Amino acid composition of trout vitellogenin. Values are expressed in per cent by weight and in residue per cent mol of total amino acids.

Amino acid	g(%) ± SEM*	mol (%)
Asp	9,05 ± 0,21	8,6
Thr	5,00 ± 0,17	5,4
Ser	5,88 ± 0,19	7,1
Pro	6,30 ± 0,81	6,8
Glu	11,31 ± 0,28	9,8
Gly	2,24 ± 0,15	3,8
Ala	8,25 ± 0,49	11,7
Cys/2	1,22 ± 0,10	1,7
Val	7,22 ± 0,09	7,8
Met	1,71 ± 0,08	1,5
Ile	6,10 ± 0,47	5,9
Leu	9,61 ± 0,10	9,3
Tyr	3,84 ± 0,08	2,7
Phe	4,93 ± 0,09	3,8
Lys	8,74 ± 0,06	7,6
His	2,54 ± 0,09	2,0
Arg	6,05 ± 0,27	4,4

* Mean of three determinations after a 24 h (× 1) or a 48 h (× 2) hydrolysis.

When comparing the present results to those obtained by others, it appears that the distribution of the main components was similar, whatever the source of VTG : mature female trout (Hara and Hirai, 1978 ; this study) estrogenized male trout (Campbell and Idler, 1980), estrogenized juvenile trout (Norberg and Haux, 1985) or vitellogenic trout receiving an n-3 deficient or non-deficient diet (Fremont *et al.*, 1984). Moreover, analyses performed during the last 5 months before ovulation did not show any change in the proportions of protein and lipid classes (Riazi and Fremont, 1988).

The present results support the hypothesis that VTG is secreted as a homogeneous complex having a definite distribution of molecular species during the course of vitellogenesis.

However, the level of some components, namely PUFA, only provided by diet, decreased when trout were fed a n-3 PUFA deficient diet during vitellogenesis (Fremont *et al.*, 1984). As phospholipids are the major PUFA vehicles in VTG, we determined fatty acid distribution in this class of structural lipids when diet supplied the required levels.

Figure 3 shows the profiles obtained by TLC and HPLC, revealing the predominance of phosphatidylcholine which accounted for 83 % of the total phospholipids (table 3). The fatty acid distribution in phosphatidylcholine (PC)

TABLE 3

Distribution of phospholipid classes in trout vitellogenin.

Phospholipid class	% of total phospholipid phosphorus
Phosphatidylcholine (PC)	83,2 ± 1,5
Lysophosphatidylcholine (LPC)	6,2 ± 0,7
Phosphatidylethanolamine (PE)	3,8 ± 0,5
Phosphatidylserine (PS)	3,2 ± 0,2
Phosphatidylinositol (PI)	0,5 ± 0,05
Sphingomyelin (SM)	3,1 ± 0,1

Data are expressed as mean ± SEM of two independant determinations. Each of them was performed on pooled samples of serum from four fish.

and phosphatidylethanolamine (PE) is reported in table 4. It is clear that in both classes, total saturated fatty acids represented less than one third of the total fatty acids. Monounsaturated fatty acids (the main one was oleic acid) were minor components when PUFA accounted for almost half of the total fatty acids (47.6 % in PC and 44.4 % in PE). The n-3/n-6 ratio was 4.5 in PC and only 2 in PE.

In a previous experiment (Fremont *et al.*, 1984), the percentage of the docosahexaenoic acid (22 : 6 n-3) relative to total VTG fatty acids were in the same range in September (23 : 3) and December (25.4) when trout were fed an n-3 containing diet. By contrast a n-3 depleted diet had a lowering effect.

The present data show that PC is the main vehicle of this essential fatty acid. The amount can be estimated from VTG composition and PC percentages in the lipid moiety. By this calculation, the PC 22 : 6 n-3 accounted for 3.9 % of the VTG weight.

It is well known that fatty acid chains of phospholipids play a key role in the structure of lipoproteins of $d < 1.21$ g/ml (Segrest *et al.*, 1974). VTG is a peculiar lipoprotein since it is characterized by a strong hydrophobicity ; however, it can be expected that, in relation with the length and degree of unsaturation, the arrangement of phospholipids and proteins also depends on the spatial organization of the acid chains. Alterations in embryonic development observed when

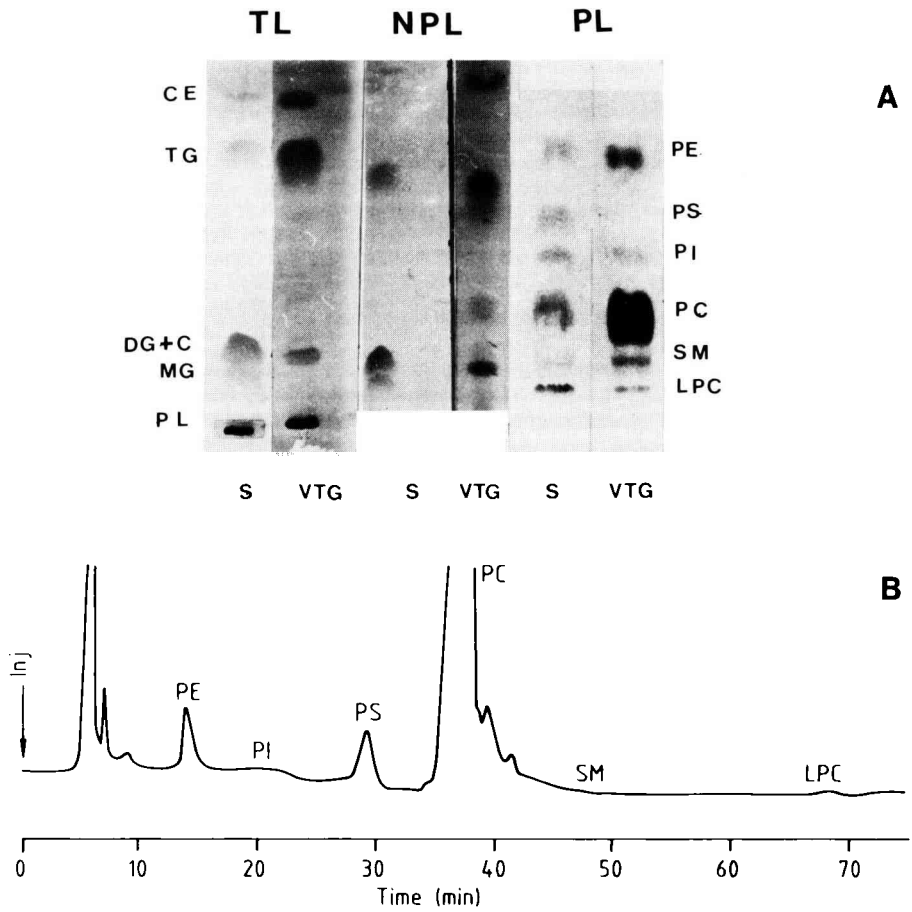

FIG. 3. — *Fractionation of lipid classes in trout vitellogenin* (VTG).

A. Thin-layer chromatography on 0.25-mm thick silica gel H plates. Total lipids (TL) and non-phosphorylated lipids (NPL) were eluted using hexane-, ethyl ether-, formic acid (80/20/2 v/v/v) as the developing system.
Standards (S) were cholesterol ester (CE), triglyceride (TG), diglyceride (DG), cholesterol (C), monoglyceride (MG), phospholipid (PL). Phospholipid classes (PL) were separated using chloroform, methanol, hexane, acetic acid, boric acid (40/20/30/10/1.8 v/v/v/v/w) as the developing system. The spots were visualized by iodine vapours.
Standards (S) were phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylcholine (PC), sphingomyeline (SM), lysophosphatidylcholine (LPC).

B. Elution profile of phospholipid classes separated by preparative high-performance liquid chromatography. The column (10 × 250 mm) was packed with silica (5 µm ultrasphere-Si). Two solvent systems described in Material and Methods were used for elution.
Abbreviations are similar to that used in A.
1-2 mg of phospholipids dissolved in 0.5 ml hexane-2 propanol (6/8 v/v) was pumped onto the column at 2.5 ml/min and fractions were eluted using the solvent systems described in Material and Methods. The relative mobilities of PI and PS were inverted in B as compared to A.

TABLE 4

Fatty acid distribution in vitellogenin phospholipids: phosphatidylcholine (PC) *and phosphatidylethanolamine* (PE), *of trout receiving a standard diet.*

Fatty acid	PC	PE
14 : 0	1,0 ± 0,01	1,4 ± 0,0
16 : 0	19,6 ± 0,4	12,6 ± 0,0
16 : 1 n-9 + n-7	1,7 ± 0,4	5,9 ± 0,5
18 : 0	9,0 ± 0,1	13,1 ± 0,1
18 : 1 n-3	10,2 ± 0,5	7,7 ± 0,2
18 : 1 n-7	2,3 ± 0,05	2,9 ± 0,0
18 : 2 n-6	3,1 ± 0,05	7,7 ± 0,2
18 : 3 ± 18 : 4 n-3	0,5 ± 0,01	0,9 ± 0,1
20 : 1 n-9	2,5 ± 0,05	5,3 ± 0,4
22 : 2 n-6	1,5 ± 0,1	1,7 ± 0,1
20 : 4 n-6	3,4 ± 0,01	4,5 ± 0,0
20 : 5 n-3	4,1 ± 0,05	3,8 ± 0,1
22 : 4 + 22 : 5 n-6	0,6 ± 0,04	0,6 ± 0,0
22 : 5 n-3	1,7 ± 0,05	1,1 ± 0,0
22 : 6 n-3	32,7 ± 0,2	24,1 ± 0,6
24 : 1 n-9	1,5 ± 0,1	0,5 ± 0,0
Other	4,6	6,2
Total n-6	8,6	14,5
Total n-3	39,0	29,9

Data are expressed as mean ± SEM of two independent determinations. Each of them was performed on pooled samples of serum from four fish.

vitellogenic trout were fed a n-3 deficient diet (Leray *et al.*, 1985) suggest a relationship with the reduction of n-3 fatty acids in plasma precursors of oocyte reserves : LP d < 1.21 g/ml as suggested by recent findings (Black and Skinner, 1987) and vitellogenin. Alterations of the embryo membranes might occur as resulting effects since changes in lipoprotein surface phospholipids influence lipid composition of cell membranes (Owen and Gillett, 1983 ; Owen, McIntyre and Gillett, 1984). Moreover, it is quite possible that VTG PUFA modulate interactions between the complex and the plasma membrane of the oocyte and affect VTG incorporation since in trout, the yolk cycle presents the features of receptor-mediated endocytosis (Busson-Mabillot, 1984). Therefore phospholipid PUFA directly involved in the metabolic utilization of VTG play a major role in the reproductive process.

Finally even if the distribution of molecular species in VTG remains constant, it is essential to regard the n-3 PUFA content of the phospholipid moiety when considering the nutritional aspects of trout reproduction.

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Résumé. *Analyse biochimique de la vitellogénine de truite Arc-en-ciel* (Salmo gairdneri). *Composition en acides gras des phospholipides.*

La vitellogénine a été obtenue à partir de sérum de truites âgées de 3 ans en fin de vitellogenèse.

Deux méthodes d'isolement du complexe ont été comparées : dialyse contre l'eau distillée et ultracentrifugation dans l'intervalle de densité 1,21-1,28 g/ml.

Les profils obtenus après filtration sur gel et électrophorèse sont identiques quelle que soit la technique de préparation. Ceci démontre que la charge et le poids moléculaire ne sont pas modifiés au cours de la préparation.

Le poids moléculaire apparent de la forme native est de 560 000 en filtration sur gel ; celui de la forme monomère est estimé à 170 000 en électrophorèse sur gel de polyacrylamide-SDS. D'autres protéines sont également présentes ; leurs poids moléculaires sont d'environ 100 000-88 000 et 78 000. Les principaux constituants de la vitellogénine sont les protéines et les lipides qui représentent respectivement 79 % et 19 % du poids sec.

La proportion des glucides est d'environ 0,3 % ; celle du phosphore protéique 0,7 % et du calcium 0,7 %.

Les phospholipides sont les lipides majeurs (70 %) ; la principale espèce moléculaire est la phosphatidylcholine dans laquelle l'acide n-3 docosahexaénoïque constitue le tiers des acides grs.

Ces résultats mettent en évidence le degré élevé d'incorporation des acides gras essentiels dans les lipides de structure qui jouent un rôle prépondérant dans le développement de l'embryon.

Références

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