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Original article

Identification of vitellogenin receptors in the ovary of a teleost fish, the Mediterranean sea bass *(Dicentrarchus labrax)*

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Summary — The characterization of vitellogenin (VTG) receptors in ovarian membranes from vitellogenic female sea bass *(Dicentrarchus labrax)* is described. Incubation of membrane proteins with radiolabeled VTG (^{125}I-VTG) after SDS-electrophoresis showed specific binding of ^{125}I-VTG to a protein band of 100 kDa. Filter binding assays showed that binding of ^{125}I-VTG to membrane receptors was saturable with increasing amounts of ^{125}I-VTG. Scatchard analysis of the saturation data revealed a single class of binding sites with an apparent K_D of 1.04.10^{-8} M. The specificity of the VTG receptors was tested in competition assays; binding of ^{125}I-VTG to ovarian membranes was completely abolished with an excess of purified sea bass VTG (cold VTG, VTG°) or plasma from estradiol (E2)- treated fish, while the addition of control male plasma (without VTG) caused negligible effect.

teleost / sea bass / *Dicentrarchus labrax* / vitellogenin / receptor

Résumé — Identification du récepteur à la vitellogénine dans l'ovaire d'un poisson téléostéen, le bar méditerranéen *(Dicentrarchus labrax)*. Les récepteurs à la vitellogénine (VTG) ont été caractérisés dans des membranes de l'ovaire de bar *(Dicentrarchus labrax)* en vitellogenèse. L'incubation des protéines membranaires avec la VTG marquée (^{125}I-VTG) après SDS-électrophorèse montre une liaison spécifique de la ^{125}I-VTG à une bande protéique de

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100 kDa. Par *filter-binding assays*, on a montré que la liaison de ^{125}I-VTG sur les récepteurs membranaires était saturable par des doses croissantes de ^{125}I-VTG. L'analyse selon l'équation de Scatchard de la courbe de saturation révèle une seule classe de sites de liaison avec une K_D de $1.04 \cdot 10^{-8}$ M. La spécificité du récepteur à la VTG a été testée par compétition ; la liaison de ^{125}I-VTG aux membranes de l'ovaire est complètement abolie par un excès de VTG purifié de bar (VTG froide, VTG°) ou du plasma de poisson traité avec de l'œstradiol (E2), tandis que l'addition de plasma de mâle (sans VTG) induit un effet négligeable.

téléostéen / bar / *Dicentrarchus labrax* / vitellogénine / récepteur

INTRODUCTION

In oviparous animals, the development of the new embryos depends on the existence of the nutritional reserves of the eggs, until the larvae are able to obtain their food supply unaided. The accumulation of these reserves into the growing oocytes occurs during the vitellogenic phase, and is mainly due to the incorporation of a plasmatic protein, vitellogenin (VTG). The VTG is a lipophosphoglycoprotein, synthesized in the liver under the influence of sex steroids, mainly estradiol-17β (E2). In the hepatocytes, the nascent VTG polypeptide is phosphorylated, glycosylated and lipidated, before being secreted into the blood stream and transported to the ovary (reviewed by Wallace, 1985; Mommsen and Walsh, 1988).

The VTG reaches the ovarian follicle through the capillaries of the theca, passes the basement membrane and intracellular gaps in the granulosa cell layer and then, along microvilli channels in the zona radiata, arrives at the perivitellin space surrounding the oocyte (Selman and Wallace, 1989; Wallace and Selman, 1990). In the oocyte membrane, the VTG is incorporated into the oocyte by receptor-mediated endocytosis, at specific areas known as coated pits (Wallace and Selman, 1990; Barber et al, 1991). In the oocyte, the VTG is progressively transferred from coated vesicles to multivesicular bodies and vitellin granules, where the VTG-receptor complex is dissociated (Opresko and Karpf, 1987; Shen et al, 1993; Van Antwerpen et al, 1993) and the VTG proteolytically cleaved to give rise to vitellin proteins (Wall and Patel, 1987).

The incorporation and proteolytical cleavage of VTG into the oocytes constitute the main source of nutritional reserves for the developing embryos. As demonstrated in birds, more than 90% of the dry mass of yolk accumulated in the growing oocytes are derived from both VTG and very low density lipoproteins (VLDL) (Schneider, 1992). In fish, it was suggested that more than 60% of the total yolk proteins present in mature oocytes are related to VTG (Tyler, 1993). By mediating the internalization of the major yolk precursor proteins, the ovarian VTG receptors play a crucial role in the development of oocyte growth in oviparous species.

In the last decade, detailed information on the VTG receptors has been accumulated for birds, *Gallus domesticus* (Woods and Roth, 1984; Stifani et al, 1988, 1990b; Barber et al, 1991; Schneider, 1992; Shen et al, 1993; Bujo et al, 1994; Lachlan et al, 1994), amphibians, *Xenopus laevis* (Opresko and Wiley, 1987; Stifani et al, 1990b) and invertebrates (Röhrkasten and Ferenz, 1986; Hafer and Ferenz, 1991; Sappington et al, 1995). In contrast, the knowledge of the VTG receptor system in fish is very scarce. Early morphological studies suggested that the presence of coated pits in the membrane of vitellogenic oocytes could indicate that the incorporation of VTG into the oocytes was mediated by specific receptors (Selman and Wallace, 1982). Selective receptor-mediated incorporation of VTG was also suggested by in vitro and in

vivo studies on VTG uptake by oocytes from salmonid species (Tyler, 1991).

The piscine receptor for VTG was characterized for the first time in the ovary of coho salmon *Oncorhynchus kysutch* (Stifani et al, 1990a). Later, specific receptors for VTG in fish were identified in another salmonid species, rainbow trout *Oncorhynchus mykiss* (Le Menn and Núñez Rodríguez, 1991; Tyler and Lancaster, 1993; Núñez Rodríguez et al, 1996) and a fresh water perciform, tilapia *Oreochromis niloticus* (Chan et al, 1991). These studies showed a relationship between the number and affinity of VTG receptors and the stage of ovarian development (Chan et al, 1991; Le Menn and Núñez Rodríguez, 1991; Lancaster and Tyler, 1994; Núñez Rodríguez et al, 1996). To date, this is all the information available for the VTG receptors in these fish species. On the other hand, nothing is known about the characteristics and regulation of VTG receptors in other fish species. Although it has been suggested that VTG receptors are highly conserved between species (Bujo et al, 1994), specific characteristics can be expected for different species, particularly in fish, which contain species with different reproductive strategies. Clearly, more detailed information on the VTG receptor system will be necessary to understand the mechanisms regulating oocyte growth in fish.

The Mediterranean sea bass *(Dicentrarchus labrax)*, a marine perciform fish, has been used as a model for studies related to the development of aquaculture of marine fish species. In the last few years, the sea bass has become one of the most important species in the European aquaculture. Nevertheless, culture of sea bass in captivity still faces major problems, mainly related to broodstock management. A consistent and predictable year-round supply of good quality eggs and larvae is one of the requirements for the expansion of sea bass aquaculture, as well as other related species.

Our laboratory has been actively involved in the study of the reproductive process of sea bass. We observed that, although sea bass spawn spontaneously in captivity, the quality of the eggs is affected by both environmental and nutritional factors (reviewed by Carrillo et al, 1995a). This problem could be related to a deficient accumulation of the yolk reserves into the growing oocytes during the vitellogenic phase. To elucidate this problem, plasma VTG levels were determined in groups of fish whose spawning characteristics were affected by different factors. We observed that, although the content of VTG in plasma was slightly affected by different manipulations, this small variation could hardly explain the dramatic loss of egg quality (Carrillo et al, 1995b). These observations suggested that deficient internalization of VTG into the oocytes might be the cause affecting egg quality. In this case, variations in the characteristics of the membrane receptors for VTG may play a critical role in regulating the rate of VTG incorporation into the growing oocytes and thus, influencing quantity and quality of the nutritional reserves accumulated into the eggs. In this framework, the present study is the first of a series of experiments directed towards the examination of VTG receptors in sea bass. Here, we present preliminary results on the identification and characterization of VTG receptors in ovarian membranes from vitellogenic female sea bass.

MATERIALS AND METHODS

Fish and preparation of ovarian membranes

Adult female sea bass *Dicentrarchus labrax* (2–3 kg body weight) at vitellogenic stages (oocyte diameter = 500–600 μm) were obtained from our fish facilities at the 'Istituto de Acuicultura de Torre la Sal' (east coast of Spain, 40 °N 0 °E). Fish were anesthetized in 0.01% MS 222 (Sigma, St Louis, MO, USA) and the ovaries collected and immediately frozen by immersion in liquid nitrogen. The ovaries were homogenized with a glass-teflon homogenizer in 25 mM Tris-HCl buffer pH 8, containing

50 mM NaCl, 2 mM $CaCl_2$ and 1 mM phenylmethylsulfonyl fluoride (PMSF, Sigma) to reduce protein degradation (buffer A). The homogenate was repeatedly rinsed with buffer A, filtered through gauze (100 μm mesh) and the extract mixed again with buffer A and homogenized using a Polytron homogenizer (2 x 20 s at 15 000 rpm and 1 x 30 s at 25 000 rpm, on ice). The homogenate was centrifuged (2 500 g for 10 min at 4 °C) to eliminate debris and the resulting supernatant centrifuged at 100 000 g (1 h at 4 °C) to precipitate membranes. The precipitate was resuspended in buffer A by aspiration through a 22-gauge needle and centrifuged again for 1 h. This step was repeated a third time, resulting in a completely transparent supernatant. The precipitate (membrane extract) was frozen in liquid nitrogen and stored at –80 °C until assayed. For the filter binding assays, this precipitate was solubilized in an appropriate volume of buffer A, after calculation of the total surface of membranes (mm^2) contained in the precipitate. For this calculation, the entire ovary was weighed and the total number of oocytes was estimated by taking a few grams of ovary and counting the total number of oocytes per gram. The membrane extract obtained with a piece of ovary was equivalent to the oocyte surface (oocyte diameter = 500–600 μm) of the oocyte number calculated. This extract was then diluted in the appropriate volume to obtain the value of 100 mm^2 of membranes per 20 μL of extract.

Radioiodination of vitellogenin

The VTG used as unlabeled ligand (cold VTG, VTG°), was purified from plasma of E2-treated male and female sea bass using a double chromatography method (Mañanós et al, 1994a). The sea bass plasmas and the specific sea bass VTG antibodies used in this study were obtained as previously described (Mañanós et al, 1994a). The VTG content in plasma samples was determined by ELISA (Mañanós et al, 1994b). Protein contents were measured according to Lowry et al (1951).

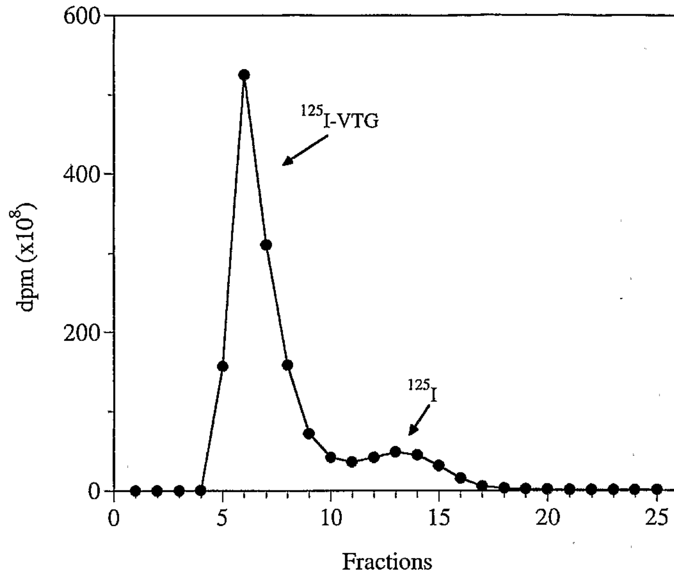
The VTG was radiolabeled with ^{125}INa using the iodogen method (Salacinsky et al, 1979) for specific activities of 216 000 dpm/pmol (485 dpm/ng of protein), assuming a molecular weight of 445 kDa for sea bass VTG (Mañanós et al, 1994a). For the iodination, 1 mL of purified sea bass VTG (3 mg) and 10 μL of ^{125}INA (44.10^6 Bq) were added to a glass scintillation vial coated with 200 μg of iodogen (Sigma). After 25 min at room temperature (gently shaken every 5 min), the mixture was applied to a 140 x 12 mm Ultrogel AcA 44 (IBF) gel filtration column, and eluted with 25 mM Tris-HCl, 2 mM $CaCl_2$ buffer pH 7.8, at a flow rate of 500 μL/min. Separation of radiolabeled VTG (^{125}I-VTG) from free ^{125}INa, in the gel filtration chromatography, is shown in figure 1. Before the purification step, the column was saturated with elution buffer containing 25% bovine serum albumin (BSA, Sigma). Fractions containing the purified ^{125}I-VTG were stored at –80 °C in 50% glycerol.

Filter binding assays

Saturation and competition studies were performed in incubation assays using ^{125}I-VTG as a tracer. Typical incubation assays were performed in 0.5 mL Eppendorf tubes, each tube containing: 20 μL of ovarian membrane extract (corresponding to 100 mm^2 of membranes), 30 μL of ^{125}I-VTG (corresponding to 50 000 dpm), 0–10 μL of 100 mM suramin (Mobay Chemical Corporation, NY, USA) and 90–100 μL of buffer A, 4% BSA. Each incubation point was distributed in five tubes: three tubes without suramin for the total binding (TB) and two tubes with suramin (10 μL) for the nonspecific binding (NSB). The ovarian membrane extract and the ^{125}I-VTG were diluted in buffer A, 4% BSA. The ^{125}I-VTG solution was previously filtered through 0.45 μm filtration units (Millipore, Bedford, MA, USA).

The ^{125}I-VTG bound to ovarian membranes was separated from free ^{125}I-VTG using the filter assay technique described by Stifani et al

Fig 1. Purification of the radiolabeled vitellogenin (^{125}I-VTG) from free ^{125}I by gel filtration on Ultrogel AcA 44. Sea bass VTG was radioiodinated with ^{125}INa by the iodogen method. The eluent buffer was 25 mM Tris-HCl, 2 mM $CaCl_2$, pH 7.8. Fractions of 500 µL were collected every minute.



(1988). Based on previous kinetic studies (Núñez Rodríguez et al, 1996), incubations were performed at 20 °C for 90 min, in order to reach equilibrium. After incubation, sample solutions were filtered through 0.45 µm cellulose acetate filters (MSI), using the Swinnex filtration system of Millipore, to separate the free ^{125}I-VTG from the ^{125}I-VTG bound to the ovarian membranes. Before the assay, filters were saturated overnight in buffer A, containing 2% non-fat dry milk. Filters were rinsed three times with the same buffer and then placed in tubes and the radioactivity counted in a Beckman gamma counter. The data obtained in the saturation assays were linearized according to Scatchard (1949). Scatchard analysis and other calculations were performed on Apple Macintosh computers using the EBDA and Ligand programs, adapted from those previously developed by McPherson (1985) and Munson and Rodbard (1988), respectively.

Autoradiography

Receptors were solubilized from the ovarian membrane extract according to Stifani et al (1988). The ovarian membrane extract was solubilized in 150 mM Tris-maleate buffer pH 6, 2 mM $CaCl_2$, 150 mM NaCl, 1 mM PMSF, containing 10 mg/mL of the non-ionic detergent OGP (*n*-octyl-β-D-glucopyranoside; Sigma). The mixture was centrifuged (100 000 g for 1 h at 4 °C) and the supernatant frozen immediately in liquid nitrogen and stored at –80 °C until assayed.

The solubilized membrane solution was subjected to SDS-electrophoresis on 7.5% polyacrylamide gels (0.1% SDS) under non-reducing conditions, according to Laemli (1970). Proteins of the gel were transferred to 0.4 µm pore size nitrocellulose paper for 2 h at 1 mA/cm^2, keeping the system refrigerated at 4 °C. Strips were saturated with buffer A containing 5% non-fat dry milk. After 2 h, ^{125}I-VTG (1.10^7 dpm/5 mL) or VTG° (600 µg/5 mL) were added in parallel 5 mL glass tubes, in order to determine total and nonspecific binding, respectively. Incubations were performed for 90 min at room temperature (constant shaking). Blots were then rinsed with buffer A (4 x 10 min) and dried for 1 h at 37 °C. Dried blots were placed on film cassettes and exposed to photographic film (Kodak X-OMAT-AR-5) in complete darkness. After 1–2 days (at –20 °C), films were developed following the manufacturer's instructions.

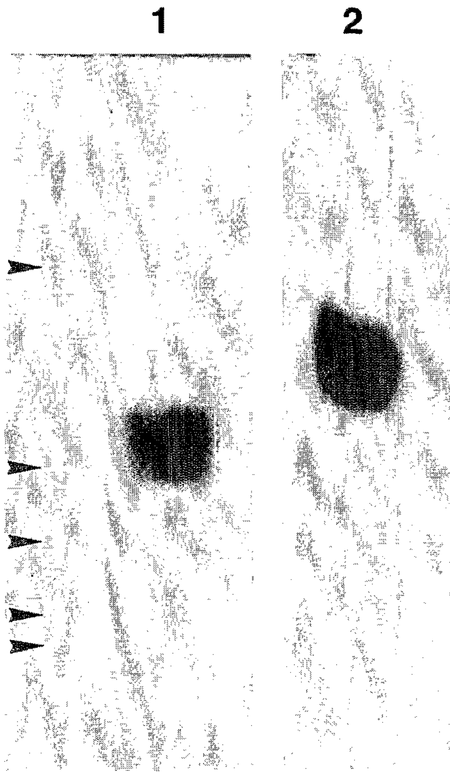


Fig 2. Visualization of the sea bass vitellogenin (VTG) receptors by ligand blotting. Membrane receptors were solubilized with octyl-β-D-glucopyranoside and run on SDS-electrophoresis (7.5% polyacrylamide gel, 0.1% SDS), under non-reducing conditions. Proteins from the gel (10 μg of proteins/lane) were transferred to nitrocellulose paper. Incubation of blots with ^{125}I-VTG (2.10^6 dpm/mL) gave rise to an autoradiographic signal associated with the VTG receptors, corresponding to a protein band of 100 kDa (lane 1). In lane 2, antibodies against sea bass VTG were run in parallel as a visual control for the signal produced by the binding of ^{125}I-VTG (lane 2). Arrowheads on the left indicate the position of the molecular weight markers (from top to bottom: 200, 94, 67, 43 and 30 kDa).

RESULTS AND DISCUSSION

Visualization of the vitellogenin receptors

To determine the existence of VTG binding sites in sea bass ovarian extracts, membrane proteins were solubilized with the non-ionic detergent OGP, and subjected to ligand blotting for visualization. After separation of the solubilized proteins by SDS-electrophoresis, proteins of the gel were transferred to nitrocellulose paper. Blots were then incubated with ^{125}I-VTG and the radioactive signal detected by autoradiography.

The ^{125}I-VTG was recognized by specific binding sites present in the solubilized membrane extract (fig 2, lane 1). These binding sites correspond to a protein band with an apparent molecular weight of 100 kDa. As a visual control of the autoradiographic signal produced by the binding of ^{125}I-VTG to proteins of the gel, a solution of sea bass VTG antibodies (Mañanós et al, 1994a) was run in parallel lanes (fig 2, lane 2). The ligand blotting technique, first used for the visualization of LDL receptors in mammals (Daniel et al, 1983), has also been used by others to study receptors for VTG. Using this technique, VTG receptors were visualized in birds *(Gallus domesticus)* as a protein band with a molecular weight of 96 kDa (Stifani et al, 1988) and in amphibians *(Xenopus laevis)* as a protein of 115 kDa (Stifani et al, 1990b). In fish, the molecular weight of the VTG receptors has been determined for coho salmon *(Oncorhynchus kisutch)* 100 kDa (Stifani et al, 1990a; Le Menn and Núñez Rodríguez, 1991), common carp *(Cyprinus carpio)* 90 kDa (Le Menn and Núñez Rodríguez, 1991) and rainbow trout *(Oncorhynchus mykiss)* 200 kDa (probably comprising two 100 kDa subunits; Tyler and Lancaster, 1993) or 113 kDa (Núñez Rodríguez et al, 1996), values close to the 100 kDa determined for the sea bass VTG receptors in the present study.

Saturability of the vitellogenin receptors

In order to identify these binding sites as the VTG receptor system, we used the filter assay technique described by Stifani et al (1988) and the ligand blotting technique developed by Daniel et al (1983). Using these techniques,

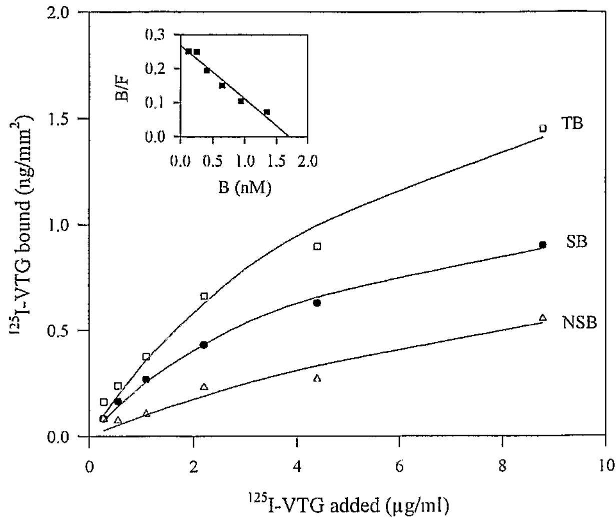


Fig 3. Saturation of the sea bass vitellogenin (VTG) receptors. In filter binding assays, incubation of a constant amount of ovarian membranes (100 mm^2 of membranes per tube), with increasing amounts of ^{125}I-VTG (20 000–640 000 dpm per tube, corresponding to 0.3–8.8 µg/mL), produced a typical hyperbolic curve of specific binding. The specific binding was obtained by substracting the nonspecific binding (tubes containing 10 µL of suramin per tube) from the total binding. Scatchard linearization of the saturation data, obtained by representation of B/F (^{125}I-VTG bound/^{125}I-VTG free) versus B, is shown in the inset. $K_D = 1.04.10^{-8}$ M, calculated by EBDA and ligand programs.

we attempted to demonstrate for the sea bass VTG receptors some general characteristics inherent to the receptor systems: (i) the binding of VTG to its receptors is saturable, (ii) the binding of ^{125}I-VTG to the receptors is displaceable by an excess of unlabeled ligand and (iii) the binding is specific for VTG.

Saturation of the VTG receptors was tested in direct binding assays. As shown in figure 3, the addition of increasing amounts of ^{125}I-VTG to a constant quantity of ovarian membranes gave rise to a typical hyperbolic curve of specific binding, reaching a plateau after 300 000 dpm/tube of ^{125}I-VTG added. The specific binding was obtained by substracting the nonspecific binding from the total binding. The nonspecific binding was obtained in parallel tubes by the addition of suramin to the incubation mixture. Suramin is a highly negative-charged hydrocarbon shown to inhibit the recognition of VTG by its receptor in birds (Stifani et al, 1988), amphibians (Stifani et al, 1990b), insects (Röhrkasten and Ferenz, 1987) and fish (Stifani et al, 1990a; Núñez Rodríguez et al, 1996).

Scatchard analysis of the saturation data (fig 3, inset) indicated the existence of a single class of VTG binding sites, with an apparent K_D of $1.04.10^{-8}$ M. This affinity was in the range of that reported for the VTG receptors in coho salmon, *Oncorhynchus kisutch*, $K_D = 1.8.10^{-7}$ M (Stifani et al, 1990a) and rainbow trout, *Oncorhynchus mykiss*, $K_D = 8.10^{-9}$ M (Tyler and Lancaster, 1993) as well as for other oviparous species, 2.10^{-7} M in birds (*Gallus domesticus*; Stifani et al, 1988), $1.3.10^{-6}$ M in amphibians (*Xenopus laevis*; Opresko and Wiley, 1987) and $4.2.10^{-8}$ M (*Locusta migratoria*; Röhrkasten and Ferenz, 1986) and $1.5.10^{-9}$ M (*Aedes aegypti*; Sappington et al, 1995) in insects. In two fish species for which data are available at different stages of the reproductive cycle, affinity of the VTG receptors ranged from 302 to 1 510 nM in *Oreochromis niloticus* (Chan et al, 1991), and from 1.3 to 3.5 nM (Lancaster and Tyler, 1994) or 10 to 30 nM (Núñez Rodríguez et al, 1996) in *Oncorhynchus mykiss*. As shown in these studies, the affinity of the receptors changes depending on the stage of oocyte growth. The functional reasons for these changes, as well as the mechanisms regulating variations in the characteristics of the VTG receptors, remain unclear.

Specificity of the vitellogenin receptors

To demontrate that these receptors recognize sea bass VTG specifically, several unlabeled ligands were used in competition assays to displace the binding of ^{125}I-VTG to the ovarian membranes, both by direct binding assays and by autoradiography. For this purpose we used, as competitors for the ^{125}I-VTG binding, purified sea bass VTG (VTG°) and plasma from E2-treated and control male sea bass. The VTG° was the same as that used for radiolabeling. Plasmas from E2-treated and untreated fish were chosen because the plasma from E2-treated fish contains high amounts of VTG, whereas plasma from control fish contains the same plasma components as those of E2-treated fish except for VTG (Mañanós et al, 1994a). Thus, any different effects of these plasma samples are caused by the presence or absence of VTG in the plasma. We showed in a previous study, by ELISA and immunoblotting, that sea bass VTG antibodies strongly reacted with plasma from E2-treated fish, while no reaction was detected with plasma from control fish, suggesting no cross-reactivity of these antibodies with other plasma components except for VTG (Mañanós et al, 1994a,b).

Figure 4 shows, by autoradiography, the competition between ^{125}I-VTG and unlabeled ligands for the binding to the VTG receptors after ligand blotting. The autoradiographic signal obtained by the binding of ^{125}I-VTG to the VTG receptor band (lane 1) was completely abolished by addition of an excess of VTG° (lane 2) or plasma from E2-treated males (lane 3), while addition of the same quantity of control male plasma caused no effect (lane 4).

Similarly, figure 5 shows the progressive displacement of the binding of ^{125}I-VTG to ovarian membranes by direct binding assays, when increasing amounts of VTG° were added. Displacement of 50% of the binding was produced by 16 µg VTG°/mL; amounts higher than 300 µg VTG°/mL produced total displacement of the binding. Increasing amounts of plasma from E2-treated fish caused similar effects as those of VTG°, although displacements were higher than those with VTG°. The addition of 8 µg of plasma proteins/mL from E2-treated fish caused 50% displacement of the binding, whereas the same amount from control fish plasma caused no effect (< 10% displacement). Total displacement (> 85%) was obtained with 300 µg of plasma proteins/mL (E2-treated fish plasma), whereas plasma from control fish at this concentration produced less than 25% of displacement. Interestingly, as shown in figure 5,

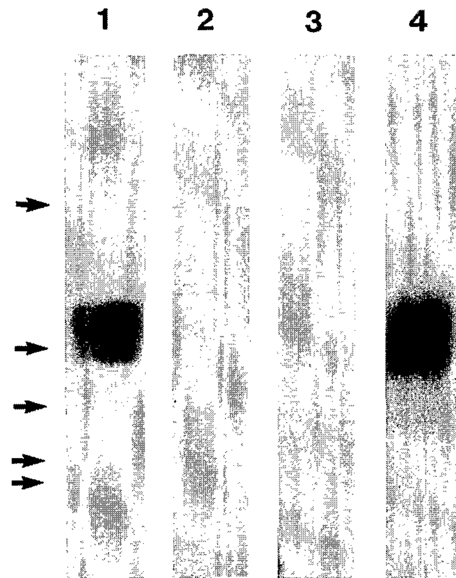


Fig 4. Specificity of the sea bass vitellogenin (VTG) receptors. The autoradiographic signal produced by the binding of ^{125}I-VTG (2.10^{6} dpm/mL) to the VTG receptor band (lane 1, 10 µg of protein per lane) is completely abolished by the addition of an excess of VTG° (120 µg of VTG/mL) (lane 2) or estradiol-treated male plasma (2 µL of plasma/mL, plasma contains 57 µg VTG/µL) (lane 3). Addition of the same quantity of control male plasma caused no effect (lane 4). Arrowheads on the left indicate the position of the molecular weight markers (from top to bottom: 200, 94, 67, 43 and 30 kDa).

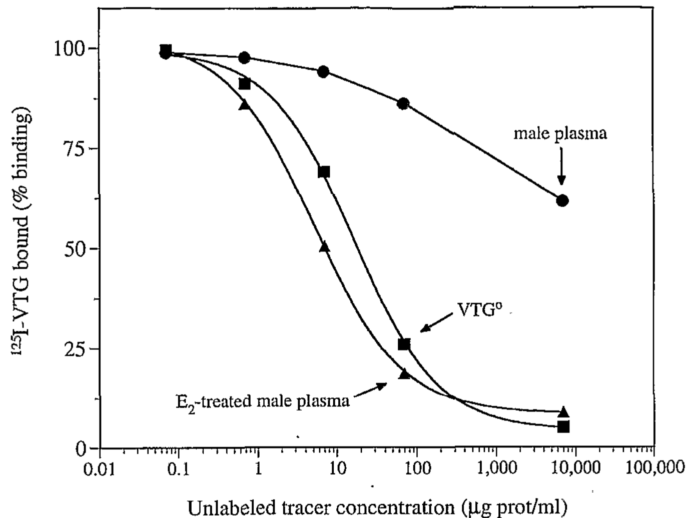


Fig 5. Competition curves obtained with several unlabeled tracers. The binding of radiolabeled vitellogenin (^{125}I-VTG; 50 000 dpm per tube) to the oocyte membranes (100 mm^2 of membranes per tube) was progressively displaced by the addition of increasing amounts (70 ng to 7 mg of VTG/mL) of cold VTG (VTG°) or plasma from estradiol (E_2)-treated males (70 ng to 7 mg of proteins/mL). The same range of protein concentrations, tested for control male plasma, caused negligible displacement.

higher concentrations of plasma from control fish (without VTG) produced some displacement of the ^{125}I-VTG binding. This, together with the fact that higher displacements were obtained with E2-treated fish plasma than with VTG°, suggested that other plasma components might be competing with VTG for the binding to the receptors. Previous studies performed in chicken showed that VTG and very low density lipoproteins (VLDL), the two major yolk protein precursors in the plasma, are internalized by the same receptor, which is very similar to the low density lipoprotein (LDL) receptor in mammals (Schneider, 1992). Although the distribution profile of the different lipoprotein classes in fish is different than that in other vertebrate species (Babin and Vernier, 1991), it is possible that a similar receptor situation is present in fish, due to the homology between fish and bird VTG receptors (Stifani et al, 1990a).

In conclusion, the results presented in this study clearly show the existence of VTG receptors in ovarian membranes from vitellogenic female sea bass. The identified receptors bound reversibly and specifically the VTG molecule. Size and binding characteristics of the sea bass VTG receptors were similar to those reported for salmonid and other fresh water fish species.

ACKNOWLEDGMENTS

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