Protein and Lipid Composition of a Vitellin Isolated from Eggs of *Sparus aurata*

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Z. Naturforsch. **59 c**, 132–134 (2004); received May 6/July 23, 2003

The protein and lipid composition of a vitellin isolated from eggs of *Sparus aurata* were characterized by SDS PAGE, N-terminal sequence analysis and lipid analysis by thin layer chromatography and gas chromatography. The lipoprotein complex contains proteins with apparent molecular weights of 69, 59, 23, 21 and 12 kDa and were characterized as vitellinogenin fragments by N-terminal sequencing. Lipid extraction and analysis indicate an association of cholesterol and phospholipids with the protein subunits. The phospholipids contain fatty acids with 14, 16 and 18 carbon atoms as determined by GC/MS.

Key words: Vitellogenin, Lipids, Fatty Acids

Introduction

The formation of yolk within the ovary of oviparous vertebrates requires the uptake from the blood of serum yolk precursor proteins, the most prevalent being vitellogenin (Wallace and Begovac, 1985). Vitellogenin, derived from liver, appears to enter the oocyte via receptor-mediated endocytosis and is proteolytically cleaved to form yolk proteins. The major part of the yolk proteins consists of conjugated lipoproteins and phosphoproteins, which include vitellogenin derivatives (lipovitellin and phosvitin) (Opresko *et al.*, 1980; Tyler *et al.*, 1988). Vitellogenins and vitellins are lipoglyco-carotenoproteins of large molecular mass (Wang *et al.*, 1983; Wyatt and Pan, 1978). The native molecular mass of vitellogenins is in the range of 250–560 kDa and vitellogenins are composed of two or more subunits with varying molecular mass of 75–130 kDa (Chang *et al.*, 1993). Vitellin is composed of two types of subunits with molecular sizes of 115 kDa and 31 kDa. Phosvitin has a molecular size of 35 kDa and high amount of serines. In addition small proteins of 14 and 19 kDa have been identified. This study is focused on the isolation and characterization of vitellin of the gilthead sea bream (*Sparus aurata*) species, the qualitative determination of phospholipids and their fatty acids, that are conjugated with the vitellin molecule.

Results and Discussion

The purification of vitellin from eggs was achieved as described by Stratakis and Pateraki (1997) with following modifications. The KBr density centrifugation step was avoided. Further vitellin was eluted with step salt gradient (100, 200, 350, 500 mM and 1 M NaCl) instead of a continuous salt gradient. SDS PAGE analysis showed that the protein fraction eluted at 200 mM NaCl contained proteins with molecular weights of 69, 59, 23, 21 and 12 kDa. (Fig. 1). The molecular mass for the native proteins were estimated using the Blue Native (BN) electrophoresis according to (Schaeger and von Jagow, 1991). The major band migrated to a position corresponding to a molecular mass

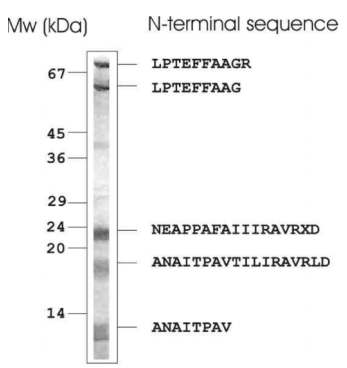

Fig. 1. SDS-PAGE (15%) and N-terminal sequence analysis of the protein fraction eluted at 200 mM NaCl of cation exchange chromatography. Proteins were stained with silver nitrate.

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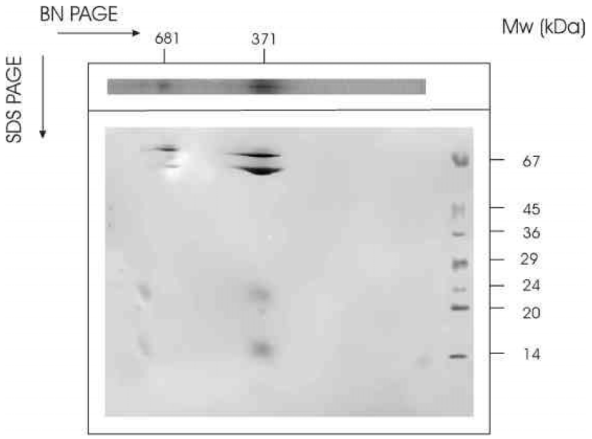

Fig. 2. Two dimensional electrophoresis of isolated protein complex at 200 mM NaCl. First, protein complexes separated by BN PAGE using 8% acrylamide, overlaid by a 3% sample gel. Second, SDS PAGE of excised vertical lanes for the separation of the subunits of the native complexes. Proteins were stained with Coomassie blue.

of 371 kDa. Further, a minor band of 681 kDa was also observed (Fig. 2). Similar molecular weights of 390 and 400 kDa have been reported for native vitellin for *Oncorhynchus nerka* and *Clupea harengus,* respectively, estimated by gel filtration experiments (Hara and Hirai, 1978). SDS electrophoresis of the native protein complex showed protein bands at 69, 59, 23 and 12 kDa (Fig. 2). In order to identify the protein subunits N-terminal sequence analysis has been performed after transfer of the proteins on a PDVF membrane. The protein subunits with molecular weights of 69, 59, 23, 21 and 12 kDa were identified as vitellogenin fragments (Fig. 1) indicating that vitellogenin is the precursor of the yolk proteins. Further, the presence of similar N-terminal sequences between 69 and 59 and 21 and 12 kDa, respectively, indicates the presence of possible proteolytic fragments.

Qualitative lipid analysis of the isolated vitellin was performed by thin layer chromatography (TLC) according to Stratakis *et al.* (1993). The spots of neutral lipids and phospholipids were visualized by iodine vapor. Based on the density cholesterol seems to be the major neutral lipid. Individual phospholipids were identified by comparison with standards (Sigma). Table I shows the various lipid classes associated with the vitellin and separated by TLC. Phosphatidyl choline was the major phospholipid while phosphatidyl serine and phosphatidyl ethanolamine gave less intense spots on TLC plates. The fatty acids were characterized by GS/MS using their corresponding methyl esters. Fatty acid content of phospholipids had 14, 16 and 18 carbon atoms and all of the carbon-carbon bonds were saturated (Table I). While in phosphatidyl choline and phosphatidyl ethanolamine the presence of all three fatty acids was observed in the case of phosphatidyl serine only the fatty acids with C16 and C18 were identified. C20 or unsaturated fatty acids were not found as has been reported for *Eurypelma californicum* indicating a variation of the fatty acids in the different species (Stratakis *et al.*, 1993).

Methods

Fertilized eggs from sea bream *Sparus aurata*, (Linnaeus 1758, Pisces: Sparidae) were collected after the formation of 16 cells. Eggs (0.5 g) were homogenized in 5 volumes of 20 mM Tris, pH = 8, containing 200 mM NaCl, 5 mM $CaCl_2$, 25 mM $MgCl_2$, 10 mM Na_2SO_4, 10 mM $NaHCO_3$ and disrupted by mechanical press. The homogenate was centrifuged at 2000 × *g* for 5 min. Supernatant was ultracentrifugated at 160000 × *g* for 120 min. The purification of vitellin from supernatant was achieved by ion exchange chromatography (Stratakis *et al.*, 1993). A 7.6 × 0.5 cm column of CM Sepharose from SIGMA was equilibrated with 100 mM sodium acetate containing 10 mM EDTA buffered at pH 5.8. After dialysis of the supernatant against 100 mM sodium acetate buffer containing 10 mM EDTA, pH 5.8 (3 h), 1.5 ml of the sample was applied to the column. Vitellin was eluted with step salt gradient (100, 200, 350, 500 mM and

	Fatty Acid Ratio		
Lipids	C14	C16	C18
Phosphatidyl choline	1.04 (± 0.07)	4.00 (± 0.06)	3.00 (± 0.07)
Phosphatidyl serine	n.d.	2.00 (± 0.07)	1.00 (± 0.09)
Phosphatidyl ethanolamine	1.02 (± 0.05)	2.00 (± 0.07)	1.00 (± 0.03)
Cholesterol	–	–	–

Table I. Major lipids and their fatty acid content of *Sparus aurata* vitellin.

( ) = Standard deviation; n = 4.
n.d. = not detected.

1 M NaCl). The purity of the fractions was assessed by SDS-polyacrylamide gel electrophoresis.

Qualitative lipid analysis of the isolated vitellin was performed by thin layer chromatography (TLC) according to Stratakis *et al.* (1993). Vitellin was precipitated with 10% TCA (w/v) and lipids were extracted with 0.5 ml chloroform/methanol (2:1, v/v). After centrifugation of the extract (12000 rpm, Eppendorf centrifuge, 5 min), the supernatant of lipids was concentrated to 50 μl. The silica gel plates were developed with petroleum ether/diethyl ether/acetic acid (70:30:1, v/v) and identified by comparison with standards. The phospholipids from the origin line were scraped into test tubes, extracted by chloroform/methanol (2:1, v/v) and separated by TLC again. The solvent system was chloroform/methanol/ammonia (60:30:2, v/v/v).

The methyl esters of fatty acids were prepared for gas chromatography by treatment of individual phospholipids with boron trifluoride/methanol according to AOAC Official Method 969.33 (1998). Analyses of methyl esters were performed as described by Schartau and Leidescher (1983).

Acknowledgements

The authors thank Dr. F. Lottspeich, Martinsried (Germany) for the N-terminal sequence analysis. The project was supported by the Greek Secretariat for Research and Technology (PENED 99 to G. T.) and the University of Crete.

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