VITELLOGENESIS IN THE TELEOST *BRACHYDANIO RERIO* (ZEBRA FISH)

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ABSTRACT

During oogenesis in teleosts, the volume of the oocyte will typically increase several hundred fold mainly due to the accumulation of yolk proteins within the ooplasm. This process is termed as vitellogenesis. In the teleost, as in other oviparous vertebrates, these yolk proteins are derived from a major precursor protein vitellogenin (Vg) produced in the liver in response to estrogen stimulation and released into the blood where it is transported to the gonad, and sequestered into the growing oocytes.

This study concerns vitellogenesis in the zebra fish (Brachydanio rerio). The major estrogen inducible protein in the zebra fish liver has been purified to homogeneity by FPLC using anion exchange chromatography (Mono-Q Pharmacia) with purification being monitored by SDS-PAGE electrophoresis. The purified protein was found to have an estimated molecular weight of 325 kilo Daltons (kD) in its native state and an amino acid composition which closely paralleled that found for Vg from other teleost species. As with Vg purified from other teleost species, on treatment with sodium dodecyl sulfate it dissociated to form several peptide species including two major peptides of about 196 and 160 kD molecular weight.

It was also found that, in accord with studies on other species, the synthesis of the putative Vg was enhanced in the liver of female zebra fish and induced in male fish by estradiol-17β (E2) stimulation. Repeated dosages of E2 (4µg/gm body weight) to female zebra fish resulted in increased content of native protein such that the putative purified Vg obtained after chromatography constituted 68%, 40% and 46% of the total protein of the serum, liver and gonad respectively.

Hepatocytes in primary monolayer cultures synthesised Vg in response to E2 stimulation as assessed by SDS-PAGE. The synthesis was dose dependent, with the most
effective dose of E$_2$ being $10^{-4}$ M and the least effective was $10^{-8}$ M. The synthesised protein was secreted into the medium after a lag period of 3 h.

Through the use of polyclonal antiserum prepared to the native protein and Western blotting techniques, Vg was identified in medium collected from stimulated hepatocyte cultures derived from E$_2$ stimulated male and female fish. Using immunohistochemical techniques, antigen was detected in the liver and gonads from E$_2$ stimulated fish with other tissues being immunonegative. The polyclonal antiserum was utilised to develop an enzyme linked immunosorbent assay (ELISA) to measure the levels of Vg in the serum of E$_2$ stimulated male and female zebra fish. In the males serum Vg levels of 0.65 mg/mg of the total serum protein were found after the final E$_2$ stimulation, compared with 0.14 mg/mg in the serum of mature females increasing to 0.84 mg/mg after the final E$_2$ stimulation. The antiserum was also found to be species specific and showed no cross reaction in the ELISA with serum obtained from verteogenic chickens a lizard (gecko) and another teleost species, the medaka.

In the other study several steroids were tested for their capacity to induce gonadal maturation in the zebra fish, of the steroids tested 17α-hydroxy-20β-dihydro-4-pregene-3-one (17α,20β-P) proved the most potent. Ovaries obtained from fish with a gonadosomatic index (GSI) higher then 16% showed an increase in the percentage of oocytes undergoing final maturation in vitro in the presence of 17α,20β-P. Oocyte size was recognised as an important factor for final maturation and oocytes with less than 600μ m in diameter failed to mature whether or not the steroid was present.
TABLE OF CONTENTS

TITLE i
DEDICATION ii
DECLARATION iii
ABSTRACT iv
ACKNOWLEDGMENT vi
TABLE OF CONTENTS vii
LIST OF TABLES xi
LIST OF FIGURES xii
ABBREVIATIONS xiv

1. LITERATURE REVIEW ......................................................... 2
   1.1 INTRODUCTION ............................................................. 2
   1.2. OVARIAN YOLK PROTEINS ............................................. 3
       1.2.1. Purification ......................................................... 3
       1.2.2. Characteristics ................................................... 3
   1.3 VITELLOGENESIS ............................................................ 4
       1.3.1. Mechanism of Vitellogenesis .................................... 4
       1.3.2. The Site of Vitellogenin Synthesis .............................. 5
       1.3.3. Hormonal Control of Vitellogenesis ......................... 6
           1.3.3.1. PITUITARY REGULATION .................................. 6
           1.3.3.2. STEROIDS ....................................................... 7
   1.4. VITELLOGENIN ............................................................. 9
       1.4.1. Isolation .............................................................. 10
       1.4.2. Quantification of Vitellogenin ................................ 10
       1.4.3. Biochemical Characterisation of Vitellogenin .............. 12
   1.5 HEPATIC EVENTS AND CHANGES RELATED TO VITELLOGENESIS .......... 13
   1.6. OVARY RELATED EVENTS ............................................... 15
       1.6.1. Steroids Production by Ovarian Follicles During Vitellogenesis ...................................................... 15
       1.6.2. Passage of Macromolecular Materials Through the Follicular Epithelium ................................................. 16
       1.6.3. Uptake of Vitellogenin by Growing Oocyte .................. 16

vii
1.7. VITELLOGENESIS : IN VITRO STUDIES .................................................. 19
  1.7.1. Estrogen Responsive Liver Cultures ........................................... 19
  1.7.2. Hepatocytes Primary Monolayer Cultures .................................... 20
1.8. OOCYTE MATURATION AND OVULATION ............................................ 21
  1.8.1. Induced Final Maturation and Ovulation .................................... 21
    1.8.1.1 Levels Of Intervention .................................................... 21
    1.8.1.2. Use of specific compounds .............................................. 22
  1.8.2. In Vitro Maturation and Ovulation ......................................... 25
    1.8.2.1. Final Maturation .......................................................... 26
    1.8.2.2. Ovulation .................................................................. 28
1.9. AIMS OF THIS STUDY .................................................................... 28

2. ISOLATION, PURIFICATION AND CHARACTERISATION OF VITELLOGENIN ......................................................... 30

2.1. INTRODUCTION ........................................................................ 30

2.2. MATERIALS AND METHODS ...................................................... 32
  2.2.1. MAINTENANCE OF FISH ........................................................... 32
    2.2.1.1. Fish ........................................................................... 32
    2.2.1.2. Prerequisites for Holding Fish ........................................... 32
  2.2.2. ESTRADIOL STIMULATION ...................................................... 33
  2.2.3. TISSUE COLLECTION AND SAMPLING .................................... 33
  2.2.4. PURIFICATION OF VITELLOGENIN BY ANION EXCHANGE CHROMATOGRAPHY .................................................. 34
  2.2.5. ISOTOPE INCORPORATION STUDY .......................................... 35
  2.2.6. ELECTROPHORESIS ................................................................. 35
    2.2.6.1. Polyacrylamide Gel Electrophoresis (PAGE) ....................... 35
    2.2.6.2. Sample Preparation, Loading and Electrophoresis .......... 36
    2.2.6.3. Staining of Electrophoresed Gels .................................... 36
  2.2.7. AMINO ACID ANALYSIS ......................................................... 37

2.3. RESULTS ................................................................................. 37
  2.3.1. CHROMATOGRAPHY OF VITELLOGENIN .................................. 37
  2.3.2. ISOTOPE INCORPORATION ...................................................... 38
  2.3.3. ANALYSIS BY POLYACRYLAMIDE GEL ELECTROPHORESIS .................. 38
    2.3.3.1. Effect of Estradiol Induction ............................................ 38
    2.3.3.2. Effect of Sequential Estradiol Dose .................................. 38
    2.3.3.3. Analysis of Purified Putative Vg ..................................... 39
  2.3.4. AMINO ACID ANALYSIS ......................................................... 39
  2.3.5. MEASUREMENT OF VITELLOGENIN ........................................... 39

2.4. CONCLUSION AND DISCUSSION .................................................. 40
3. PRIMARY MONOLAYER CULTURES OF HEPATOCYTES AND SYNTHESIS OF VITELLOGENIN

3.1. INTRODUCTION

3.2. MATERIALS AND METHOD

3.2.1. PREPARATION OF FISH FOR OBTAINING TISSUE

3.2.2. ISOLATION OF HEPATOCYTES AND REPERATION OF PRIMARY CULTURE

3.2.2.1. Collection of Tissue

3.2.2.2. Preparation Of Cell Suspension And Cell Harvesting

3.2.2.3. Lysis Of Erythrocytes

3.2.2.4. Cell Count And Viability

3.2.2.5. Coating Of Culture Plates

3.2.2.6. Seeding Density For Primary Monolayer Cultures

3.2.3. CULTURE CONDITION

3.2.4. IN VITRO VITELLOGENIN INDUCTION

3.2.4.1. Estradiol Stimulation

3.2.4.2. 3H-Leucine Incorporation

3.2.5. SDS-PAGE ELECTROPHORESIS

3.2.5.1. Silver Staining Of SDS-PAGE

3.3. RESULTS

3.3.1. ISOLATION AND VIABILITY

3.3.2. MORPHOLOGY OF ISOLATED AND CULTURED HEPATOCYTES

3.3.3. CELL ATTACHMENT WITH TIME IN CULTURE

3.3.4. EFFECT OF SUBSTRATE ON HEPATOCYTE CULTURE

3.3.5. KINETICS OF 3H-LEUCINE INCORPORATION

3.3.6. EFFECT OF INSULIN ON PROTEIN SECRETION IN CULTURE

3.3.7. EFFECT OF ESTROGEN ON HEPATOCYTES IN CULTURE

3.4. CONCLUSION AND DISCUSSION

4. IMMUNOLOGICAL LOCALISATION AND IDENTIFICATION OF VITELLOGENIN AND RELATED YOLK PROTEINS

4.1 INTRODUCTION

4.2 MATERIALS AND METHOD

4.2.1. ANTIBODY PRODUCTION

4.2.2. ASSAY FOR ANTIBODY ACTIVITY
4.2.3. PROTEIN TRANSFER AND ANALYSIS BY WESTERN
BLOTTING ................................................. 76

4.2.4. IMMUNOHISTOCHEMISTRY .................................. 77

4.2.4.1. Elimination of Endogenous Peroxidase Activity ....... 78

4.2.5. ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)... 78

4.2.5.1. General Methodology .................................. 78

4.2.5.2. Measurement Of Enzyme Activity Of Alkaline
Phosphatase Conjugate ......................................... 79

4.3. RESULTS .......................................................... 80

4.3.1. IMMUNODIFFUSSION ........................................ 80

4.3.2. WESTERN BLOT ANALYSIS .................................. 80
4.3.2.1. Immunological Identification of Estradiol Induced
Polypeptides ......................................................... 80

4.3.2.2. Polypeptide Pattern of Native Vitellogenin ......... 81

4.3.2.3. Identification of Vitellogenic Polypeptides
Produced by Estradiol ....... 81

4.3.3 IMMUNOHISTOCHEMICAL LOCALISATION OF
VITELLOGENIN .................................................. 81

4.3.4. DEVELOPMENT OF ELISA FOR VITELLOGENIN ..... 83

4.3.4.1. Concentration of Antigen for Coating the
Microtiter Plates ................................................ 83

4.3.4.2. Standardising the Antibody Working Dilution ..... 83

4.3.4.3. Verification of Antigen Specificity .................. 83

4.3.4.4. Species Specificity .................................... 84

4.3.4.5. Measurement of Ig ...................................... 84

4.4. CONCLUSION AND DISCUSSION ............................... 85

5. INDUCED OVULATION AND MATURATION OF THE
OOCYTES IN VITRO ............................................. 104

5.1 INTRODUCTION .................................................. 104

5.2 MATERIALS AND METHODS ....................................... 105

5.2.1. SPAWNING (BREEDING) .................................. 105
5.2.1.1 Fish ....................................................... 105

5.2.1.2. Breeding Tank Conditions ............................... 106

5.2.1.3. Collection of Eggs .................................. 106

5.2.2. IN VITRO FERTILIZATION (ARTIFICIAL
INSEMINATION) ............................................... 107

5.2.2.1 Collection Of Male and Female Gametes ............... 107

5.2.2.2. Fertilization and Incubation of the Embryos ..... 107

5.2.3. IN VIVO HORMONAL INDUCTION .......................... 108

5.2.3.1 Selection Of Fish ...................................... 108
5.2.3.2 Induction .................................................................... 108
5.2.3.3 Induced Spawning ..................................................... 109
5.2.4 IN VITRO MATURATION ................................................ 109
5.2.4.1 Collection Of Oocytes ............................................... 109
5.2.4.2 Culture Medium ....................................................... 109
5.2.4.3 Steroids ................................................................. 110
5.2.4.4 Culture Of Oocytes ................................................... 110
5.2.5 CLEARING OF THE OOCYTES ...................................... 110
5.2.5 IN VITRO OVAULTATION AND FERTILIZATION ........... 110

5.3 RESULTS ........................................................................... 111
5.3.1 INDUCED OVULATION AND SPAWNING ...................... 111
5.3.1.1 Effects of hCG Dose and Time of Induction on
Ovulation ........................................................................ 111
5.3.1.2 Effect of hCG in Presence of Male fish ...................... 111
5.3.2 IN VITRO MATURATION AND OVAULTATION ............. 112
5.3.2.1 Effect of size of oocytes on maturation ...................... 112
5.3.2.2 Effect of Incubation Medium on Maturation .............. 112
5.3.2.3 Effect of Concentration of various Steroids ............... 113
5.3.2.4 Effect of Time Exposure to 17α,20β-P ....................... 113
5.3.2.5 Effect of Gonadotrophic Index on Steroid
Maturation ....................................................................... 113
5.3.2.6 Effect of Prostaglandin F2α on Ovulation ................. 114

5.4 CONCLUSIONS AND DISCUSSION .................................. 114
6 GENERAL DISCUSSION AND CONCLUSIONS ....................... 124

BIBLIOGRAPHY ...................................................................... 129