Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 95, No. 2, April 1986, pp. 171–179.

Histochemical studies on the vitellogenesis in a fairy shrimp *Streptocephalus dichotomus* Baird (Crustacea: Anostraca)

N MUNUSWAMY and T SUBRAMONIAM

Unit of Invertebrate Reproduction, Life Sciences Building, Department of Zoology, University of Madras, Guindy Campus, Madras 600025, India

MS received 20 July 1985; revised 31 December 1985

Abstract. Histochemical characterisation of ooplasm and yolk granules in different stages of oocyte maturation in the ovary was made in the freshwater fairy shrimp *Streptocephalus dichotomus*. In stage I oocyte, the ooplasm is reactive to basic and acidic groups of protein, aromatic groups such as tryptophenyl and tyrosyl and sulphur containing substances. The other organic compounds include acid mucopolysaccharide and acidic lipids. In stage II oocyte, sudanophilic lipoidal substances appear coinciding with yolk formation. Stage III oocytes are characterised by the abundance of yolk granules which are rich in sulphated acid mucopolysaccharide as well as glycogen. Besides these, the presence of acid phosphatase activity in the ooplasm and nurse cell is also noticed and its functional role in relation to vitellogenesis is discussed.

Keywords. Yolk granules; sulphated AMP; auto-heterosynthesis.

1. Introduction

Studies on oogenesis in Crustacea is limited mainly to malacostracans (Blinski 1979; Zerbib 1980; Adiyodi and Subramoniam 1983). During vitellogenesis, various yolk components appear sequentially. For many decapods, yolk is histochemically characterised (Zerbib 1980; Varadarajan and Subramoniam 1980). The present paper reports on the origin of various yolk components, as revealed by histochemical characterization in a freshwater anostracan *Streptocephalus dichotomus* Baird. Ultrastructural aspects of oogenesis in this crustacean is reported elsewhere (Munuswamy and Subramoniam 1985).

2. Materials and methods

The ovaries of mature female *S. dichotomus*, were dissected out and fixed in 5% neutral buffered formalin for protein and carbohydrates. For lipid, fresh as well as formol calcium fixed tissues were used.

Histochemical procedures to detect and characterize the various yolk components such as proteins, carbohydrates, lipids and the acid and alkaline phosphatase activity were adopted from Lillie (1965), Pearse (1968), Chayen *et al* (1973), Bancroft and Stevens (1975) and Pearse (1972).

3. Results

3.1 *Stage I*

The oocytes gave positivity for protein tests such as *p*-dimethyl amino benzaldehyde and Millon's, indicating the presence of tryptophan and tyrosyl groups respectively.

Acidic and basic groups of protein were markedly abundant in the nucleus and cytoplasm, as revealed by mercuric and aqueous bromophenol blue. Sulphydryl and disulphide groups were also detected by a positive reaction to ferric ferricyanide and performic acid alcian blue with appropriate controls. Presence of DNA in the nucleus and RNA in the cytoplasm was revealed from the staining reactions of Feulgen test and methyl green pyronin G respectively (table 1).

The periodic acid schiff test (PAS) positivity may suggest the presence of a variety of carbohydrate components. The PAS positivity was considerably reduced after the diastase treatment suggesting the presence of glycogen in the cytoplasm.

The positive reaction of PAS after diastase treatment showed the presence of acid mucopolysaccharide (AMP). Further characterisation of AMP with alcian blue and toluidine blue indicated its carboxylated nature (table 2).

The presence of lipid in the cytoplasm of the oocyte was demonstrated by Sudan black B, and oil red 'O'. The appearance of blue colour with Nile blue sulphate indicated the presence of acidic lipids in the cytoplasm, but the negativity to acid haematin suggested the absence of phospholipids (table 3).

3.2 *Stage II*

A gradual decrease in basophilia in the nucleus and later in the ooplasm was evident in stage II, increased staining at this stage with aqueous bromophenol blue shows the basic protein nature of yolk. With Mallory's triple stain, the oogonial cells and the nuclei of the oocytes stain blue, whereas the yolky ooplasm stained orange red, indicating the presence of basic protein.

Ooplasm revealed the presence of basic and acidic groups; tyrosyl, tryptophanyl groups, sulphydryl and disulphide groups. Acid phosphatase was also localised in the ooplasm as well as nurse cells in the second stage of oocyte maturation. Besides this, the follicle cells in the ovarian wall showed intense positivity to acid phosphatase. The histochemical tests revealed the presence of glycogen and AMP in the cytoplasm. In this stage the glycogen appears and continues to accumulate in the cytoplasm during the growth of the egg until yolk granules appear in the oocytes (tables 1 and 2).

Phospholipids formed the prominent lipid component of yolk granules (table 3). The cytoplasm of the stage II oocyte showed high RNA content. Sudanophilic lipoidal substances made their appearance in the yolk granules. Nurse cells which are prominent in the first and second stages of oocyte development showed positive reactions to protein, DNA and RNA tests.

3.3 *Stage III*

In stage III, yolk granules were abundant and the mature oocytes were ready to be released into the lateral pouch of oviduct. Histological stains, haematoxylin eosin and Mallory's triple stain gave eosinophilic and red colour to the yolk granules respectively. The yolk consisted predominantly of basic proteins, rich in aromatic, disulphide and sulphydryl groups (table 1).

Glycogen accumulation in the granules increased with the onset of yolk formation. The histochemical tests with appropriate controls further indicated the presence of

sulphate groups and phosphate groups in the AMP. The outer layer of oocyte showed positivity to alkaline phosphatase activity as inferred from Gomori test (table 2).

Coarse sudanophilic lipid granules were characteristic of all growing oocytes. Intensity to acid haematin after the pyridine treatment is of considerable interest because intense staining may be due to the presence of protein rather than lipids. The results of performic acid-schiff and its control revealed the presence of unsaturated fatty acid in the oocytes (table 3).

4. Discussion

The cytochemical observations made on the growing oocytes and nurse cells of *S. dichotomus* have revealed the sequential deposition of different deutoplasmic substances in the oocytes. In early stages of ovarian maturation the ooplasm is devoid of any yolk granule and the cytoplasm shows much affinity for basic dyes such as haematoxylin. This basophilia is gradually replaced by acidophilia in the ooplasm as vitellogenesis advances. Concomittant with this change in the staining property of ooplasm, small glycoprotein granules appear in the perinuclear region. This granulation intensifies further and fills up the entire cytoplasm. The mature granules stain for glycolipoprotein. In *Streptocephalus dichotomus* the granules are of the same type unlike the granules in the oocyte of *Chirocephalopsis bundyi*, where two types have been reported histochemically (Linder 1959). The changing histochemical property of the yolk components has also been reported in many decapod oocytes (Blinski 1979; Zerbib 1980).

Anostracans are ideal crustaceans to study oocyte-nurse cell association during vitellogenesis. The presence of nurse cells in the early phase of oocyte development suggests an initial autosynthetic mode of yolk formation in *S. dichotomus* as the nurse cells supply only RNA at this time. In the brine shrimp *Artemia*, the nurse cells become polyploid by endomitosis and extrude DNA from the nucleus into the cytoplasm before being phagocytosed by the oocyte (Fautrez-Firlefyn 1951). However, the process of vitellogenesis is not purely internal to oocyte. The histochemical observations on the yolk granules of *S. dichotomus* reveal both auto and heterosynthetic mode of yolk formation. This condition may be comparable to an anomuran crab *Clibanarius clibanarius* in which the yolk formation takes place by heterosynthesis to supplement the initial autosynthesis (Varadarajan and Subramoniam 1980).

In addition to the nurse cells RNA contribution to the vitellogenic oocyte comes from the nucleolus. Besides this, presence of basic protein in the nucleoplasm suggest the metabolic role of the nucleolus (Fautrez-Firlefyn and Fautrez 1953).

Interestingly, the localization of acid phosphatases in the stage II oocyte as well as in the follicle cells suggest their involvement in the process of vitellogenesis. Acid phosphatase is an important hydrolytic enzyme mediating the degradation and transfer of nurse cell products to vitellogenic oocyte in a hemipteran insect, *Gerris remigis* (Cone and Eschenberg 1981).

Table 1. Histochemical reactions for proteins in the oocytes during vitellogenesis.

Tests	Stage I Nucleus	Stage I Cytoplasm	Stage II Nucleus	Stage II Cytoplasm	Stage III Yolk globules	To detect
Mercuric bromophenol blue	+	+ +	+	+ +	+ +	General proteins
	B	DB	B	DB	DB	
Aqueous bromophenol blue (ABB)	+	+	+	+	+ +	Basic proteins
	B	B	B	B	DB	
Deamination + ABB	−	−	−	−	−	Removal of amino groups
Fast green at pH 1	±	+	−	+ +	+	Acidic proteins
	G	G		G	G	
Fast green at pH 8	−	±	−	±	+ +	Basic proteins
		G		G	G	
Toluidine blue (TB)	+	+	+	+	+ +	Acidic proteins
	B	B	B	B	B	
Methylation + TB	−	−	−	−	−	Removal of acidic groups
Ninhydrin	+	+	+	+	+ +	Amino groups
	B	B	B	B	B	
Millon's	−	+	−	+	+ +	Tyrosine
		BR		BR	BR	
Bromination + Millon's	−	−	−	−	−	To block-OH group
p-Dimethyl amino benzaldehyde	−	+	−	+	+ + +	Tryptophan
(DMAB)		BC		BC	BC	
40% Formalin + DMAB	−	−	−	−	−	To block tryptophenyl groups

Ferric ferricyanide (FFC)	–	+ PB	–	+ PB	+ + PB	-SH groups
Mercuric chloride + FFC	–	–	–	–	–	To block-SH groups
Performic acid alcian blue (PFAB)	+ B	+ B	+ B	+ B	+ + B	
						-S-S- groups
Performic acid Schiff	+ M	+ M	+ M	+ M	+ M	
Thioglycollate + PFAB	–	–	–	–	–	To block-S-S-groups
Feulgen's	+ M	–	+ M	–	+ M	DNA
Methyl green pyronin G (MGP)	+ G	+ G	+ G	+ G	+ G	RNA
10%Cold perchloric acid + MGP	–	–	–	–	–	To extract RNA
Gomori's	–	±	–	+ Br	+ + Br	Acid phosphatase
Gomori's	–	–	–	+ Br	+ + Br	Alkaline phosphatase

B, blue; Bb, bluish black; Br, brown; BR, brick red; DB, dark blue; G, green; M, Majenta; Pb, prussiun blue; R, red.
–, absent; ±, doubtful; +, + +, + + +, degree of positive reaction intensity.

Table 2. Histochemical reactions for carbohydrates in the oocytes during vitellogenesis in *S. dichotomus*.

Tests	Stage I Nucleus	Stage I Cytoplasm	Stage II Nucleus	Stage II Cytoplasm	Stage III Yolk globules	To detect
Schiff alone	–	–	–	–	–	Free aldehydes
Periodic acid schiff (PAS)	–	+	–	+	+ + +	Glycogen, 1–2 glycol
		M			M	group, unsaturated fatty acid and acid mucopolysaccharide
Diastase + PAS	–	±	–	+	+ +	Removal of glycogen
		M		M	M	
Acetylation + PAS	–	±	–	±	±	Removal of 1, 2 glycol
		M		M	M	group
Deacetylation + PAS	–	+	–	+	+	1,2 glycol group
		M		M	M	
Bromination + PAS	–	–	–	–	–	To block C-C groups
Best's carmine (BC)	±	+	±	+ +	+ +	Glycogen
Aldehyde fuchsin	–	+	–	+	+ +	Sulphated and non-sulphated acid
		P		P		muco substances

Alcian blue Critical electrolyte concentration (CEC)						
0.2 M	–	+++ B	+++ B	+++	+	Carboxylated muco substances
0.4 M	–	++	–	++ B	++ B	Phosphated muco substances
0.6 M	–	+++ B	–	++	+++ B	
1.0 M	–	–	–	–	+++ B	Strongly sulphated muco substances
Toluidine blue at different hydrogen ion concentration						
pH 2	–	++	–	++ B	+++ V	Sulphated muco-polysaccharide
pH 3	–	+++ B	–	++ B	+++	
pH 4	–	+++ B	–	+++	++	Phosphated and carboxylated mucosubstances
pH 5	–	+++ B	–	++ B	+ B	Carboxylated substances
Benzidine		–	–	–	++ B	Sulphate group

P, pink; V, violet.

Table 3. Histochemical reactions for lipids in the oocytes during vitellogenesis in *S. dichotomus*.

Tests	Stage I Nucleus	Stage I Cytoplasm	Stage II Nucleus	Stage II Cytoplasm	Stage III Yolk globules	To detect
Sudan black B (SBB)	± Bb	+ Bb	± Bb	+ Bb	+ + Bb	General lipids
Chloroform/methanol + SBB	−	± Bb	−	± Bb	± Bb	Removal of lipids
Hot acetone + SBB	−	± Bb	−	+ Bb	± Bb	Bound lipids
Nile blue sulphate 1% (NBS)	+ B	+ B	+ B	+ B	+ + B	Acidic and neutral lipids
Chloroform/methanol + NBS	−	−	−	−	−	To remove lipids
Oil red 'O'	−	+ R	−	+ R	+ R	Neutral lipids
Acid haematin (AH)	−	+ R	−	+ + R	+ + + R	Phospholipids
Chloroform/methanol + AH	−	−	−	−	−	To remove lipids

For abbreviation and symbols see table 1.

Acknowledgement

We are thankful to Prof. K Ramalingam for the facilities provided for this research work. One of us (NM) is grateful to University Grants Commission, New Delhi for the award of a fellowship.

References

Adiyodi R G and Subramoniam T 1983 Oogenesis, oviposition and oosorption in Crustacea; in *Reproductive biology of Invertebrates*, (eds) K G Adiyodi and R G Adiyodi (New York: John Wiley and Sons.) 443–495

Bancroft J D and Stevens A 1977 *Theory and Practice of Histochemical Techniques* (Edinborough: Churchill)

Blinski S 1979 Ultrastructural study of yolk formation in *Porcellio scaber* Latr (Isopoda); *Cytobios* **26** 123–130

Chayen T, Bitensky L and Butcher R G 1973 *Practical Histochemistry* (London: John Wiley and Sons)

Cone V and Eschenberg K M 1981 Histochemical localization of acid phosphatase in the ovary of *Gerris remigis* Say (Hemiptera); *J. Exp. Zool.* **161** 337–352

Fautrez-Firlefyn N 1951 Etude Cytochimique des acides nucliques au cours de la gamétogènese et des premiers stadis du development embryonnaire chez *Artemia salina* L; *Arch. Biol.* **62** 391—438

Fautrez-Firlefyn N and Fautrez T 1953 Intra nuclear proteins of the oocytes in *Artemia salina* L; *Nature (London)* **172** 163—164

Lillie R D 1965 *Histopathologic Technic and Practical Histochemistry*, 3rd edition (New York: McGraw Hill)

Linder H J 1959 Studies on the freshwater fairy-shrimp *Chirocephalopsis bundyi* (Forbes). 1. Structure and histochemistry of the ovary and accessory reproductive tissues; *J. Morphol.* **104** 1–60

Munuswamy N and Subramoniam T 1984 Egg envelopes of *Streptocephalus dichotomus* Baird - A structural and histochemical study; *Hydrobiologia* **114** 17–28

Munuswamy N and Subramoniam T 1985 Oogenesis and shell gland activity in a freshwater fairy shrimp *Streptocephalus dichotomus* Baird (Crustacea: Anostraca) *Cytobios* **44** 137–147

Pearse A G E 1968 *Histochemistry: Theoretical and Applied* (London: J and A Churchill Ltd.) Vol. 1

Pearse A G E 1972 *Histochemistry: Theoretical and Applied* (London: Churchill-Livingstone) Vol. 2.

Varadarajan S and Subramoniam T 1980 Histochemical investigation on vitellogenesis of an anomuran crab, *Clibanarius clibanarius*; *Int. J. Invertebr. Reprod.* **2** 47–58

Zerbib G H 1980 Ultrastructural observation of oogenesis in the crustacean amphipoda *Orchestia gammarellus* (Pallas); *Tissue Cell* **12** 47–62