

BIOCHEMICAL ANALYSIS OF PROTEINS IN THE ANDROGENIC GLAND OF EYESTALK ABLATED ANIMALS IN BARYTELPHUSA GUERINI

Abstract

Androgenic gland is a part of male reproductive system situated at the terminal portion of vas deferens. It secretes hormones responsible for the differentiation of the male and female genital apparatus and the secondary sexual characteristics. The proteins of androgenic gland exhibit variations in relation to the reproductive cycle, on eyestalk ablation and eyestalk extract injection. This was studied in Barytelphusa guerini. It showed the signs of increased secretory activity on eyestalk ablation.

INTRODUCTION

Androgenic gland is a part of male reproductive system situated at the terminal portion of vas deferens. It is widely accepted that the AG of decapod crustaceans secretes hormones responsible for controlling the differentiation of the male and female genital apparatus and the secondary sexual characteristics in *Orchestia gammarellus* (Charniaux-Cotton, 1954, 1964); *Paratelson hydromous* (Adiyodi and Adiyodi, 1970; Fingerman, 1997); *Pontastacus leptodactylus* (Payen, 1973); *Procambarus clarkii* (Taketomi *et al.*, 1990, 1996; Taketomi and Nishikawa, 1996); *Cherax destructor* (Fowler and Leonard, 1999); *Cherax quadricarinatus* (Khalaila *et al.*, 1999, 2001).

To elucidate the chemical nature, AGH was isolated and the investigations encompassing ultrastructural and histological studies suggested that AGH was a protein and supports the possibility of a peptidergic proteinaceous secretion, *Pachygrapsus crassipes* (King, 1964); *Oniscoides superieus* (Malo and Juchault, 1970); *Porcellio scaber* (Radu and Craciun, 1976); *Procambarus clarkii* (Miyawaki and Taketomi, 1978; Taketomi, 1986); *Macrobrachium rosenbergii* (Sagi, 1988; Awari and Dube, 1999; Piera *et al.*, 2000; Zhang *et al.*, 2000; Okumara and Hara, 2004; Ventura *et al.*, 2009, 2011); *Portunus trituberculatus* (Qing *et al.*, 2010) and *Cherax quadricarinatus* (Rosen *et al.*, 2013).

The protein content was studied in the testis and androgenic gland in normal and eyestalk ablated prawns in *Portunus pelagicus* and *Portunus sanguinolentus* (Radhakrishnan, 1979); *Scylla serrata* (George and Gopakumar, 1987); *Macrobrachium rosenbergii* (Sheen and Amaro, 1991; Anonymous, 1999); *Charybdis smithii* (Balasubramanian and Suseelan, 2001) and *Scylla tranquebarica* (Thirunavukkarasu, 2005); *Macrobrachium rosenbergii* (Revathi *et al.*, 2013).

The total protein content in testis and androgenic gland shows variation in relation to the reproductive cycle and seasons in both normal and eyestalk ablated crabs. The eyestalk ablation induced the biochemical changes in different reproductive tissues such as testis and androgenic gland in *Macrobrachium rosenbergii* (Revathi *et al.*, 2013).

Effects of eyestalk ablation was studied in *Carcinus menas* (Demeusy and Veillet, 1958; De Meusy, 1967), *Pandalus platyceros* (Brockenbrough-Foulks and Hoffman, 1974); *Portunus pelagicus* and *Portunus sanguinolentus* (Radhakrishnan, 1979); *Scylla serrata* (George and Gopakumar, 1987; Prasad and Neelakantan, 1989); *Macrobrachium rosenbergii* (Sheen and Amaro, 1991; Anonymous, 1999); *Armadillidium vulgare* (Okuno *et al.*, 1997, 1999; Martin *et al.*, 1999; Sagi and Khalaila, 2001); *Charybdis smithii* (Balasubramanian and Suseelan, 2001); *Cherax quadricarinatus* (Khalaila *et al.*, 2001, 2002); *Parapenaopsis hardwickii* (Kulkarni *et al.*, 1984); *Scylla tranquebarica* (Thirunavukkarasu, 2005); *Macrobrachium rosenbergii* (Revathi *et al.*, 2013).

The protein content increased in the androgenic gland in ablated prawns in *Portunus pelagicus* and *Portunus sanguinolentus* (Radhakrishnan, 1979); *Scylla serrata* (George and Gopakumar, 1987); *Macrobrachium*

rosenbergii (Sheen and Amaro, 1991); *Charybdis smithii* (Balasubramanian and Suseelan, 2001) and *Scylla tranquebarica* (Thirunavukkarasu, 2005); *Macrobrachium rosenbergii* (Revathi *et al.*, 2013).

The main objective of the present investigation is to determine quantitatively the variation in the protein content in the androgenic gland in the three periods of annual reproductive cycle of the eyestalk ablated animals of fresh water male crab *Barytelphusa guerini*.

MATERIALS AND METHODS

Total proteins were estimated by Biuret method (Gornall *et al.*, 1949) in the three periods of the annual reproductive cycle, in the androgenic gland of eyestalk ablated animals.

RESULTS

Table : 1

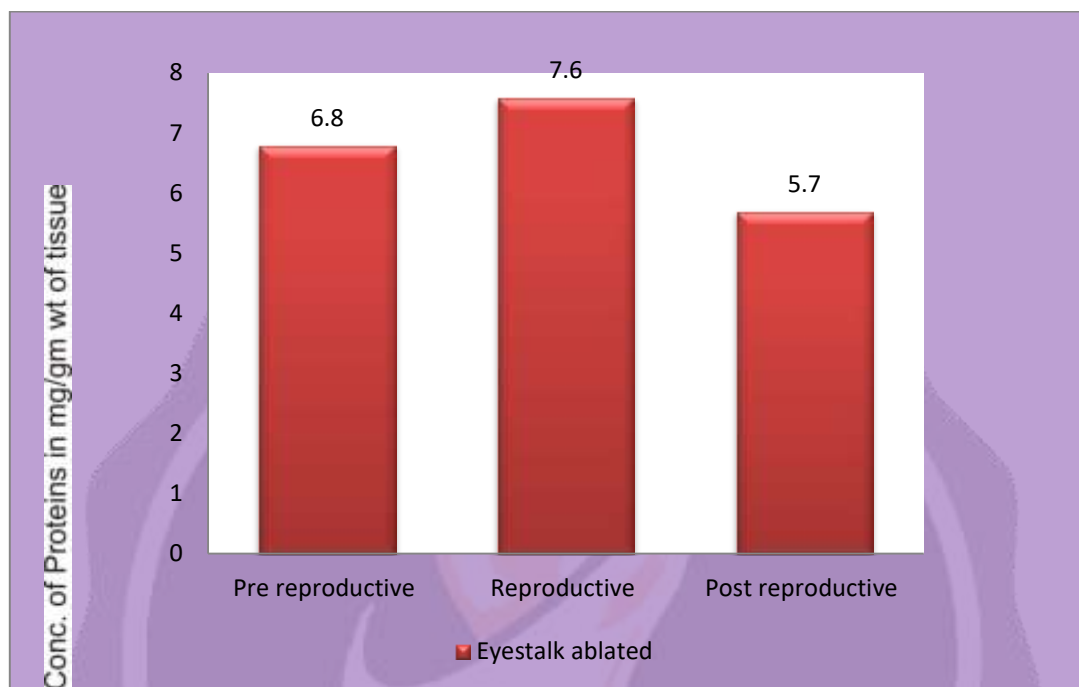
Showing the concentration of Proteins in the Androgenic Gland in mg/gm wt. of tissue in different periods of the annual reproductive cycle in Eyestalk ablated animals

Sample	Pre-reproductive period	Reproductive period	Post-Reproductive period
1	6.9 mg/gm wt of tissue	7.7 mg/gm wt of tissue	5.8 mg/gm wt of tissue
2	6.8 mg/gm wt of tissue	7.5 mg/gm wt of tissue	5.5 mg/gm wt of tissue
3	6.8 mg/gm wt of tissue	7.6 mg/gm wt of tissue	5.7 mg/gm wt of tissue
4	6.7 mg/gm wt of tissue	7.4 mg/gm wt of tissue	5.7 mg/gm wt of tissue
5	6.8 mg/gm wt of tissue	7.5 mg/gm wt of tissue	5.7 mg/gm wt of tissue
6	6.9 mg/gm wt of tissue	7.7 mg/gm wt of tissue	5.8 mg/gm wt of tissue
sum	40.9	45.4	34.2
Mean	6.82	7.57	5.70
S.D.	0.075277	0.121106	0.10954
Variance	0.005667	0.014667	0.012

The protein content was expressed as mg/ gram weight of tissue. The protein content of the androgenic gland in eyestalk ablated animals were:

Protein concentration in pre-reproductive period was 6.82 mg/gram weight of tissue, in reproductive period it was 7.57 mg/ gram weight of tissue and in post-reproductive period it was 5.70 mg/ gram weight of tissue.

Graphical representation of Proteins in the AG in the normal, eyestalk ablated and eyestalk extract injected animals during the three periods of the annual reproductive cycle



DISCUSSION

Total proteins were determined in the AG of eyestalk ablated male crabs. The protein content in the AG was maximum in the reproductive period and minimum in the post reproductive period of eyestalk ablated animals.

In *Barytelphusa guerini* the eyestalks removal tends to increase the length and size of the androgenic gland in all the periods of the annual reproductive cycle, the number, size and activity of the AGH secreting cells were also increased, AG showed hypertrophy as suggested by *Cherax quadricarinatus* (Kulkarni *et al.*, 1983; Khalaila *et al.*, 2001; Khalaila *et al.*, 2002; Ventura *et al.*, 2009) and hyperactivity *Carcinus menas* (Demeusy and Veillet, 1958; *Potamon dehaani* (Otsu, 1963); De Meusy, 1967); *Orconectes nais* (Carpenter and De Roos, 1970); *Scylla serrata* (Rangnekar *et al.*, 1971); *Rithropanopeus harrissi* and *Callinectes sapidus* (Payen *et al.*, 1971); *Lysmata seticaudata* (Touir, 1977); *Macrobrachium lamarrei* (Sarojini Gyananath, 1985); *Scylla paramamosain* (Liu *et al.*, 2008); *Macrobrachium rosenbergii* (Ventura *et al.*, 2009, Revathi *et al.*, 2013) when compared to the normal animals of the different periods of the reproductive cycle and correspondingly there was an increase in the activity of the AG.

In *Barytelphusa guerini* eyestalk ablation affects the AG, protein synthesis of specific polypeptides representing androgenic factors as in *Armadillidium vulgare* (Okuno *et al.*, 1997, 1999; Martin *et al.*, 1999; Sagi and Khalaila, 2001), indicated by an increase in protein content and over expression of AG polypeptides as in *Cherax quadricarinatus* (Khalaila *et al.*, 2001, 2002).

Eyestalk ablation directly stimulated the protein level in *Portunus pelagicus* and *Portunus sanquinolentus* (Radhakrishnan, 1979); *Scylla tranquebarica* (Thirunavukkarasu, 2005).

In the eyestalk ablated animals the androgenic activity is increased thereby there was an increase in the protein synthetic activity which further increased the production of the AGH.

Thus the eyestalk ablated animals showed increased androgenic gland activity in all the three periods of reproductive cycle namely, pre-reproductive period, reproductive period, and post-reproductive period with more production of AGH.

Among the eyestalk ablated animals there was a progressive increase in the protein level from pre-reproductive period to the reproductive period and decreased in the post-reproductive period.

Upon ablation the AG in both pre-reproductive as well in the post-reproductive period shows the characteristics as that of the reproductive period.

REFERENCES

- Allan G. Gornall, Charles J. Bardawill and Maxima M. David. Determination of Serum Proteins by means of the Biuret reaction. The journal of biologicalchemistry.1949
- Adiyodi, K.G. and Adiyodi, R.G. 1970. Endocrine control of reproduction in decapod crustacea. Biol. Rev., 45: 121-165.
- Anonymous, S. 1999. Crustaceans, crab, blue, cooked, moist heat. Blue crab-nutrition. Results from the USDA. Nutrient Database for Standard Reference, pp: 1-3.
- Awari, S.A. and Kiran Dube. 1999. Histological and histochemical study of the androgenic gland of *Macrobrachium rosenbergii* (De Man). Journal of Aquaculture in the Topics, 14: 101-112.
- Balasubramanian, C.P. and C., Suseelan. 2001. Biochemical composition of the deepwater crab *Charybdis smithii*. Indian Journal of Fish, 48: 333-335.
- Brockenbrough-Foulks, N. and Hoffman, D.L. 1974. The effects of eyestalk ablation and B-ecdysone on RNA synthesis in the androgenic gland of the protandric shrimp, *Pandalus platyceros* Brandt. Gen. Comp. Endocrinol., 22: 439-447.
- Carpenter, M.B. and DeRoos, R. 1970. Seasonal morphology and histology of the androgenic gland of the crayfish, *Orconectes nais*. Gen. Comp. Endocrinol., 15: 143-157.
- Charniaux-Cotton, H. 1954. Decouverte chez un crustace amphipode (*Orchestia gammarella*) d'une gland endocrine responsable de la differentiation de caracteres sexual primaires et secondaries male. CR. Acad. Sci., Paris, 239: 780-782.
- Charniaux-Cotton, H. 1964. Endocrinologie et genetique du sexe chez les crustaces superieurs. Ann. Endocrinol., 25: 36-42.
- Demeusy, N. and Veillet, A. 1958. Influence de L'ablation des *Pedoncules oculaires* sur la glande androgene de *Carcinus maenas* L. Compt. Rend. Acad. Sci., 246: 1104-1107.
- Demeusy, N. 1967. Modalites d'action du controle inhibiteur pregonculaire exerce sur des caractres sexuelles externes males du Decapode Brachyoure *Carcinus maenas* Hebol.
- Fingerman, M. 1997. Roles of neurotransmitters in regulating reproductive hormone release and gonadal maturation in decapod crustaceans. Invert. Reprod. Develop, 3147-54.
- Fowler, R.J. and Leonard, B.V. 1999. The structure and function of the androgenic gland in *Cherax destructor* (Decapoda: Parastacidae). Aquaculture, 171: 135-148.
- George, C. and K., Gopakumar, 1987. Biochemical studies on crab *Scylla serrata*. Fisheries. Technology, 24: 57-61.
- Khalaila, I., Weil, S. and Sagi, A. 1999. Endocrine balance between male and female components of the reproductive system in intersex, *Cherax quadricarinatus* (Decapoda: Parastacidae). J. Exp. Zool., 283: 286-294.
- Khalaila I, Katz T, Abdu U, Yehezkel G, Sagi A. 2001. Effects of implantation of hypertrophied androgenic glands on sexual characters and physiology of the reproductive system in the female red claw crayfish, *Cherax quadricarinatus*. Gen Comp Endocrinol .,121:242-249.
- Khalaila I, Manor R, Weil S, Granot Y, Keller R, Sagi A. 2002. The eyestalk-androgenic gland-testis endocrine axis in the crayfish *Cherax quadricarinatus*. Gen Comp Endocrinol., 127:147-156.

- King, D.S. 1964. Fine structure of the androgenic gland of the crab, *Pachygrapsus crassipes*. Gen. Comp. Endocrinol., 4: 533-544.
- Kulkarni, G.K., Nagabhushanam, R. and Joshi, P.K. 1983. Neuroendocrine control of reproduction in the male penaeid prawn, *Parapenaeopsis hardwickii* (Miers) Crustacean, Decapoda, Penaeidae). Hydrobiologia., Volume 108: No. 3, pp 281-289.
- Kulkarni, G.K., R., Nagabhushanam. and P.K., Joshi. 1984. Neuroendocrine control of reproduction in the male penaeid prawn, *Parapenaeopsis hardwickii* (Miers) (Crustacea, Decapoda, Penaeidae) Hydrobiologia, vol.108, no:3, 281-289.
- Liu Hong, Kwok-Chu Cheung. and Ka-Hou Chu. 2008. Cell structure and seasonal changes of the androgenic gland of the mud crab, *Scylla paramamosain* (Decapoda: Portunidae). Zoological studies .,47(6): 720-732.
- Malo, N. and Jachault, P. 1970. Contribution a l'etude des variations ultrastructurales de la glande androgene des *Oniscoides superieus* (Crustaces Isopodes), a la suite de la decerebration. CR. Acad. Sci. Ser D 271: 230-232.
- Martin, G., O., Sorokine, M., Moniatte, P., Bulet, C., Hetru, and A., Van Dorsselaer. 1999. The structure of a glycosylated protein hormone responsible for sex determination in the isopod, *Armadillidium vulgare*. Eur. J. Biochem., 262:727-736.
- Miyawaki, M. and Taketomi, Y. 1978. The occurrence of an extended perinuclear space in androgenic gland of the crayfish, *Procambarus clarkii*. Cytologia. 43: 351-355.
- Okumura, T. and Hara, M. 2004. Androgenic gland cell structure and spermatogenesis during the molt cycle and correlation to morphotypic differentiation in the giant freshwater prawn, *Macrobrachium rosenbergii*. Zool., Sci., 21: 621-628.
- Okuno, A., Hasegawa, Y., Hiromichi Nagasawa. 1997. Purification and properties of androgenic gland Hormone from the terrestrial isopods, *Armadillidium vulgare*, in Zoological Science, 14(5): 837-842.
- Okuno, A., Hasegawa, Y., Ohira, T., Katakura, Y. and Nagasawa, H. 1999. Characterization and cDNA cloning of androgenic gland hormone of the terrestrial isopod *Armadillidium vulgare*. Biochem. Biophys. Res. Commun. 264: 419-423.
- Otsu, T. 1963. *Bihormonal control of sexual cycle in the freshwater crab, Potoman dehaani*. Embryologia, 81-20.
- Payen, G.G., Costlow, J.D. and Charniaux-Cotton, H. 1971. Etude comparative de l'ultrastructure des glandes androgenes de Crabes normaux et pedonculectomises pendant la vie larvaire ou apres le puberte chez les especes: *Rhithropanopeus harrisii* (Gould) et *Callinectes sapidus* Rathbun. Gen. Comp. Endocrinol., 17: 526-542.
- Payen, G. G. 1973. Etude descriptive des principales etapes de la morphogenese sexuelle chez un crustace decapode a developpement condense, l'Ecrevisse *Pontastacus leptodactylus*. (Eschscholtz, 1823). Ann. Embryol. Morph., 6: 179-206.
- Piera . S. Sun , Weatherby, T.M., Dunlap, M.F., Arakaki, K.L., Sacarias, D.T. and Malecha, S.R. 2000. Developmental changes in structure and polypeptide profile of the androgenic gland of the freshwater prawn, *Macrobrachium rosenbergii*. Aquacult. Int. 8: 327-334.
- Prasad, P.N. and B., Neelakantan 1989. Fecundity of the mud crab, *Scylla serrata* (Forsk.) Mahasagar, 22: 23-28.
- Qing.SU., ZHU Dong – fa., Yang Ji – fen. and QI Yi-zhou. 2010. Microstructure and Ultrastructure of Androgenic gland in swimming crab *Portunus trituberculatus*. Fisheries science., 04.
- Radhakrishnan, C.K. 1979. Studies on portunid crabs of porto Novo (Crustacea: Decapod: Brachyura) D Thesis, Annamalai University, India, pp: 129.
- Radu. V.Gh. and C.Craciun. 1976. The Ultrastructure of the Androgenic gland in *Porcellio scaber* Latr. (Terrestrial isopod) Cell and Tissue research by Springer Verlag., 175:245-263.

- Rangneker, P.V., Madhyastha, M.N. and Latey, A.N. 1971. Hormonal control of reproduction in the male crab, *Scylla serrata* (Forsk.). J. Anim. Morphol. Physiol., 18(1): 17-29.
- Revathi, P. P., Iyapparaj, L. Arockia Vasanthi, S. Jeyanthi, S. Sankaralingam, R. Ramasubburayan, S. Prakash. and M. Krishnan. 2013. Impact of Eyestalk Ablation on the Androgenic Gland Activity in the Freshwater Prawn *Macrobrachium rosenbergii* (De Man). World Journal of Fish and Marine Sciences 5 (4): 373-381.
- Rosen, O., Rivka Manor., Simy Weil., Eliahu, D., Aflalo., Anna Bakhrat., Uri Abdu. and Amir Sagi. 2013. An Androgenic gland membrane- anchored gene associated with the crustacean insulin- like androgenic gland hormone in *Cherax quadricarinatus*. J. Exp. Bio., jeb.biologists.org/ jeb.080523. abstract.doi: 10.1242.
- Sagi, A. 1988. The androgenic gland in crustacea—with emphasis on the cultured freshwater prawn *Macrobrachium rosenbergii*—a review. Israeli J. Aquacult. (Bamidgeh), 409-16.
- Sagi, A. and Isam Khalaila. 2001 . The Crustacean Androgen: A Hormone in an Isopod and Androgenic Activity in Decapods . American Zoologist 2001 41(3):477-484.
- Sarojini. R. and G Gyananath. 1985. Histochemical analysis of the androgenic gland cells showed the presence of cystines, protein bound amino acid groups in Androgenic gland of *Macrobrachium lamerrii*. Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 94: No.5, pp 503-508.
- Sheen, S.S. and L.R. D' Aramo. 1991. Response of juvenile freshwater, *Macrobrachium rosenbergii*, to different levels of cod liver/corn oil mixture in a semi-purified diet. Aquaculture, 93: 121-134.
- Taketomi, Y. 1986. Ultrastructure of the androgenic gland of the crayfish, *Procambarus clarkii*. Cell. Biol. Int. Rep. 10: 131-136.
- Taketomi, Y., Murata, M. and Miyawaki, M. 1990. Androgenic gland and secondary sexual characters in the crayfish, *Procambarus clarkii*. J. Crust. Biol., 10: 492-497.
- Taketomi, Y. and Nishikawa, S. 1996. Implantation of androgenic glands into immature female crayfish, *Procambarus clarkii*, with masculinization of sexual characteristics. J. Crust. Biol., 16: 232:239.
- Taketomi, Y., Nishikawa, S. and Koga, S. 1996. Testis and androgenic gland during development of the external sexual characteristics of the crayfish, *Procambarus clarkii*. Journal of Crustacean Biology, 16: 24-34.
- Thirunavukkarasu, N. 2005. Biology, Nutritional evaluation and utilization of mud crab *Scylla tranquebarica* (Fabricius, 1798). PhD Thesis, Annamalai University, India, pp: 126.
- Touir, A. 1977. Fonnes nouvelles concernant l'endocrinologie sexuelle des Crustaces Decapodes Natantia hermaphrodites et gonochoriques. I. Maintien des glandes androgenes et role de ces glandes dans le controle des gametogeneses et des caracteres sexuels externes males. Bull. Soc. Zool. Fr., 102: 375-400.
- Ventura, T., Manor, R., Aflalo, E.D., Weil, S., Raviv, S., Glazer, L. and Sagi, A. 2009. Temporal silencing of an androgenic gland-specific insulin-like gene affecting phenotypical gender differences and spermatogenesis. J. Endocrinology. 150 (3): 1278-1286.
- Ventura, T., Manor, R., Aflalo, E.D., Weil, S., Khalaila, S., Rosen, O. and Sagi, A. 2011. Expression of an androgenic gland-specific insulin-like peptide during the course of prawn sexual and morphotypic differentiation. ISRN Endocrinology. Vol. 2011: 11 pages.
- Zhang, Y. H., Y., Xu, J., Zhang. and R. H., Lu. 2000. Separation and identification of isopoda AGH analogue in *Macrobrachium rosenbergii*. Acta Hydro. Sinica, 24:167-171.