Carotenoids and retinoids metabolites as precursors of receptors-specific bioactive compounds. Advances in shrimp[1]

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ABSTRACT: Additional efforts need to be devoted to understanding of the bioactive forms of non-produced de novo metabolites such as carotenoids and their derived metabolites, retinoids in crustaceans. Dietary carotenoids are the sole biological precursos of retinoids in animal systems. The results obtained in survival, gonadosomatic and hepatosomatic index in treatments with carotenoids and retinol, demonstrated the importance of carotenoids to yield retinal and retinol needed for significicantly better gonad development. A rough correlation exists between the rate of retinal formation and the biological activity of the administered carotenoids. The presence of 9-cis and 13-cis retinol in reproductive tissue of *P. vannamei* shrimp points-out their role in the RXR receptors due to the previously reported function of these isomers in the binding domains fo genes which induces growth and development by controlling the production of local morphogenic signals by a nuclear receptor signaling pathway.

KEY WORDS: bioactive additives, carotenoids, retinoids, shrimp

INTRODUCTION

The use of bioactive substances such as nutritional additives to improve the yields of cultured shrimp is a topic that has received relatively little attention. Feeding of cultured marine crustaceans is limited to artificial diets lacking bioactive metabolites considered important for growth and survival of shrimp. These conditions may lead to nutritional imbalances, deficiencies and diseases. Few studies, however have investigated the dietary pigments as precursors of highly bioactive molecules involving biological functions in shrimp. Carotenoid function in crustaceans has normally been ascribed to pigmentation, antioxidant functions, and as a source of dietary pro-vitamin A (Meyers and Latscha, 1997). The bioactive roles of carotenoids in crustaceans requires further research in order to clarify the functions of these pigments (other than for pigmentation). Dietary carotenoids in shrimp are derived primarily from the intake of carotenoids. Perhaps the best known function for certain carotenoids in animal systems is their ability to be metabolically converted to biologically active compounds called retinoids (Brody, 1999). Some evidence suggests that the bioconversion roles of these metabolites into retinoids occurrs in shrimp. Retinoids are a class of molecules derived from vitamin A considered crucial for many growth, developmental, and homeostatic processes in the vertebrates (Mangelsdorf *et al*. 1995), but the functions of retinoids in the Penaeidae is unclear. Studies in a number of vertebrate systems argue that at physiological concentrations, retinoids such as retinoic acid induces growth and development by controlling the production of local morphogenic signals by a nuclear receptor signaling pathway (Duester, 1996). Concerning crustaceans studies must be undertaken in order to help define wheter effect attributed to the carotenoids are due to their vitamin A activity or are an intrinsic property of the carotenoid molecule itself. The properties of retinoids as hormonal compounds through their effects on

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hormonal nuclear receptors could have important implications for successful aquaculture of economically important crustaceans, such as shrimp.

We are interested in examining the roles of carotenoids as precursors of retinoids and their possible effect on their nuclear receptors during the maturation process of *Penaeus vannamei.*

MATERIAL AND METHODS.

Farmed (reproductively exhausted broodstocks) *Penaeus vannamei* in gonadic condition II and IV were injected with different concentration of carotenoids and retinoids (Table 1). Once attained the maturation, organisms were collected throughout the experimental period. Carotenoids and retinoids characterization and quantification was undertaken in reproductive tissues of shrimp.

Carotenoids were extracted from the crude homogenate with acetone/methanol (2:1) and treated according to Schiedt *et al.* (1993). In all the cases, analysis were carried-out under dim light and using HPLC grade solvents. Polar cartenoids were fractionated by DMSO. The hexane phase was saponified with KOH in ethanol (10 %). The non polar carotenoids were extracted in ether/hexane (1:1). Pure analytical standards of carotenoids and retinoids as well as initial measurements were check-out and scanned in a diode-array spectrophotometer (Hewlett-Packard, HP 8453). Retinoids were analyzed according to the method proposed by Napoli and Horst (1989). Tissues were homogenate in a buffer solution at 4 ^{o}C (0.5 % ascorbic acid, 0.5 mM EDTA in PBS buffer at 7.3 pH). Retinoids was extracted in ethyl acetate:methyl acetate (8:1) and in butilaldehyde hydroxytolueno). Supernatant phase was resuspended in 50 ml methanol. Analysis of retinoids was carried-out by high performance liquid chromatography in a Waters HPLC (370 nm in a silica column).

Analysis of the presence of retinoids isomers associated to functional receptors was carried-out and related to shrimp organ, mainly in ovaries.

RESULTS AND DISCUSSION

As seen in table 1, higher survival, gonadosomatic and hepatosomatic index were registered in *P. vannamei* subjected to retinol palmitate and slightly lower values were obtained in treatments with astaxanthin and beta-carotene. The treatment based on retinoic acid gives the lower survival percentage as well as the lower response in the hepatosomatic and gonadosomatic index.

Table I. Effects of carotenoids and retinoids in survival, gonadosomatic and hepatosomatic index.

Treatment	Dose (µg/g)	Survival (%)	GSI (%)	HSI (%)
β–carot	18.6	100	2.51±0.19	2.16±0.16
asta	18.6	100	3.05±0.34	2.23±0.22
RA 1	133.0 *	89	0.98±0.34	2.13±0.06
RA 2	266.0 *	64	1.40±0.51	2.27±0.04
RA 3	400.0 *	64	0.99±0.23	2.12±0.34
RP 1	5.3	100	3.24±0.42	2.24±0.57
RP 2	10.6	100	3.69±0.49	2.42±0.45
RP 3	16.0	100	4.70±0.46	2.24±0.43
Control		100	1.70±0.33	2.40±0.38

* ng/g

When analyzing retinoids effects in ovaries, important changes were noticed according to the level of oocyte development (Fig 1). The level IV of oocyte maturation when comparing to the level II (Fig. 1), shows a higher profile and concentrations of isomeric forms of retinal and retinol, mainly the 9-cis and 13-cis isomers. At the same time, carotenoids pathways response was activated as observed from the esterified and free astaxanthin increases.

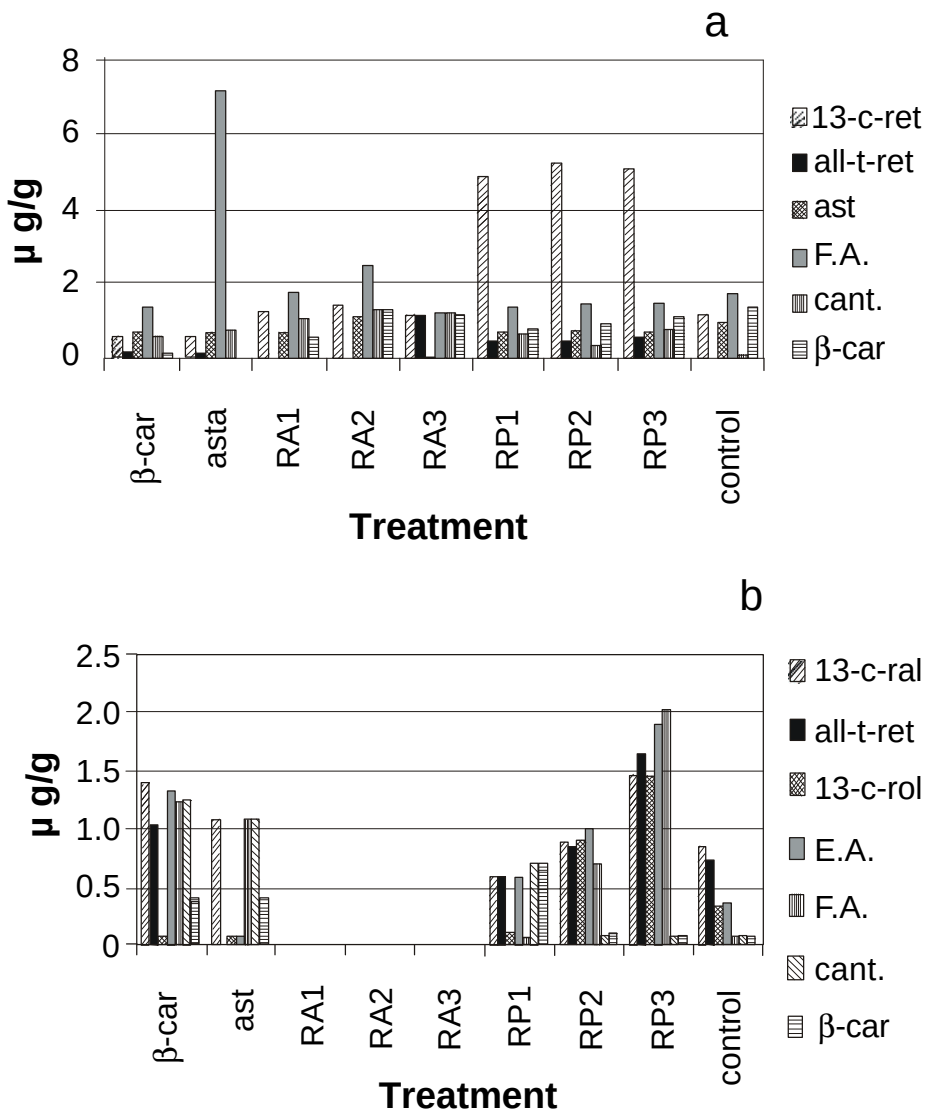


Fig. 1. Carotenoids and retinoids profile in ovary of *P. vannamei* at level II (a) and IV (b) of maturation**.**

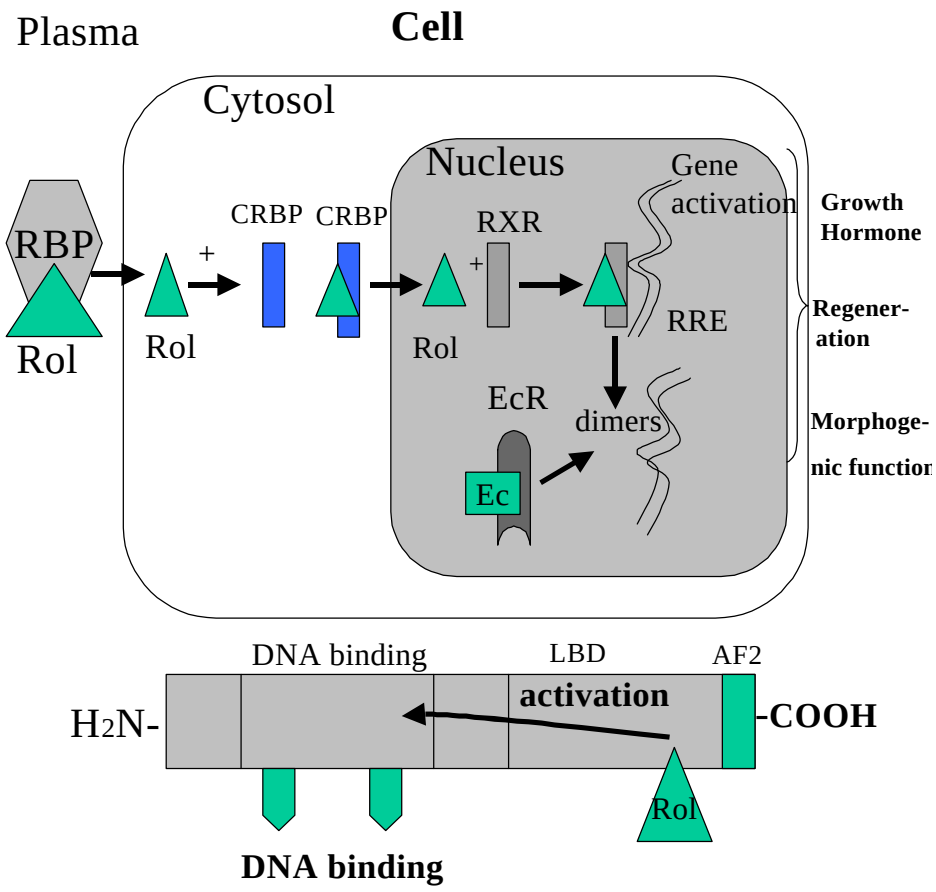
The positive effects of retinoids in shrimp can be related to the reported role of this metabolite, essential for normal health and life functions, such as growth, development, and reproduction in animals. However, few applications on this topic exist in marine invertebrates (Alava *et al*. 1993; Takeuchi *et al*. 1998). Recent findings on the roles of retinoids as nuclear hormonal receptors activating genes can be associated to the obtained results in this work.

However, we can suggest that both carotenoids and retinoid forms possess the bioactivity required for the enhancement of basic physiological process, such as the observed improvement of gonadosomatic and hepatosomatic index.

It appears that the carotenoids were sufficient to maintain survival; however for significantly better gonad development, both retinol and carotenoids (beta-carotene and astaxanthin) are necessary. These

findings demonstrate that vitamin A is an essential nutrient for the gonadal development of *Penaeus vannamei*. The negative effects of retinoic acid were first noticed by the unusual white color of shrimp exoesqueleton as a consequence of altered metabolic pathway of carotenoids and intermediary retinoids biosynthesis. We believe that the applied dose was higher than the physiological concentration needed to activate bioactive functions. In this way, retinoic acid could exert an inhibitory instead a bioactive effect at the level of ovaries as evidenced from the poor survival and significantly reduced gonadosomatic index. This in fact could be antagonic with the normal pathways of other natural precursor metabolites such as carotenoids. It has been reported that the mechanism of action of retinoic acid (the active derivative of vitamin A) is closely similar to that of steroid hormones and thyroxine, involving activation of the expression of specific genes, and thus placing retinoids in the category of hormones regulating growth, differentiation, and embryonic development. (Wolf, 1990). Alava *et al*. (1995) reported that crustaceans build-up vitamin A reserves during maturation, which are then transferred to the oocytes.

It is clear from recent studies that vitamin A (retinol) regulates growth, development, and epithelial maintainance in vertebrates by conversion to an active form, retinoic acid (Lakshman *et al.* 1993). Few references on this subject exist in crustaceans. Durica *et al*. (1999) reported the expression of the nuclear receptors genes encoding the ecdysteroid receptor at early stages of blastemal development by exposure of *Uca pugilator* to retinoids. Our hypothesis is that retinol isomeric forms are playing a similar role in shrimp functioning as a ligand controlling a nuclear receptor-signaling pathway. Two families of retinoid receptors have been identified, i.e., the retinoic acid receptor (RAR), and the retioid X receptor (RXR) families. Since we were unable to detect retinoic acid, and we registered isomers of retinol most probable receptors of this kind of family were associated to active functions in ovaries. In most instances it appears that the active receptor in an RAR/RXR heterodimer which binds DNA regulatory sequences and regulates gene transcription in response to ligand binding (Mangelsdorf *et al*., 1995). RAR binds all-trans-retinoic acid and the closely related isomers 9-cis-retinoic acid, whereas RXR binds only 9-cis isomers, suggesting that it may play a different role in retinoid signaling than RAR (Petkovich, 1992). Fig. 2, illustrates the functions of retinoids as components of nuclear hormonal receptors and the derived role of retinoids in the transcriptional activation domain of the gene. In support to this theory, important improvementes in *Penaeus vannamei* yields are expected as a consequence of positive responses in growth hormone and activation of morphogenic and embryogenesis functions. Our understanding of how retinol is physiologically activated to form the ligand for this signaling pathway is being corroborated in experiments *in vitro* and *in vivo.*



RBP =retinol binding protein; Rol = retinol; CRBP= cell retinoid binding protein; RXR= retinoid X receptor; AF2 = ligand-dependent trascriptional activation domain; RRE =retinoid response element; ECR = ecdysteroid receptor ; Ec =acdysteroid;LBD = ligand binding dominain.

Fig. 2. Schematic diagram of working hypothesis on the genomic actions of retinal presumed in shrimp.

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