Chemical composition of carotenoprotein from Penaeus sp.

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Abstract

Carotenoids are exogenously derived isoprenoid compounds which are responsible for pigmentation in crustaceans. While implants and bacteria are able to synthesize these carotenoids de novo, shrimps like other animals entirely depend on their dietary supply. The carotenoprotein found in most crustacean tissues and responsible, e.g. for the typical colour of penaeids (Penaeus spp.) is a combination of an apoprotein and a carotenoid pigmnet. With increasing industrialization in shrimp farming there is a growing demand for synthetic, nature-identical carotenoids, not only for pigmentation,but also for the maintenance of growth and fertility. In this work we have tried to establish the composition of carotenoprotein in shrimp exoskeleton.

Keywords: Carotenoprotein, carotenoids, astaxanthin, shrimps exoskeleton.

Introduction

Interactions between carotenoids and proteins are of great physiological importance in living organisms. The carotenoids are usually much more stable ***in vivo*** than when they are isolated, and can be transported and function in a predominantly aqueous environment. In photosynthetic systems, the carotenoids are located, with chlorophylls or bacteriochlorophylls, in pigment-protein complexes to ensure the precise positioning and orientation that are essential for efficient energy transfer. In these complexes, the electronic structure of the carotenoid chromophore shows little change [1]. This work concentrates on the carotenoprotein, which are commonly found in marine invertebrate animals and are responsible for green, purple or blue colours, in contrast to the yellow, orange or red of the free carotenoid. A familiar and striking example is provided by the shrimps (*Penaeus spp*). which, when alive, is slate-blue in colour of the exoskeleton but, on cooking, becomes bright orange-red[2]. The red colour is due to the carotenoid (astaxanthin or canthaxanthin) *which* is liberated when the cooking process denatures the natural blue pigment of the carapace, the carotenoprotein complex[3].

Experimental section

The test material was shrimp (Penaeus spp.) which was purchased from the local market. All materials were washed under tap water. The exoskeleton of shrimps was removed, weighed and used immediately for further processing. The samples were homogenized in a laboratory blender for 2 minutes at 1500 r.p.m. with distilled water to increase the surface area for efficient enzyme treatment. From 500 g of raw shrimps were obtained 100 g of fresh skinned material (exoskeleton). The homogenized material was placed in a large beaker with 60 mL of sodium citrate solution 1%. Pretreated and homogenized materials were stored at 5^0 C for 24 h. After this time the solution was removed and replaced with 100 mL of EDTA buffer (pH=6,8) and mixed in a magnetic stirrer for 6 hours. The homogenate was vacuum filtered through on Whatman no 42 filter obtaining about 40 mL of a green-blue colour solution. From that solution was measured the electronic spectra (UV-VIS) on a Cary-50 spectrofotometer(fig.1). In figure 2 is presented the corresponding interval for carotenoid absorbance.

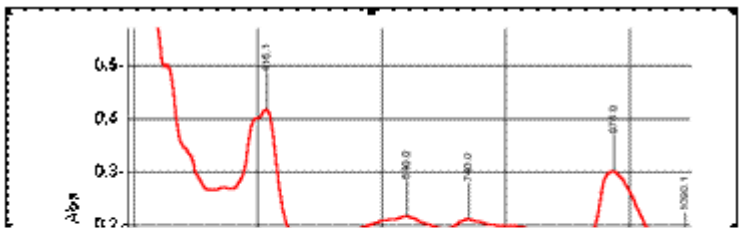


Figure 1. Electronic spectra of carotenoprotein solution (pH=6,8)

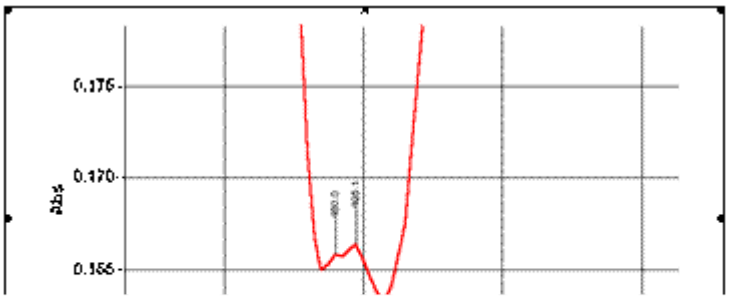
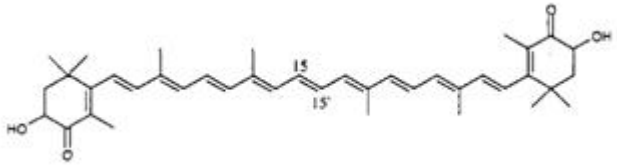


Figure 2. The spectral width between 450-550 nm for carotenoid absorbance

For extraction and identification of carotenoid, 50 g of fresh shrimp was hydrolyzed with CH_3COOH normal solution for 30 min. After this time 20 g of homogenate were extracted in a Soxhlet continue with CH_2Cl_2 : metanol (2 :1) for 2 h. The cooled solution was kept over night at 4^0 C to eliminate the presence of sterols. The pigment solution was used for IR (fig. 4) and UV-VIS (fig. 5) spectral analysis. The identity of pigment was confirmed by the presence of carboxylic groups and by comparison with a standard solution. The both comparison of spectra (UV-VIS and IR) indicate the presence of astaxanthin. The thin layer chromatography show that there are no other pigments in the extract.



(3,3'-dihydroxy-P,P-carotene-4,4'-dion*(eI)*)

Figure 3. Chemical structure of astaxanthin

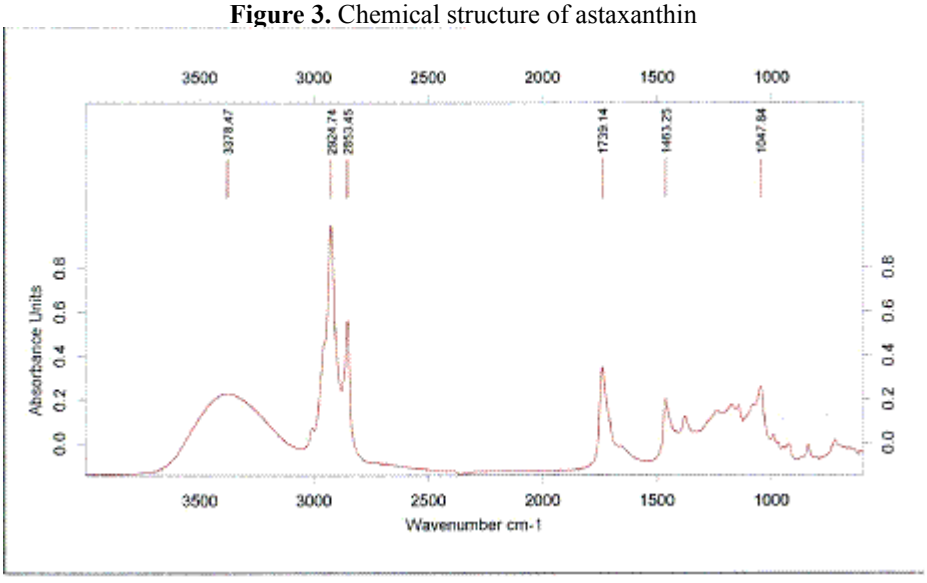


Figure 4. IR spectrum of astaxanthin (KBr pell.)

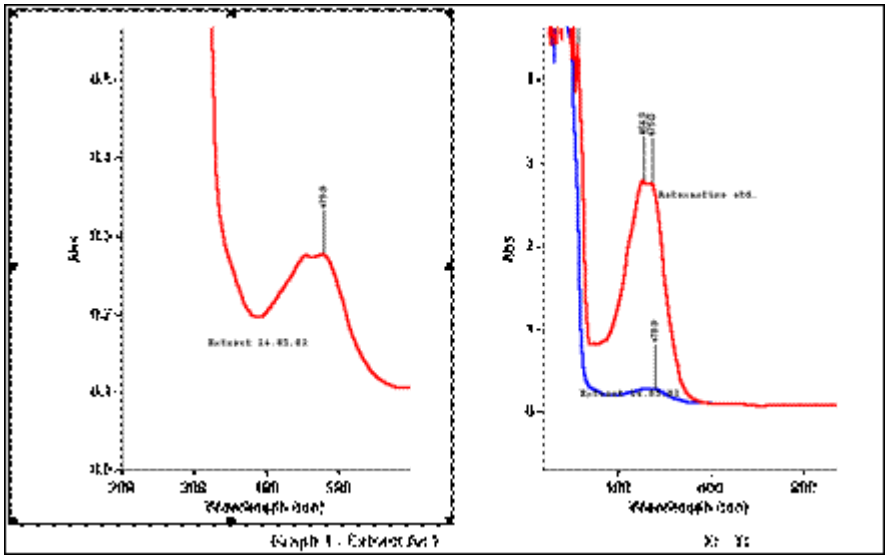


Figure 5. The comparative UV-VIS spectra of astaxanthin (left –standard astaxanthin ; right-astaxanthin from carotenoprotein compared with standard)

For extraction and caracterization of the apoprotein, a small fraction of buffer extract was colected and was examined by (polyacrylamide gel electrophoresis) PAGE. The fraction was hydrolyzed in the presence of trypsine (Fluka) with a 9000 U/mg activity during 1 h at 25^0 C and pH=7.6. The solution was than filtered on a Millipore filter (50 kDa) under vacuum to obtain the protein separation. Separation by electrophoresis was made in a minicell in polyacrylamide gel (10% with 3.3.mL acrylamide 30%) 1,5 mm. The samples was located in site A for test solution, site B –an molecular weight marker (Biorad) and the caotenoprotein previously extracted in site C. The composition of weight marker is presented in table 1.

Table 1. The composition of weight marker (Biorad)

Marker	MW(kDa)
Myozine	209.0
ß-galactosidaze	130.0
Bovine serum albumine	87.0
Carbonic anhydraze	43.9
Trypsin inhibitor (soy)	32.1
lisozime	18.1

The migration buffer was composed from Tris-Glicin (Sigma) pH=8,3. After 30 min. 70 V (60 mA, 3 W) the separation by electrophoresis was made for 110 min. at 120 V and 80 mA. The PAGE minigel was treated with Coomasie Blue 1% 30 min and then with EtOH : CH_3COOH (40% :10%) for 4 h. The coresponding bands were scanned by densitometry (Unscanit) and are presented in figure 6.

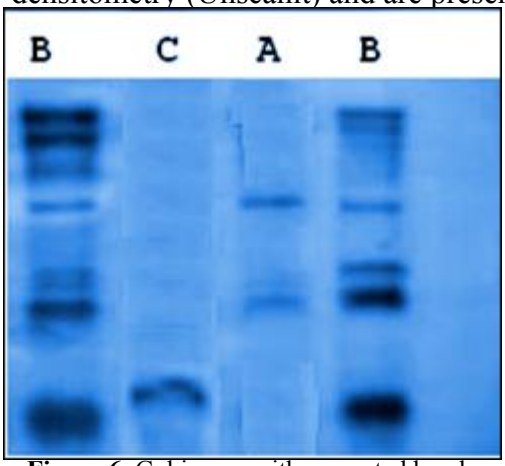


Figure 6. Gel image with separated bands

Results and Discussions

The carotenoprotein from shrimp (*Penaeus spp.)* contain astaxanthin as a pigment and a protein unit as apoprotein. The carotenoprotein, as whole was interpreted based on the Rf values AT 220 kDa and is presented

in table 2.

Table 2. The Rf values for carotenoprotein and the corresponding subunits

SAMPLE	R_f	MW (kDa)
Carotenoprotein	0.125	220
Subunit 1	0.581	118
Subunit 2	0.610	45

The protein from complex was separated by PAGE and shows to have two subunits, one with 118 kDa and the second with 45 kDa.

References

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