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Studies on carotenoproteins in animals. X. *Euphausia superba* Dana 1852 (*Crustacea, Euphausiacea*)

ABSTRACT: Carotenoprotein compounds containing astaxanthin as a prosthetic group were isolated from the body of the Antarctic krill — *Euphausia superba*.

Key words: Antarctic, *Euphausia superba*, carotenoproteins, amino acids

1. Introduction

In the search for animal protein resources, to meet the growing needs of the mankind, attention of the researchers is paid to krill, a swarming crustacean, *Euphausia superba* that occurs in masses in the Antarctic waters.

There are various publications treating on that crustacean and viewing the matter under different aspects: biological studies (Dzik and Jażdżewski 1968, El-Sayed and Hampton 1980, George 1980, Makarov and Maslennikov 1981), analyses of micro- and macro-elements in krill (Kikuchi and Kano 1975), oxygen consumption (Rakusa-Suszczewski and Opaliński 1978), studies on vitamin B_{12} (Hirano 1964), prostaglandins (Mężykowski and Ignatowska-Świtalska 1981) and various carotenoids found in krill (Czeczuga and Kłyszejko 1977, 1978, Czerpak, Jackowska and Mical 1980, Jackowska, Czerpak and Mical 1980).

Euphausia superba contains large amounts of ketocarotenoids and among them astaxanthin, which gives this crustacean its red colour. As it is well known (Zagalsky 1976, Britton et al. 1982) astaxanthin often forms in crustaceans complexes with protein giving pigmentation to the body of these animals and their carapaces in particular.

It was therefore of some interest to find out whether astaxanthin, the dominant carotenoid in *Euphausia superba* is the prosthetic group of

the carotenoprotein complexes and what is amino acid composition of the protein in the carotenoprotein complex present in this crustacean.

2. Material and methods

The material for analyses consisted of 250 g of homogenized specimens (whole body) of *Euphausia superba* caught during the 1978 Antarctic Expedition on the r/v "Profesor Siedlecki". The material was collected near King George Island during the Antarctic springtime and kept in cold storage (−10°C).

The material was dissected, homogenized and centrifuged under refrigeration in a Janetzki K-24 centrifuge and suspended in EDTV solution. The carotenoprotein complexes were precipitated with ammonium sulphate (Zagalsky, Ceccaldi and Daumas 1970). The precipitate was centrifuged again and dissolved in 0.05M phosphate buffer (pH 7). After an overnight dialysis, also under refrigeration, in presence of phosphate buffer (pH 7) the material was centrifuged once more and then it was purified by means of the ion-exchange chromatography with a DEAE-cellulose carrier. Elution was made with phosphate buffer (pH 7) using linear concentration gradient of 0.02 — 0.35 M. The measurements of extinction in the eluent were taken in the range of 300—800 nm, using a "Spectromom" spectrophotometer Model 203.

Ketocarotenoid was identified as the prosthetic group of carotenoproteins examined by means of the thin-layer chromatography of the extracted carotenoid, alone or admixed with a ketocarotenoid (astaxanthin) standard (Hoffman-La Roche and Co. Itd., Basle), on the thin-layer of silica gel-G with 15% acetone in petroleum ether (Zagalsky, Cheesman and Ceccaldi 1967).

Carotenoids were liberated from carotenoproteins with acetone (Shone, Britton and Goodwin 1979).

Samples for the analysis of the amino acid composition were prepared after the methods described by Zagalsky, Cheesman and Ceccaldi (1967). They were hydrolyzed for 36 hrs, at the temperature of 110°C.

Amino acids were separated in a two-column system, using a JEOL JLC-6 AH automatic amino acids analyser, under the standard conditions recommended by the makers (JEOL Instructions, Tokyo).

The columns were filled with ICR-2 resin, separation temperature −52°C, 0.8 ml samples of the material being used for the analyses. The speed of flow of buffer solutions was 25.2 ml/hr and of the aninhidrine dye 12.6 ml/hr.

The alkaline amino acids (Lys, His, Arg) were separated in 8 × 150 mm column, in 0.35 Na-citrate buffer solution, at pH 5.28, under the pressure of approximately 8 atm.

The acid and neutral amino acids were separated on 8 × 500 mm column, in buffer solution No. 2:0.2 n Na-citrate, at pH 4.2, under the pressure of approximately 20 atm.

The results were calculated on the basis of the data from a two-channel integrator. The standard amino acid solution, produced by Pierce Ltd., U.S.A., was used.

The carotenoids found in the examined material were determined with the method described earlier (Czeczuga and Krywuta 1981a).

Carotenoid pigments were extracted with 96% acetone in a dark room. Saponification was made with 10% KOH in ethanol, at temperature of about 20°C, for 24 hrs, in nitrogenous atmosphere in a dark chamber.

The column and thin-layer chromatography, described in detail in an earlier paper (Czeczuga and Krywuta 1981a), were used for the analysis of various carotenoids.

Pigments were identified by the following methods: a) behaviour in the column chromatography; b) adsorption spectra of the pigments in various solvents recorded in a Beckmann spectrophotometer Model 2400 DU; c) partition characteristic of the carotenoid between hexane and 95% methanol; d) comparison of the R_f values in the thin-layer chromatography; for identification of β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and astaxanthin, co-chromatography with identical carotenoids was used (Hoffman-La Roche, Basle); e) presence of allylic hydroxyl groups as determined with acid chloroform; f) epoxide test.

Quantitative determinations of the concentrations of carotenoid solutions were made by means of the quantitative adsorption spectra. These determinations were based on the extinction coefficient, $E^{cm}_{1\%}$, at the wavelength of the maximum absorption in petroleum ether or hexane.

3. Results

By means of the ion-exchange chromatography, a carotenoprotein complex of a yellowish colour was isolated from the examined material. In ammonium sulphate this carotenoprotein complex gave two adsorption maxima, this first at 498 nm and the second at 710 nm (Fig. 1).

The carotenoid isolated from the carotenoprotein complex gave only one adsorption maximum in various solvents, e.g. in petroleum ether at 468 nm, in ethanol at 478 nm, in acetone at 480 nm, at benzene at 482 nm. The epi-hypophase ratio of this carotenoid was 20:80. This indicates that astaxanthin is a ketocarotenoid of the prosthetic group in the carotenoprotein complex. It was also confirmed by the thin layer chromatography with an astaxanthin standard (Hoffman-La Roche and Co. Ltd., Basle). Astaxanthin predominates among carotenoids identified in the *Euphausia superba* material (Table I Fig. 2).

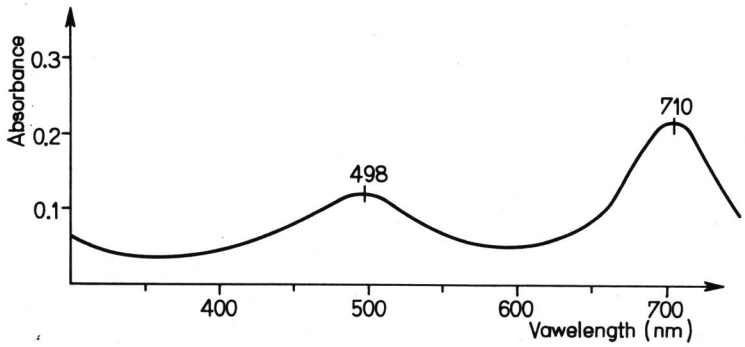

Fig. 1. Absorption spectrum of carotenoproteins from *Euphausia superba* (in 0.05 M phosphate buffer, pH 7)

Table I.

Carotenoids in *Euphausia superba*

Carotenoid	Structure (see Fig. 2)	Semisystematic name	Content (in %)
β — carotene	A — X — A	β,β — carotene	0.8
β — cryptoxanthin	A — X — B	β,β — caroten — 3 — ol	13.5
canthaxanthin	D — X — D	β,β — carotene — 4,4′ — dione	23.4
lutein	B — X — C	β,ε — carotene — 3,3′ — diol	2.6
zeaxanthin	B — X — B	β,β — carotene — 3,3′ — diol	12.0
astaxanthin	E — X — E	3,3′ — dihydroxy — β,β — carotene — — 4,4′ — dione	42.5
flavoxanthin	C — Y — F	5,8 — epoxy — 5,8 — dihydro — β,ε — — carotene — 3,3′-diol	5.2
Total amount in μg/g of fresh weight material			72.5

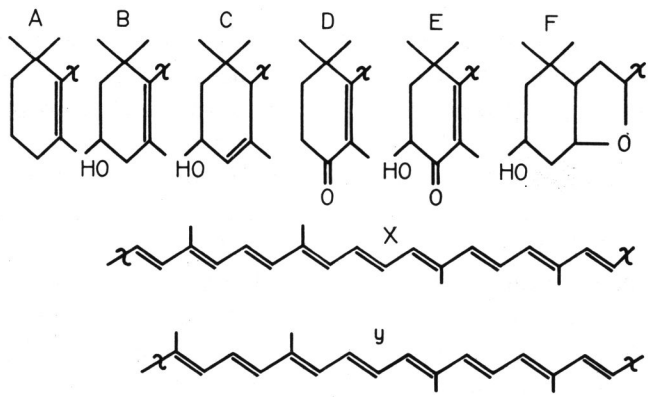

Fig. 2. Structural features of carotenoids from *Euphausia superba* specimens

Table II.

Amino acid composition of the protein moiety of *Euphausia superba*

Amino acid	Amount (mole%)
Lys	9.10
His	1.66
Arg	3.90
Asp	10.88
Thr	3.77
Ser	4.98
Glu	19.37
Pro	5.19
Gly	7.03
Ala	11.38
Cys/Cys	trace
Val	5.94
Met	5.88
Ileu	3.86
Leu	8.81
Tyr	1.33
Phe	2.15

The amino acid composition of the protein forming with astaxanthin the carotene-protein complex is given in Table II. This protein contains mostly glutamine, alanine, and asparagine. The comparatively very high content of glutamine — 19.37 mole is of particular interest; cysteine, tyrosine, and histidine occured in extremely small quantities.

4. Discussion

As described by other authors (Fisher, Kon and Thompson 1955, Herring 1972, Czeczuga and Kłyszejko 1977, 1978, Ržavskaja and Meniaeva 1981) the dominant carotenoid in the crustaceans was astaxanthin or another ketocarotenoid (Aakagava and Kayama 1975). It has been stablished that astaxanthin is the prosthetic group in the carotenoprotein complexes in marine crustaceans, such as: *Homarus gammarus* (Cheesman, Lee and Zagalsky 1967, Zagalsky 1982); *Plesionika edwardsi* (Zagalsky, Cheesman and Ceccaldi 1967); *Acanthephyra* sp., *Gigantocypris* sp. *Lepas* sp. and *Pollicipes polymerus* (Zagalsky 1976). Astaxanthin enters also into the composition of carotenoprotein complexes in numerous fresh-water crustaceans (Czeczuga and Krywuta 1981a-c, Czeczuga 1982), such as: *Orconectes limosus, Gammarus lacustris* and *Eudiaptomus amblyodon.*

The analysis of the amino acid composition of the protein, which forms with astaxanthin the carotenoprotein complex isolated from the *Euphausia superba* material, shows that this protein contains mainly glutamine. Similar

observations were reported by other authors. Glutamine was found to be the dominant amino acid of the protein of the carotenoprotein complexes in *Homarus gammarus, Cancer pagurus* (Zagalsky and Herring 1972), *Pagurus bernhardus, Pagurus prideauxi* and in individuals of the *Lepas* sp. (Zagalsky 1976), and *Eriphia spinifrons* (Zagalsky, Ceccaldi and Daumas 1970), whereas cysteine occurred in extremely small quantities, just as in the *Euphausia superba* material.

The question arises what biological significances the presence of the carotenoprotein complex in *Euphausia superba* may have. The present studies show that, apart from the carotenoprotein complex of a yellowish colour isolated from the examined material, the dominant carotenoid in *Euphausia superba* is astaxanthin which gives to this crustacean its red colour. Studies on the protein-astaxanthin complexes have shown that they may be blue or red in colour; both, these colours protect the crustaceans from the excessive light radiation (Herring 1967, 1972, 1973). This in turn enables *Euphausia superba* to feed in the upper layers of the waters during the Antarctic spring and summer, i.e. in the water layers where large quantities of phytoplankton are to be found.

5. Резюме

Из особей крыля *Euphausia superba*, собранного у берегов Антарктиди, изолирован каротинопротеиновый комплекс жёлтоватого цвета, содержащего астаксантин в виде простетической группы.

Белковая часть этого комплекса содержала 17 аминокислот, среди которых доминировали глютамин, аланин, аспаргин, лизин, а также леуцин, а в самых небольших количествах присутствовали цистеин, тырозин, а также — гистидин.

Автор анализирует биологическое значение этого каротинопротеинового комплекса у особей *Euphausia superba*.

6. Streszczenie

Z osobników kryla *Euphausia superba* z wód Antarktyky wyizolowano kompleks karotenoproteinowy o odcieniu żółtawym, którego grupą prostetyczną okazał się ketokarotenoid — astaksantyna.

Część białkowa tego kompleksu zawierała 17 aminokwasów, wśród których w największych ilościach wystąpiły glutamina, alanina, aspargina, lizyna oraz leucyna, zaś w minimalnych ilościach — cysteina, trozyna oraz histydyna.

Autor omawia znaczenie biologiczne tego kompleksu karotenoproteinowego u osobników *Euphausia superba*.

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