Folia biologica (Kraków), vol. **53** (2005), No 3-4

Carotenoids and Carotenoproteins in *Asellus aquaticus* L. (Crustacea: Isopoda)

Bazyli CZECZUGA, Ewa CZECZUGA-SEMENIUK and Adrianna SEMENIUK

Accepted September 6, 2005

CZECZUGA B., CZECZUGA-SEMENIUK E., SEMENIUK A. 2005. Carotenoids and carotenoproteins in *Asellus aquaticus* L. (Crustacea: Isopoda). Folia biol. (Kraków) **53**: 109-114.

Column (CC), thin-layer (TLC), high-performance liquid (HPLC) and ion-exchange chromatography (IEC), were used to investigate corotenoid and carotenoprotein complexes in *Asellus aquaticus* specimens from the Narew river. The following carotenoids were found: α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, diadinoxanthin, mutatoxanthin, crustaxanthin, echinenone, hydroxyechinenone, phoenicoxanthin, canthaxanthin and astaxanthin. Astaxanthin (37.5%), canthaxanthin (21.4%) and phoenicoxanthin (12.3%) were found in the largest amounts. The total carotenoid content was 13.824 μg g^{-1} of dry mass. Carotenoprotein complexes containing astaxanthin as the prosthetic group were purified from *Asellus aquaticus*. The carotenoprotein complexes belonged to the crustacyanins group as α- and γ-crustacyanin. The protein forming the α-crustacyanin contained large amounts of such amino-acids as asparic acid, glutamic acid and leucine, whereas the protein of the γ-crustacyanin contained primarily glutamic acid, glycine and lysine.

Key words: Crustacea, Isopoda, carotenoids, carotenoproteins, crustacyanins, amino acids.

Bazyli CZECZUGA, Department of General Biology, Medical University, Kilińskiego 1, 15-089 Białystok, Poland.
bazzylio@poczta.onet.pl
Ewa CZECZUGA-SEMENIUK, Department of Gynecological Endocrinology, Medical University, M. Skłodowskiej-Curie 24a, 15-276 Białystok, Poland.
Adrianna SEMENIUK, Student of Medical Faculty, Medical University, Kilińskiego 1, 15-089 Białystok, Poland.

Asellus aquaticus is one of the commonest Isopoda crustaceans in European waters. The carapace of this crustacean is of a grey colour which suggests the presence of a carotenoprotein complex.

Studies concerning carotenoprotein complexes in marine representatives of Isopoda of the *Idothea* genus (LEE 1966a,b; LEE & GILCHRIST 1972) and of the *Mesidotea* genus (CZECZUGA 1996) showed that they contain carotenoprotein complexes, the prosthetic group of which does not consist of astaxanthin, as in other such complexes in other crustacean species, but of a different ketocarotenoid, canthaxanthin.

It was in view of this, and the fact that no investigations of carotenoprotein pigments in fresh-water representatives of the Isopoda have been carried out, that this study was undertaken in the hope that the results would enrich our knowledge of these complexes in the Isopoda.

Material and Methods

Asellus aquaticus L. individuals (approx. 100 g of dry weight) were collected in May and June of 1999 from the river Narew near Suraż. The material was kept in the freezer compartment of a refrigerator until removed for chromatographic analysis.

Investigation of carotenoids

The presence of respective carotenoids in *Asellus aquaticus* L. specimens was determined using column chromatography (CC), thin-layer chromatography (TLC) with different systems of solvents (CZECZUGA 1984a) and high-performance liquid chromatography (HPLC). Prior to chromatography, the material was homogenized with acetone under nitrogen in dark glass bottles and the extracts kept in a refrigerator until analysis. Saponification was carried out with 10% KOH in ethanol at 20^{o} C for 24 h in the dark under nitrogen. Column and thin-layer chromatography (CZECZUGA 1984a; KRAUS & KOCH 1996) were used to separate the carotenoids, which were identified by comparison with standard compounds by (a) the behaviour on column chromatography; (b) their UV-VIS spectra (Beckman 2400 spectrophotometer); (c) their

partition between *n*-heksane and 95% ethanol; (d) their R_f-values on thin-layer chromatography; (e) the presence of allylic OH-groups determined by the acid $CHCL_3$ test; (f) the epoxide test and (g) the mass spectrum (cf. VETTER *et al.* 1971). Carotenoid pigments were also determined by ion – pairing, reverse – phase HPLC. To 1000 μl of the clear extract, 300 μl of ion – pairing reagent was added according to MANTOURA & LLEWELLYN (1983). The HPLC equipment consisted of a Shimadzu LC – 6A double – system pump, driven by a gradient programmer Shimadzu SCL-6B and Rheodyne 7125 injector equipped with a 20 μl loop. Detection was performed by a Shimadzu SPD – 6AV UV – VIS spectrophotometric detector set on 440 nm and Shimadzu RF – 535 fluorescence detector.

Carotenoid pigment standards were obtained from Hoffman – La Roche Company, Switzerland, International Agency for ^{14}C Determinations, Denmark and Sigma Chemical Company, USA.

Quantitative determinations were performed by UV, VIS spectroscopy according to the Davies method (CZECZUGA 1985). For the structures of carotenoids see STRAUB (1987) and CZECZUGA (1988).

Investigation of carotenoproteins

The material was dissected, homogenized and centrifuged under refrigeration in a Janetzki K-24 centrifuge and suspended in EDTV solution. The carotenoprotein complex was precipitated with ammonium sulphate (ZAGALSKY *et al.* 1970). The precipitate was centrifuged again and dissolved in 0.05 M phosphate buffer (pH 7.0). After an overnight dialysis, also under refrigeration, in the presence of phosphate buffer (pH 7.0), the material was centrifuged once and then purified by means of ion-exchange chromatography with a DEAE-cellulose carrier. Elution was made with phosphate buffer (pH 7.0) using a 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 "Spectroma" spectrophotometer Model 203.

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

Carotenoids were liberated from carotenoproteins with acetone (SHONE *et al.* 1979).

Samples for the analysis of the amino acid composition were prepared after the methods described by ZAGALSKY *et al*. (1967). They were hydrolyzed for 36 hrs, at a temperature of 110°C.

Amino acids were separated on 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 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 0.33 Na-citrate buffer solution, at pH 5.28, under the pressure of approximately 8 atm.

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

Table 1

Carotenoids (%) in the body of *Asellus aquaticus* (total content 13.824 μg g^{-1})

Carotenoid	Summary Formula	Structure (see Fig. 1)	Semisystematic name	%
1. α-carotene	$C_{40}H_{56}$	A - R - B	β,ε-Carotene	0.8
2. β-carotene	$C_{40}H_{56}$	A - R - A	β,β-Carotene	3.7
3. β-cryptoxanthin	$C_{40}H_{56}O$	A- R - C	β,β-Caroten- 3-ol	4.1
4. lutein	$C_{40}H_{56}O_2$	C - R - D	β,ε-Carotene-3,3'-diol	2.7
5. zeaxanthin	$C_{40}H_{56}O_2$	C - R - C	β,β-Carotene-3,3'-diol	2.8
6. mutatoxanthin	$C_{40}H_{56}O_3$	F - R_1 - G	5,8-Epoxy-5,8-dihydro-β,β-carotene-3,3'-diol	0.8
7. crustaxanthin	$C_{40}H_{56}O_4$	H - R - H	Trans-β,β-carotene-3,4,3',4'-tetrol	6.5
8. diadinoxanthin	$C_{40}H_{54}O_3$	E - R_1 - G	5,6-Epoxy-7',8'-didehydro-5,6-dihydro-β,β-carotene-3,3'-diol	2.4
9. echinenone	$C_{40}H_{54}O$	A - R - I	β,β-Caroten-4-one	1.2
10. hydroxyechinenone	$C_{40}H_{54}O_2$	A- R - K	3-Hydroxy-β,β-caroten-4-one	3.8
11. phoenicoxanthin	$C_{40}H_{52}O_3$	I - R - K	3-Hydroxy-β,β-caroten-4-,4'-dione	12.3
12. canthaxanthin	$C_{40}H_{52}O_2$	I - R - I	β,β-Carotene-4,4'-dione	21.4
13. astaxanthin	$C_{40}H_{52}O_4$	K - R - K	3,3'-Dihydroxy-β,β-carotene-4,4'-dione	37.5

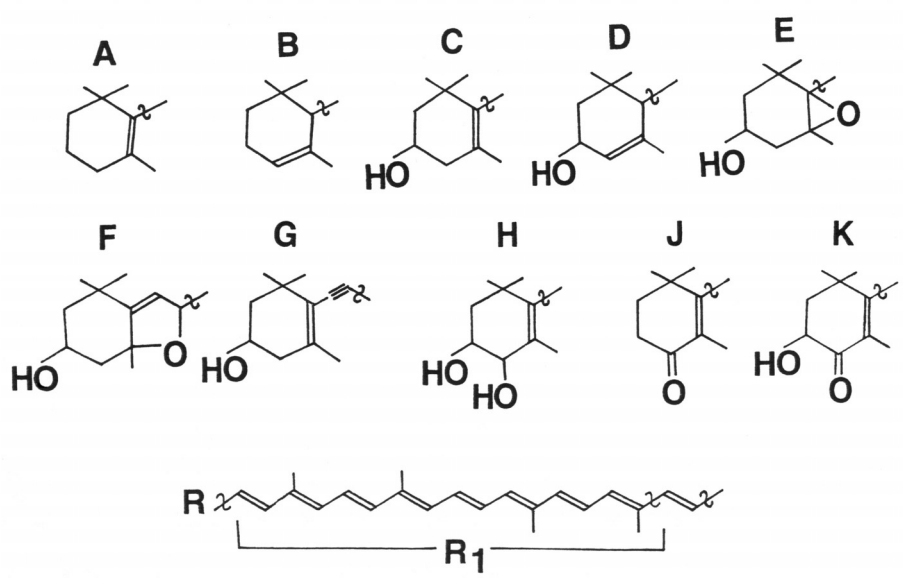

Fig. 1. Structural features of carotenoids from investigated materials (see Table 1).

Table 2

Amino acid composition of the carotenoproteins of *Asellus aquaticus*

Amino acid	Amount in mol %	
	α-crustacyanin	γ-crustacyanin
Lysine	5.45	8.52
Histidine	3.07	2.06
Arginine	5.11	6.24
Asparic acid	12.78	3.48
Threonine	5.79	2.24
Serine	4.77	5.01
Proline	5.96	2.47
Glutamic acid	10.22	12.63
Glycine	6.98	8.92
Alanine	7.16	6.12
Valine	7.50	4.19
Cystine/Cystine	1.19	0.84
Methionine	1.17	3.07
Isoleucine	5.62	4.26
Leucine	8.18	6.18
Tyrosine	3.41	1.86
Phenylalanine	5.62	4.11

Table 3

Comparison of composition (mol %) of carotenoproteins in some species of Isopoda

Amino acid sets	*Asellus aquaticus**	*Idothea resecata*	*Mesidotea entomon*
Apolar residues (A): Val, Leu, Ileu, Phe, Met	25.0	27.3	26.4
Polarity index (P): sum of values of Asp, Thr, Ser, Glu, Lys, His, Arg	43.7	46.0	50.2
P/A ratio	1.75	1.70	1.90
Small amino acids: Ala, Gly	14.6	15.2	14.3
Charged amino acids: Asp, Glu, Arg, Lys	32.3	31.1	30.4

* mean for sum of α-crustacyanin and γ-crustacyanin

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

Results

Chromatographic analysis revealed the presence of 13 carotenoids in *Asellus aquaticus* individuals, with the largest amounts of astaxanthin, canthaxanthin and phoenicoxanthin. The total carotenoid content was 13.824 $\mu g\ g^{-1}$ of dry mass (Table 1, Fig. 1).

A carotenoprotein complex was isolated from specimens of *Asellus aquaticus* which, in a phosphate buffer at pH 7.0, gave two fractions, absorption maxima first fraction at 608 and the second fraction at 636 nm. The carotenoid separated from these fractions was identified as astaxanthin. The proteins of the first fraction contained large amounts of amino-acids such as asparic acid, glutamic acid and leucine. Considerable amounts of valine, alanine and glycine were also found. Methionine and cysteine constituted the smallest proportions. Proteins of the second fraction contained large amounts of glutamic acid, glycine and lysine (Table 2).

Discussion

Three basic groups of carotenoproteins can be distinguished in aquatic animals. The first group includes blue-coloured crustacyanins, a combination of carotenoid and protein found mainly in crustacean armour. BUCHWALD and JENCKS (1968) demonstrated that crustaceans contain three types of crustacyanine: α-, β-,and γ-, all with astaxanthin as the prosthetic group. Presently it is known that the prosthetic group of crustacyanins may include other ketocarotenoides such as canthaxanthin and phoenicoxanthin (CZECZUGA & CZECZUGA-SEMENIUK 1998). Crustacyanins can be found both in marine crustacean species and fresh water ones. α-Crustacyanine was detected in *Eudiaptomus amblyodon* and *Palaemon adspersus*, β-crustacyanine in *Gammarus lacustris* and *Palaemon adspersus* and γ-crustacyanine in *Crangon crangon* and *Cyclops kolensis* (CZECZUGA 1997; CZECZUGA *et al.* 2000). Carotenoproteines such as crustacyanines were also found in other marine (*Euphausia superba, Mesidotea entomon*) and fresh water (*Chirocephalus diaphanus*, *Daphnia magna*, *Orconectes limosus*) crustacean species (ZAGALSKY *et al.* 1983; CZECZUGA 1984a,b; 1996; CZECZUGA & KRYWUTA 1981a; CZECZUGA & CZECZUGA-SEMENIUK 1998). The second group contains ovoverdins, a combination of carotenoid and glycolypoprotein, which gives a green pigmentation to crustacean eggs and ovaries. The third group consists of red ovorubins, a combination of carotenoid and glycoprotein, found in the albumen gland and shellfish eggs (CHEESMAN *et al.* 1967; KE 1971; ZAGALSKY 1976; LEE 1977; NAKAGAWA 1978; BRITTON *et al.* 1982; RENSTRØM *et al.* 1982; GOODWIN 1984). According to ZAGALSKY *et al.* (1990) and KEEN *et al*. (1991), the large molecule of the carotenoprotein complex consists of a number of smaller protein subunits, each cup-shaped with edges formed by a coiled protein chain, in which a pigment (astaxanthin) molecule may be attached to the hydrophobic part of the protein and situated on the cup bottom. Apart from grey, green and a variety of red shades, the most common colour is blue, an effect of crustacyanins, particularly α-crustacyanin. A blue colour of carotenoproteids due to crustacyanins also occurs in marine species of coelenterates (ZAGALSKY & HERRING 1977; ZAGALSKY & JONES 1982), starfish (CZECZUGA 1983; CLARK *et al.* 1990) and Tunicata representatives (HERRING 1978).

α-Crustacyanine is more common in crustaceans (HOISCHEN *et al.* 1998). The same individual may contain more than one protein-carotenoid complex simultaneously: red (MILICUA *et al.* 1985), pink (GOMEZ *et al.* 1988), yellow (ZAGALSKY 1982; MILICUA *et al*. 1986 a,b) and blue (GARATE *et al.* 1984, RIVAS *et al.* 1988). This also refers to the eggs of *Cyclops vernalis* females. The colour can be grey, brown, green, purple, blue and even black (DU PRAW 1958). Different proportions of these protein-carotenoid complexes result in various colours of crustacean armour. A greyish colour due to a carotenoprotein complex in isopods produces protective coloration to environments in which they live (LEE 1966a,b; CASTILLO *et al.* 1982; CZECZUGA 1996).

In view of the absorption maxima of the carotenoprotein complex isolated from *Asellus aquaticus*, it seems that two varieties of the same complex occur in these specimens. The literature (JENCKS & BUTEN 1964; BUCHWALD & JENCKS 1968; CHEESMAN *et al.* 1966) indicates that absorption maxima at 608 and 636 nm are characteristic of crustacyanin. This type of crustacyanin has already been noted in the fresh-water crustacean, *Gammarus lacustris* (CZECZUGA & KRYWUTA 1981b). The absorption maximum at 608 nm indicates the presence of γ-crustacyanin in *Asellus aquaticus*, whereas the absorption maximum at 636 nm indicates α-crustacyanin. The absorption maximum of γ-crustacyanin is within 603-616 nm. Absorption maxima approaching 636 nm were noted by other authors in studies of other crustaceans. CECCALDI & ALLEMAND (1964a,b) iso-

lated a carotenoprotein complex with maximum absorption within the range of 625-635 nm from *Homarus gammarus* individuals and from the shrimp *Aristeus antennatus* (CECCALDI and ZAGALSKY 1967). In both cases, the authors identified this complex as α-crustacyanin, with astaxanthin as the prosthetic group.

In this study the main maximum was at 636 nm, it can be assumed that this crustacyanin was the main component of the complex. It would therefore seem more advisable to compare the amino acid composition of the protein in the complex contained in the *Asellus aquaticus* with that of α-crustacyanin of other crustaceans. According to BUCHWALD and JENCKS (1968), the protein of α-crustacyanin from *Homarus gammarus* consisted mainly of asparic acid, glutamic acid and tyrosine. In the material examined in this study, the first two constituted the greatest proportions of the amino acid composition whereas tyrosine, on the other hand, constituted one third of the quantity of asparic- and glutamic acids. It should be emphasized that in eight species of crustaceans belonging to various systematic classes, the tyrosine content in protein of the carotenoprotein complexes was found to be approximately 3 mol% (ZAGALSKY 1976). Furthermore, in certain fresh-water crustaceans in which crustacyanin was determined, tyrosine was found to constitute 1.80-2.42 mol% (CZECZUGA & KRYWUTA 1981a,b,c).

When the proteins of the complexes isolated from *Asellus aquaticus* are compared with those obtained from other species of Isopoda (Table 3), it was found that the P/A ratio and the smaller index are the same as those noted in *Idothea resecata* (LEE 1977) and *Mesidotea entomon* (CZECZUGA 1996). On the other hand, the apolar index and the polarity index and sum of values of charged amino acids are specific for *Asellus aquaticus* specimens.

Unlike that of the marine representatives of the Isopoda, the carotenoprotein complex of *Asellus aquaticus* contained an astaxanthin prosthetic group. It should be noted that astaxanthin was found to occur in large amounts in the *Asellus aquaticus* throughout a one year cycle of investigations concerning the presence of various carotenoids on a population in the River Narew (CZECZUGA 1975). Astaxanthin is also the dominant carotenoid in land crustaceans belonging to isopods (CZECZUGA 1975).

References

BRITTON G., ARMITT M., LAU S. Y. M., PATEL A. K., SHONE C. C. 1982. Carotenoproteins. (In: Carotenoid Chemistry and Biochemistry. G. Britton & T.W. Goodwin eds. Pergamon Press, Oxford, New York): 237-251.

BUCHWALD M., JENCKS W. P. 1968. Properties of the crustacyanins and the yellow lobster shell pigment. Biochemistry **7**: 844-859.

CASTILLO R., NČGRE-SADARGUES G., LENEL R. 1982. General survey of the carotenoids in Crustacea. (In: Carotenoid Chemistry and Biochemistry, G. Britton, T.W. Goodwin eds. Pergamon Press, Oxford, New York): 211-224.

CECCALDI H. J., ALLEMAND H. 1964a. Carotenoproteides. I-Spectres d'absorption dans le visible des caroténoprotéides du homard *Homarus gammarus* (L.) Rec. Trav. Mar. End. Bull. **32/48**: 59-64.

CECCALDI H. J., ALLEMAND H. 1964b. Carotenoproteides. III-Extraction du pigment bleu de la carapace du homard *Homarus gammarus* (L.) Rec. Trav. Mar. End. Bull. **35/51**: 3-7.

CECCALDI H. J., ZAGALSKY P. F. 1967. Studies on the carotenoprotein from the stomach wall of *Aristeus antennatus*. Comp. Biochem. Physiol. **21**: 435-438.

CHEESMAN D. F., LEE W. L., ZAGALSKY P. F. 1967. Carotenoproteins in invertebrates. Biol. Rev. **42**: 132-160.

CHEESMAN D. F., ZAGALSKY P. F., CECCALDI H. J. 1966. Purification and properties of crustacyanin. Proc. Roy. Soc. **164B**: 130-151.

CLARK R. J. H., RODLEY G. A., DRAKE A. F., CHURCH R. A., ZAGALSKY P. F. 1990. The carotenoproteins of the starfish *Linckia laevigata* (Echinodermata: Asteroideae): a resonance Raman and circular dichroism study. Comp. Biochem. Physiol. **95B**: 847-853.

CZECZUGA B. 1975. The presence of carotenoids in the "centipede" *Oniscus asellus* (Crustacea:Isopoda). Zool. Pol. **24**: 223-228.

CZECZUGA B. 1983. Carotenoprotein complexes in four species of Echinodermata from the Adriatic Sea. Biochem.System. Ecol. **11:** 123-125.

CZECZUGA B. 1984a. Carotenoprotein and carotenoids in *Daphnia magna* Straus.Pol. Arch. Hydrobiol. **31**: 91-97.

CZECZUGA B. 1984b. Studies on carotenoproteins in animals. *Euphausia superba* Dana 1852 (Crustacea, Euphausiacea). Pol. Polar. Res. **5**: 121-127.

CZECZUGA B. 1985. Carotenoids in representatives of the Cladoniaceae. Biochem. Syst. Ecol. **13**: 83-88.

CZECZUGA B. 1988. Carotenoids (In: Handbook of Lichenology, vol.3. M. Galun ed. CRC Press, Boca Raton, Florida): 25-34.

CZECZUGA B. 1996. Carotenoprotein complexes in *Mesidotea entomon* (L.) (Crustacea:Isopoda) from Baltic sea. Folia biol. (Kraków) **44**: 73-77.

CZECZUGA B. 1997. Crustacyanins in two species of shrimp from the Baltic sea. Folia biol. (Kraków) **45**: 79-82.

CZECZUGA B., CZECZUGA-SEMENIUK E. 1998. Carotenoprotein complexes in *Chirocephalus diaphanus* Prevost (Crustacea:Anostraca). Folia biol. (Kraków) **46**: 197-201.

CZECZUGA B., KOZŁOWSKA M., CZECZUGA-SEMENIUK E. 2000. Adaptative role of carotenoids and carotenoproteins in *Cyclops colensis* Lilljeborg (Crustacea:Copepoda) specimens to extremely eutrophical conditions. Folia biol. (Kraków) **48**: 77-84.

CZECZUGA B., KRYWUTA S. 1981a. The presence of carotenoproteins in the carapace of *Orconectes limosus* (Raf.). Comp. Biochem. Physiol. **68B**: 339-343.

CZECZUGA B., KRYWUTA S. 1981b. Presence of crustacyanins in *Gammarus lacustris* G.O. Sars. Comp. Biochem. Physiol. **70B**: 665-667.

CZECZUGA B., KRYWUTA S. 1981c. Blue astaxanthin-proteins of *Eudiaptomus amblyodon*. Biochem. Syst. Ecol. **9**: 339-340.

DU PRAW E. J. 1958. Analysis of eggs color variation in *Cyclops vernalis*. J. Morph. **103**: 31-63.

GARATE A. M., URRECHAGA E., MILICUA J. C. G., GOMEZ R., BRITTON G. 1984. A blue carotenoprotein from the carapace of the crab *Carcinus maenas*. Comp. Biochem. Physiol. **77B**: 605-608.

GOODWIN T. W. 1984. Carotenoid-protein complexes. (In: The Biochemistry of Caroptenoids, vol. 2. Animals. T. W. Goodwin ed. Chapman and Hall, London): 1-21.

GOMEZ R., MANZANO I., GARATE A. M., BARBON P. G., MACARULLA J. M., MILICUA J. C. G. 1988. A purple carotenoprotein from the carapace of *Galatea strigosa.* Comp. Biochem. Physiol. **90B**: 53-57.

HERRING P. J. 1978. A blue carotenoprotein, containing an unusual chromophore, isolated from *Salpa cylindryca* (Tunicata:Salpidae). Comp. Biochem. Physiol. **61B**: 391-393.

HOISCHEN D., COLMENARES L. U., LIU J., SIMMONS C. J., BRITTON G., LIU R. S. H. 1998. Fluorinoprotein analogs of the carotenoprotein, α-crustacyanin. Bioorg. Chem. **25**: 365-372.

JENCKS W. P., BUTEN B. 1964. The denaturation of crustacyanin. Arch. Biochem. Biophys. Acta **107**: 511-520.

KE B. 1971. Carotenoproteins. (In: Methods in Enzymology A. S. Pietro ed. Academic Press, New York): 624-636.

KEEN J. N., CACERES I., ELIOPOULOUS E., ZAGALSKY P. F., FINDLAY J. B. 1991. Complete sequence and model for the A_2 subunit of the carotenoids pigment complex, crustacyanin. Eur. J. Biochem. **197**: 407-417.

KRAUS L., KOCH A. 1996. Dünnschichtchromatographie. Springer Verlag, Berlin.

LEE W. L. 1966a. Pigmentation of the marine isopod *Idothea montereyensis* (Maloney). Comp. Biochem. Physiol. **18**: 17-36.

LEE W. L. 1966b. Pigmentation of the marine isopod *Idothea glanulosa* (Rathke). Comp. Biochem. Physiol. **19**: 13-27.

LEE W. L. 1977. Carotenoproteins in animal coloration. Dowdens, Hutchinson and Ross, Stroudsburg.

LEE W. L., GILCHRIST B. M. 1972. Pigmentation, color change and the ecology of the marine isopod *Idothea resecata* (Stimpson). J. Exp. Mar. Biol. Ecol. **10**: 1-27.

MANTOURA R. F. C., LLEWELLYN C. A. 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography. Anal. Chim. Acta **151**: 297-314.

MILICUA J. C. G., ARBERAS I., BARBON P. G., GARATEA A. M., GOMEZ R. 1986a. A yellow carotenoprotein from the carapace of the crayfish *Astacus leptodactylus*. Comp. Biochem. Physiol. **85B**: 615-619.

MILICUA J. C. G., GARATE A. M., BARBON P. G., GOMEZ R. 1986b. Relatedness between α-crustacyanin from the lobster *Homarus americanus* and the blue carotenoprotein from the crayfish *Procambarus clarkii.* Comp. Biochem. Physiol. **85B**: 621-626.

MILICUA J. C. G., GOMEZ R., GARATE A. M., MACARULLA J. M. 1985. A red carotenoprotein from the carapace of the crayfish, *Procambarus clarkii*. Comp. Biochem. Physiol. **81B**: 1023-1025.

NAKAGAWA H. 1978. Carotenoproteins. (In: Carotenoids of Aquatic Animals, Japan Soc. Sci. Fish. Koseisha-Koseikaku Ed., Tokyo): 90-107.

RENSTRØM B., RØNNEBERG H., BORCH G., LIAAEN-JENSEN S. 1982. Further studies on the carotenoproteins crustacyanin and ovoverdin. Comp. Biochem. Physiol. **71B**: 249-252.

RIVAS J. D. L., MILICUA J. C. G., GOMEZ R. 1988. Futher studies on the blue carotenoprotein from *Astacus leptodactylus*. Comp. Biochem. Physiol. **89B**: 65-68.

SHONE C. C., BRITTON G., GOODWIN T. W. 1979. The violet carotenoprotein of the starfish *Asterias rubens*. Comp. Biochem. Physiol. **62B**: 507-513.

STRAUB O. 1987. Key to carotenoids. Birkhauser Verlag, Basel.

VETTER W., ENGLERT G., RIGASSI N., SCHWIETER U. 1971. Spectroscopic methods. (In: Carotenoids, O. Isler ed. Springer Verlag, Basel): 189-266.

ZAGALSKY P. F. 1976. Carotenoid-protein complexes. Pure appl. Chem. **47**: 103-120.

ZAGALSKY P. F. 1982. A study of the yellow astaxanthin-proteins of lobster carapace. Comp. Biochem. Physiol. **71B**: 243-247.

ZAGALSKY P. F., CECCALDI H. J., DAUMS R. 1970. Comparative studies on some crustacean carotenoproteins. Comp. Biochem. Physiol. **34**: 579-607.

ZAGALSKY P. F., CHEESMAN D. F., CECCALDI H. J. 1967. Studies on carotenoid-containing lipoproteins isolated from the eggs and ovaries of certain marine invertebrates. Comp. Biochem. Physiol. **22**: 851-871.

ZAGALSKY P. F., ELIOPULOS E. E., FINDLAY J. B. C. 1990. The architecture of invertebrate carotenoproteins. Comp. Biochem. Physiol. **97B**: 1-18.

ZAGALSKY P. F., GILCHRIST B. M., CLARK R. J. H., FAIRCLOUGH D. P. 1983. The canthaxanthin-lipovitellin of the brine shrimp, *Artemia* (Crustacea, Anostraca): a resonance Raman and circular dichroism study. Comp. Biochem. Physiol. **74B**: 647-652.

ZAGALSKY P. F., HERRING P. J. 1977. Studies of the blue astaxanthin-proteins of *Velella velella* (Coelenterata: Chondrophora). Philos. Trans. R. Soc. London **299B**: 289-326.

ZAGALSKY P. F., JONES R. 1982. Quaternary structures of the astaxanthin-proteins of *Velella velella*, and of β-crustacyanin of lobster carapace, as reyealed in electron microscopy. Comp. Biochem. Physiol. **71B**: 237-242.