CAROTENOIDS IN THE CELLS OF THE ALGA *TRENTEPOHLIA GOBII* MEYERBAZYLI CZECZUGA¹, OLEG M. MAXIMOV²¹Department of General Biology, Medical Academy,
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ABSTRACT

Column-, and thin-layer chromatography revealed the presence of the following carotenoids in the cells of the species *Trentepohlia gobbii* Meyer from cobbles in the river Urmi in the Badzhal mountains of Khabarovsk Territory in the Far East: β , β -carotene, β , ϵ -carotene, β , β -carotene-2-ol, β , ϵ -carotene-2-ol, β , β -carotene-2, 2'-diol, 5,6-epoxy-5,6-dihydro- β , β -carotene-2-ol, 5,6-epoxy-5,6-dihydro- β , ϵ -carotene-2-ol and 5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro- β , β -carotene-2,2'-diol.

KEY WORDS: algae, *Trentepohlia*, carotenoids.

INTRODUCTION

Algae, representatives of the family *Trentepohliaceae* belonging to the *Chlorophyceae*, present numerous peculiarities. Some of the species lead a free mode of life, while others constitute a phycobiontic part of lichen (Ahmadijan 1967). Algae of the genus *Trebouxia* together with those of the *Trentepohlia* occur as phycobionts in approximately 70% of lichen species. About 60 free-living species of the genus *Trentepohlia* are usually found on wet soil, rocks, tree bark and leaves in tropical and subtropical countries (Starmach 1963). However, in a moderate climate only few species of algae of this genus have been reported (Kadłubowska 1975).

The cells of the *Trentepohlia* species combine by means of plasmodesmes, the phenomenon which is uncommon among the *Chlorophyceae*. It was observed at the end of the previous century already (Zopf 1892) that these cells contain a large number of carotenoids which give the cells of free-living species orange or even reddish colour.

The carotenoid contents has been investigated exclusively in the cells of two species of the genus *Trentepohlia*: *T. aurea* and *T. iolitus*. It has appeared that β , ϵ - and β , β -carotenes stain *Trentepohlia aurea* cells red (Tischer 1936, Jeremias 1963, Czygan and Kalb 1966). The studies of the *Trentepohlia iolitus* cells have found specific and rare carotenoid such as β , β -carotene-2-ol and β , ϵ -carotene-2-ol (Kjlsen et al. 1972, Buchecker et al. 1974 and Nybraaten et al. 1974a, b).

In the mountains of Asia, *Trentepohlia gobbii* Meyer can be quite frequently observed on rocks and stones on the borderline of land environment and aquatic environment. It gives the banks of streams and rivers specific orange and red colouring.

The present study is concerned with carotenoid content in the cells of *Trentepohlia gobbii* collected from cobbles in the river Urmi in the Badzhal mountains in the Far East.

MATERIALS AND METHODS

The cells of *Trentepohlia gobbii* Meyer were collected in July 1991 from cobbles in the river Urmi in the Badzhal mountains of Khabarovsk Territory in the Far East (Russia).

The cells were cleaned of all organic debris, placed in dark glass bottles and macerated with acetone. The air above the fluid in the bottle was replaced with nitrogen to ensure an anaerobic atmosphere. Samples were kept in a refrigerator until analysed for their carotenoid content by column and thin-layer chromatography. Carotenoid pigments were extracted with 95% acetone in a dark room. Saponification was carried out with 10% KOH in ethanol at about 20°C for 24h in the dark, in a nitrogen atmosphere.

Column and thin layer chromatography, described in detail by Czczuga (1980), were used to separate various carotenoids. These were identified by performing replicate chromatography with standard carotenoids (Hoffman-La Roche and Co. Ltd., Basel, Switzerland, and Sigma Company, USA), except epoxy and diepoxy carotenoids. Pigments were identified on the basis of: (a) their behaviour on column chromatography; (b) their absorption spectra in various solvents (Beckman spectro-photometer model 2400); (c) their partition between hexane and 95% methanol; (d) their R_f values (TLC); (e) the presence of allylic hydroxyl groups, determined by the acid-chloroform test; (f) the epoxide test, and (g) the mass spectrum of end groups (see Vetter et al. 1971 for basic

methodology). Concentration of carotenoid solutions were determined from the absorption spectra, on the basis of the extinction coefficient $E\ 1\% \text{ cm}^{-1}$ at the wavelengths of maximal absorbance in petroleum ether or hexane (Davies 1976). The structures of the carotenoids have been reported previously by Straub (1987).

RESULTS

Eight carotenoids were detected in the cells of *Trentepohlia gobic* (Table 1); worth special noting is the finding of β , β -carotene-2-ol, β , ϵ -carotene-2-ol and a few derivatives. One of the derivatives of β , β -carotene-2-ol found in the studies – 5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro- β , β -carotene-2,2'-diol has never been observed in the cells of other species of the genus *Trentepohlia* and in general. The *Trentepohlia gobic* cells studied contained proportionally largest amounts of β , β -carotene (37.8%) and its derivative β , β -carotene-2-ol (30.4%). 5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro- β , β -carotene-2,2'-diol constituted only 1.5% of all the carotenoids.

DISCUSSION

At the end of the previous century, it was thought that red-dish colour is given to the cells of certain species of the genus *Trentepohlia* by carotenoids (Zopf 1892), such as β , ϵ -, β , β -carotene, zeaxanthin and not fully identified xanthophylls (Karrer and Jucker 1948). Later studies confirmed only the presence of the carotenes, while zeaxanthin was not found (Jeremias 1963). The studies, using the modern chromatographic methods, apart from the carotenes mentioned above, in the cells of *Trentepohlia iolithus* revealed the presence of xanthophylls, new to the genus *Trentepohlia*, such as β , β -carotene-2-ol, β , ϵ -carotene-2-ol, β , β -carotene-2,2'-diol and epoxy forms of the first two mentioned (Kjlsen et al. 1972, Buchecker et al. 1974, Nybraaten et al. 1974a, b). These are rare carotenoids in the world of flora (Goodwin 1980, Straub 1987). As it is well known, xanthophylls originate from carotenes mainly through the addition of hydroxyl group to carbon in the position 5 and 5' of ionone ring. In the case of carotene-2-ols, the addition of hydroxyl group occurs

Fig. 1. Structural features of carotenoids from investigated materials.

at the position 2 and 2'. In this lies the peculiar nature of carotenes and their derivatives (Fig. 1 – E, F).

Various shades of red cells of algae caused by the presence of a large number of certain carotenoids have already been described. Goodwin and Jamikorn (1954), Droop (1955), Santos and Mesquita (1984) observed intensive colouring of the *Haematococcus pluvialis* cells. The authors suggested that the accumulation of large amounts of astaxanthin in the cells of this alga is promoted by a small amount of nitrogen in the environment and by intensive insolation. This assumption was confirmed by Czygan (1970) who observed blood-rain and blood-snow due to the red cells of *Haematococcus pluvialis* and *Chlamydomonas nivalis* containing large amounts of astaxanthin. According to Boussiba and Vonshak (1991), the accumulation of large amounts of astaxanthin in the cells of *Haematococcus pluvialis* is significantly influenced by stressful environmental factors. Astaxanthin also cause red colouring of the *Euglena rubida* cells which in turn induce red flourishing of water (Czeczuga 1974), and its the main carotenoid in some of sea algae, e.g. *Acetabularia mediterranea* (Czeczuga 1986d). Red colouring of alga cells of certain species was also observed due to the accumulation of large amounts of β , β -carotene (Aasen et al. 1969), which was found, among others, in the cells of *Dunaliella* genus (Ben-Amotz et al. 1989, Borowitzka 1993, Vorst et al. 1994). The

TABLE 1. List of carotenoids from investigated cells of *Trentepohlia gobic* (total content $38,422\mu\text{g}^{-1}$ dry wt.)

Carotenoid	Scture (see Fig. 1)	Absorbion maximum (nm)	Partition coefficient	Rf	Infrared absorption spectrum (KBr pallet cm^{-1})	% of total content
β , β -carotene	A–R–A	425,451,476a	epiphase	0.95	–	37.8
β , ϵ -carotene	A–R–B	420,443,472e	epiphase	0.98	–	13.5
β , β -caroten-2-ol	A–R–C	430,452,479a	86:14	0.68	1070,1040 (OH)	30.4
β , ϵ -caroten-2-ol	B–R–C	425,446,475a	86:14	0.70	1070,1037 (OH)	5.1
β , β -caroten-2,2'-diol	C–R–C	425,452,479a	12:88	0.59	1070,1030 (OH)	0.6
5,6-epoxy-5,6-dihydro- β , β -caroten-2-ol	A–R–D	423,443,472e	hypophase	0.42	–	6.7
5,6-epoxy-5,6-dihydro- β , ϵ -caroten-2-ol	B–R–D	419,441,470e	hypophase	0.44	–	4.4
5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro- β , β -caroten-2,2'-diol	D–R–D	418,442,466e	hypophase	0.21	–	1.5

a – acetone, e – ethanol

following question arises: Of what biological significance is the accumulation of such a large number of carotenoids in the cells of certain algae, including species of the genus *Trentepohlia*?

The data obtained up to the present have indicated that carotenoids in algae act, on the one hand, as additional antennas to light-harvesting, unobtainable by chlorophyll, from the environment. On the other hand, carotenoids serve as a protective barrier against high-energetic ultraviolet rays and against excessive insolation (Hagen, Braune 1993). There is some direct and indirect evidence for the existence of additional antenna function. Indirect evidence lies in the increasing content of carotenoids and chlorophyll in alga cells in poor light conditions, which has been demonstrated in a variety of species of unicellular aquatic algae (Czeczuga 1977, 1986a, Czeczuga et al. 1980), in land algae (Czeczuga 1986b) and in multi-cellular algae (Czeczuga 1986b, c; 1988).

Carotenoids have been assigned a protective function against excessive insolation in plants, including alga cells (Krinsky 1971, Goodwin 1980). The protection refers mainly to the photosynthetic apparatus and non-photosynthesizing tissues. This protective function of carotenoids has been revealed in a number of *Chlorophyceae* species of the genus *Chlamydomonas*, *Chlorella*, *Euglena* (Goodwin 1980) and *Dunaliella* (Ben-Amotz et al. 1989, Sukenik et al. 1990). It should be assumed that the presence of a large amount of β , ϵ -carotene and β , β -carotene, as well as their derivatives in the cells of *Trentepohlia gobbii* may be connected with a protective role of these pigments. Free-living species of the genus *Trentepohlia* occur in the land conditions exclusively, which are characterized by considerably larger insolation. *Trentepohlia gobbii* was collected for the analysis from stones in a river valley at an altitude of 1200-1500 m, where insolation is also very high.

The above assumption can be confirmed by the lichen species, whose phycobionts are algae of the genus *Trentepohlia*. These are crustaceous lichens that live mainly on stony rocks. The group of lichens with these algae as phycobionts includes over several hundred species (Tschermark-Woess 1988). The descriptions of the thalli of these lichens show (Nowak and Tobolewski 1975) that the cells of algae of the genus *Trentepohlia* found in these thalli do not cause their reddish colouring but rather varied shade of grey or greenish, since a relatively thick bark layer already fulfils a protective function. Only several lichen species of the genus *Porina*, with very thin thalli which cannot be protective, have *Trentepohlia* phycobiont cells containing a vast number of carotenoids. Therefore, their thalli assume peach-blood-red colour.

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WYSTĘPOWANIE KAROTENOIDÓW W KOMÓRKACH GLONU *TRENTEPOHLIA GOBII* MEYER

STRESZCZENIE

Stosując chromatografię kolumnową i cienkowarstwową badano występowanie karotenoidów w komórkach glonu *Trentepohlia gobbii* zebranych z otoczek rzeki Urmi na Dalekim Wschodzie.

Stwierdzono następujące karotenoidy: β , β -karoten; β , ϵ -karoten; β , β -karoten-2-ol; β , ϵ -karoten-2-ol; β , β -karoten-2,2-diol; 5,6-epoksy-5,6-dihydro- β , β -karoten-2-ol; 5,6-epoksy-5,6-dihydro- β , ϵ -karoten-2-ol; 5,6,5',6'-diepoksy-5,6,5',6'-tetrahydro- β , β -karoten-2,2-diol. Karotenoid 5,6,5',6'-diepoksy-5,6,5',6'-tetrahydro- β , β -karoten-2,2-diol jest nowym karotenoidem dla glonów.

Dominującym karotenoidem okazał się β , β -karoten (37.8%) oraz jego pochodny β , β -karoten-2-ol (30.4% wszystkich karotenoidów).

SŁOWA KLUCZOWE: glony, *Trentepohlia*, karotenoidy.