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Phycobiliprotein, chlorophyll, carotenoid pigments and phytochromes in the thallus of *Nephroma arcticum* from biotopes in the North

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ABSTRACT

By means of column (CC), thin-layer (TLC), high-performance liquid (HPLC) and ion exchange chromatography (IEC), the authors investigated the photosynthesizing pigments (chlorophylls, carotenoids, phycobiliproteins) and phytochromes in the thallus of *Nephroma arcticum* from biotopes in the North. Three groups of pigments absorbing rays of PAR beams from the environment are present in thallus of *Nephroma arcticum*. Chromatic adaptation can exist in the conditions of the Arctic and it is characterized by relatively poor solar energy.

KEYWORDS: *Nephroma arcticum*, lichens, chlorophylls, carotenoids, phytochromes, chromatic adaptation

INTRODUCTION

Nephroma arcticum is a common lichen species in the northern hemisphere, playing an important role in the ecosystems there. Light is the major environmental factor in biotopes inhabited by this species [1]. For most of the year, the light factors are limited (for a few months substantially), and the so-called northern summer is characterized by considerably more red and far-red light than in the moderate zone.

In this context, we decided to investigate the content of phycobiliprotein pigments in the thalli of *Nephroma arcticum*, as some of them are known to absorb the red light [2]. Moreover, we found the presence of chromoproteids, i.e. phytochromes, in the thalli of this species, which are known to play a role in physiological processes in lichens [3]. They also determine the content of chlorophylls and carotenoids.

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MATERIAL AND METHODS

The thalli of *Nephroma arcticum* (L.) Torss. were collected for analysis from 4 various sites (Canada, Greenland, Kamchatka and Spitsbergen) (Table 1).

The content of phycobiliprotein pigments and the presence of phytochromes were determined in the thalli dried at room temperature. The phycobiliproteins were separated from cyanobacteria (*Nostoc* sp.) according to the earlier methods with ammonium sulphate [4]. After centrifugation, the material was dissolved in a 0.1 M phosphate buffer at pH 7 and purified by ion exchange chromatography (IEC) using a Sephadex G-100 column. Elution was carried out in a phosphate buffer at pH 7 using a linear gradient of concentration within the range 0.005 - 0.1 M. The identification of the phycobiliproteins moiety was achieved by a visible absorption and fluorescence emission maxima [5]. Relative amounts of particular phycobiliproteins were determined by the method of Bennett and Bogorad [6].

Method of separation of phycobiliproteins is described in detail in our previous paper [4, 7].

Table 1. Sites collected of *Nephroma arcticum*.

Collected from	Locality	Altitude in m
1. Canada	Northwest Territories - Tuktoyaktuk Peninsula	20
2. Greenland	Narssaq Tugtugtoq - Moistdwarfscrub heath	10
3. Kamchatka (Russia)	Ganalski hills - stones	25
4. Spitsbergen	Northwest part - stones	75

The phytochrome proteins were isolated using the method according to Tokuhisa *et al.* [8], described by López-Figueroa *et al.* [9]. The extraction of the phytochrome protein was performed according to Lindemann *et al*. [10] with 50% ethylene glycol and 2 nM Triton X-100. After removal of contaminated material with polyethylenamine, the desired protein fraction was concentrated with ammonium sulphate (45% saturation). Relative amounts of the photoreversible protein were determined by measurement of the absorption of difference spectrum of A_{340} nm – A_{650} nm determined after saturating red irradiation, minus the difference A_{540} nm – A_{650} nm determined after saturating green irradiation. The amount of the photoreversible protein in green alga (*Coccomyxa* sp.) were determined by measurement of the absorption of difference spectrum of A_{660} nm – A_{730} nm determined after saturating far- red irradiation, minus the difference A_{660} nm – A_{730} nm determined after saturating red irradiation [11]. Absorption spectra was recorded with a Beckman spectrophotometer model 2400 DU. Further purification on a column chromatography (CC) from hydroxyapatite was achieved with the extract from investigated material. Sodium dodecyl sulphate gel electrophoresis with 10% polyacrylamide gel (0.75 nm) and immunoblotting were performed as described by Schneider-Poetsch *et al*. [12].

The presence of the respective chlorophylls and carotenoids in the specimens of *Nephroma arcticum* assayed was identified by column (CC) and thin-layer chromatography (TLC) with different solvent systems [13] as well as high-performance liquid chromatography (HPLC). Prior to chromatography, the material was homogenized and hydrolyzed in nitrogen, at room temperature. The extract was subsequently placed on an Al_2O_3 – filled Quickfit Co. column. The individual fractions were eluted using various solvent systems. The eluent was evaporated, and the residue was dissolved in an appropriate solvent to draw the maximum absorption. In addition to CC, an acetone extract was divided into fractions with TLC Silicon gel covered glass plates (Merck Co.) and various solvent systems were used. The R_f values were established according to commonly accepted criteria [14].

Pigments were also determined by ion-pairing in reverse-phase HPLC according to Mantoura and Llewellyn [15]. The HPLC equipment consisted of a Shimadzu SCL-6B gradient programmer and a Rheodyne 7125 injector. Detection was achieved in a Schimadzu SPD-6AV UV-VIS spectrophotometric detector set at 440 nm and a Shimadzu RF-535 fluorescence detector. CC, TLC, and HPLC are described in detail in Czeczuga *et al*. [16].

Carotenoids were identified by comparison with standards from: a) the behavior in CC; b) their UV-VIS spectra; c) their partition between n-hexane and 95% ethanol; d) their R_f-values in TLC; e) the presence of the allylic OH group determined by the acid $CHCl_3$ test; f) the epoxide test; g) the mass spectrum [17].

Carotenoid pigment standards were purchased from the Hoffman-La Roche Co., Switzerland; the International Agency for ^{14}C Determinations, Denmark, and Sigma Chemical Co., USA.

The structure of particular carotenoids was described by Straub [18] and Czeczuga [19].

RESULTS

Among the phycobiliprotein pigments found in the thalli of *Nephroma arcticum*, C-phycocyanin

predominated in the thalli collected from Spitsbergen (49.34%), in comparison to allophycocyanin in the thalli obtained from the other sites (41.74 - 77.79%). The total content of phycobiliprotein pigments ranged from 0.436 (Kamchatka) to 0.776 mg g^{-1} of dry mass (Greenland) (Table 2).

The study revealed the presence of phytochrome both in the cells of the green alga *Coccomyxa* sp. being the phycobiont of *Nephroma arcticum* and in the cyanobacterium *Nostoc* sp. in cephalodia (Table 3). The presence of chlorophylls and carotenoids in thallus of *Nephroma arcticum* has been shown in the Table 4.

DISCUSSION

The thallus of *Nephroma arcticum* consists of three parts [20], i.e. heterotrophic mycotic element and two autotrophic components. Inside the thallus, there is the green alga *Coccomyxa* sp. as the phycobiont [21, 22], whereas the cyanobacterium *Nostoc* sp. is found in bulges on the bottom side of the thallus called cephalodia [23, 24]. According to Jordan and Rickson [25] - *Nephroma arcticum* may have two distinct morphological forms of blue-green algae (cyanobacteria) in the same thallus and occasionally in the same cephalodium. The total content of phycobiliprotein pigments found in the

Table 2. Biliprotein distributions for investigated specimens of *Nephroma arcticum*.

Collected from	Total content mg g^{-1} dry weight	Particular biliproteins in %*		
		CPC	CPE	APC
1. Canada	0.527	5.79	25.63	68.59
2. Greenland	0.776	21.81	0.40	77.79
3. Kamchatka	0.436	37.16	21.10	41.74
4. Spitsbergen	0.458	49.34	14.41	36.25

*CPC= C-phycocyanin; CPE= C-phycoerythrin; APC= allophycocyanin
Visible absorption and fluorescence emission of particular biliproteins were according to Czeczuga *et al.* [47].

Table 3. Presence of phytochrome in autotrophic partners of *Nephroma arcticum*.

Photoconvertible protein	*Nostoc* sp. (cyanobacteria)	*Coccomyxa* sp. (green alga)
Pg form - induces by 650 nm	+	
Pr form - induces by 540 nm	+	
Pr form - induces by 670 nm		+
Pfr form - induces by 710 nm		+

Table 4. Chlorophylls and carotenoids content in thallus of *Nephroma arcticum*.

Collected from	Chlorophyll mg g^{-1} dry weight		Ratio chl.*a* / chl.*b*	Carotenoids µg g^{-1} dry weight	Major carotenoid
	a	*b*			
Canada	1.12	0.31	3.61	12.48	Violaxanthin
Greenland	0.91	0.28	3.25	8.92	Zeaxanthin
Kamchatka	0.81	0.31	2.74	25.81	Lutein epoxide
Spitsbergen	1.09	0.35	3.11	20.43	Antheraxanthin

cephalodia of *Nephroma arcticum* appeared to be relatively high (0.436 – 0,776 mg g^{-1} of dry mass), in comparison to their content in the thalli of five species of the genus *Stereocaulon*, containing the cyanobacterium *Nostoc* in cephalodia (0.027 - 0.144 mg g^{-1} of dry mass) [26]. The total contents of chlorophylls and carotenoids were also high in the thalli of *Nephroma arcticum*. The chlorophyll *a* to *b* ratio was found to be high, which could be explained by the fact that the former is present both in the cells of green alga and cyanobacterium, whereas the latter only in green alga *Coccomyxa* sp. Worthy of note is the finding of relatively large amounts of carotenoid pigments in the thalli of *Nephroma arcticum*, which take part in the uptake of light beams that are not captured by chlorophylls. These carotenoids include β-carotene [27], lutein [28], zeaxanthin [29], antheraxanthin [30], and violaxanthin [31]. In algae, they are known to act as antennas capturing short PAR beams. In the thalli of *Nephroma arcticum* examined in the current study, they were detected at all study sites. It should be mentioned that in the thalli of *Nephroma expallidum* (Nyl.) Nyl. collected from a peatbog in Iceland [32], the total carotenoid content was 42.2 μg g^{-1} of dry mass and the carotenoids mentioned above occurred in high amounts.

Thus, the high contents of all the three groups of pigments involved in the absorption of light beams in the photosynthesis of the autotrophs of the thalli of *Nephroma arcticum*, are associated with poor insolation in lichen habitats. This is a well-known phenomenon in lower aquatic [33] and terrestrial autotrophs [34], as well as in aquatic and terrestrial seed plants [35], which in the thalli of *Nephroma arcticum* and others, in the autotrophic partners, occurs as the process of adaptation to specific environmental conditions of the Arctic [36]. This refers to both photosynthesis and absorption of atmospheric nitrogen by the cyanobacterial partner [1]. The process of photosynthesis may occur in minus temperatures and in the first few hours of the light factor action [37-39]. In the process of atmospheric nitrogen assimilation by the cyanobacteria *Nostoc* sp. in the cephalopodia of *Nephroma arcticum*, the main enzyme nitrogenase becomes activated soon after a few hours of thermal stress (cold) [40]. Nitrogen fixation is possible below the freezing point [41] and nitrogenase activity of lichens was observed during snow-melt [42, 43] and even under snow cover [44]. Both the phycobiont green alga and cyanobacteria in cephalopodia contain phytochromes. The presence of phytochrome in the cells of the green alga *Coccomyxa* sp. was first detected by Vicente [3], whereas in the cells of *Nostoc* sp., a lichen symbiont, by Czeczuga *et al*. [45]. Björn and Björn [46] reported on the occurrence of phytochromes in free-living cells of *Nostoc muscorum* in the 70s of the previous century. Czeczuga *et al*. [47] described the role of phytochromes in chromatic adaptation of cyanobacteria in the northeastern waters of Poland, including a few species of the genus *Nostoc*.

Phytochromes are present both in higher plants, i.e. seed plants and in lower cryptogams [48]. One plant may contain a few types of phytochromes of the same molecular mass but induced by light beams of varied wavelength. Therefore, it is often said that both seed plants [49] and cryptogams [50] contain phytochrome “families”. In lower plants, phytochromes have been observed in free-living cyanobacteria [51], in green algae [52], brown algae [53] and red algae [54], as well as in representatives of Characeae [55]. Phytochromes have been found in the autotrophic partners of lichens [3, 45], in liverworts [56], mosses [16] and pteridophytes [57]. It has been demonstrated that phytochromes regulate morphogenesis in plants [58], biosynthesis of chlorophylls [53, 54], anthocyanins [59] and flavonoides [60]. In lichens, phytochromes have been found to play a role in the formation of aplanospores [61], to activate adenylate cyclase [3] and to control the activity of the enzyme nitrate reductase [62] known to have a major part in nitrogen metabolism [1].

It should be emphasized that the thalli of the lichen *Nephroma arcticum*, having two autotrophic partners (the photobiont - green alga *Coccomyxa* sp.; cyanobacteria *Nostoc* sp. in cephalodia) that contain three groups of pigments absorbing rays of PAR beams from the environment (chlorophylls, carotenoids and phycobiliprotein pigments) and capable of chromatic

adaptation can exist in the conditions of the Arctic, characterized by relatively poor solar energy.

Our many years of studies showed that the content of phycobiliprotein pigments in free-living [47] and in symbiotic cyanobacteria [45] in their chromatic adaptation is different. It depends on the spectral content of sun rays, in particular ecological niches. In shadowed places, in which the short rays predominate, the free-living [47] and symbiotic [63, 64] cyanobacteria have enlarged cell size and contain more bilisomes [65] and phycobilin pigments, especially C- phycoerythrin [64, 66]. Symbiotic forms of cyanobacteria occur in algae [67], lichenes [66], some species of lower mosses [68], water ferns from *Azolla* genus [69] and in seed plants - both gymnosperms and angiosperms [70]. The cyanobacteria occurring in all mentioned taxonomic groups differ especially morphologically [71]. That is why for many years they have been considered to be a different cyanobacteria species. Among lichenes, the representatives of particular families include cyanobacteria which differ morphologically, although they all belong to *Nostoc* group. Earlier they have been considered to be a different cyanobacteria species belonging to a different genera than *Nostoc*. We have been observing the morphological differences in cyanobacteria in species of such lichen genera from different continents as *Lobalia* [72], *Collema* [73], *Leptogium* [74], *Pseudocyphellaria* [75], *Peltigera* [66, 76, 77], *Stereocaulon* [26, 78] and also in many other lichen specimens living in symbiosis with cyanobacteria [79]. A plant in which cyanobacteria lives with surrounding environment [80] modify the morpho-physiological condition of cells of particular cyanobacteria species. It has been confirmed by many investigations using the newest genetical methods [81-84]. The culturing of isolated cyanobacteria show interchangeably their nostocacean character.

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