Accumulation of Photoassimilatory Products by Phycobiliprotein-Containing Algae with Special Reference to *Cyanidium caldarium*

Bruno P. Kremer and Guido B. Feige

Botanisches Institut der Universität Köln, Gyrhofstr. 15, D-5000 Köln 41

Z. Naturforsch. **34 c**, 1209–1214 (1979); received August 9, 1979

Photosynthesis, Assimilates, Chemotaxonomy, Phycobiliprotein-Containing Algae

Representatives of phycobiliprotein-containing algae such as *Anabaena cylindrica, Anacystis nidulans, Gloeotrichia echinulata* (Cyanophyceae), *Chroomonas* spec., *Hemiselmis rufescens, Rhodomonas* spec. (Cryptophyceae), *Porphyridium cruentum, Rhodella violacea* (Rhodophyceae) along with the unicellular *Cyanidium caldarium* (unspecified systematic status) have been investigated for their typical photoassimilatory accumulation products. While the red algal species synthesize a rather specific heteroside, 2-O-D-glycerol-α-D-galactopyranoside (= floridoside), not encountered in the other species analyzed, blue-green algae accumulate fructose, glucose, and sucrose, while the cryptomonads accumulate only glucose and, to a lesser extent, fructose. *Cyanidium* synthesizes neither disaccharides, nor a heteroside, but shows rapid ^{14}C-labelling of fructose and glucose. These results are compared with further biochemical and structural findings and are discussed with emphasis on chemotaxonomic implications. *Cyanidium caldarium* is proposed as an endocyanome consisting of a single endocyanella providing the functions of a chromatophore in a colourless (apoplastidal algal?) host cell.

Introduction

Phycobiliprotein-containing algae are exclusively associated with three major taxonomic units among the lower plants including representatives of the Cyanophyceae, Cryptophyceae, and Rhodophyceae. Apart from their striking similarity in pigmentation, these algal classes are rather well defined by a variety of structural and morphological properties as well as by several biochemical features. While a variety of red algal species have already been characterized with special regard to their low-molecular weight carbohydrates from a chemotaxonomic point of view [1, 2], little information is available on the photosynthetic carbon metabolism of blue-green algae [3, 4] and no work on the carbohydrate constituents of cryptomonad algae has hitherto been performed to the authors' knowledge. Nevertheless some considerations have already been given to a possible phylogenetic relationship between the Cyanophyceae and the Rhodophyceae [5]. The notable overall similarity in pigment composition gives rise to the question as to whether the members of the three phycobiliprotein-containing algal classes show further biochemical properties in common, *e. g.* with respect to their photoassimilatory accumulation products, or if they are distinctly different in this special regard.

Reprint requests to Dr. B. P. Kremer.
0341-0382/79/1200-1209 $ 01.00/0

The acidophilic and thermophilic unicellular alga, *Cyanidium caldarium*, which contains phycocyanin and allophycocyanin, present some particular taxonomic and systematic problems. This enigmatic alga has hitherto rather inconsistently been placed into the Chlorophyceae [6, 7], the Cyanophyceae [8, 9], the Cryptophyceae [10, 11], and further in the Rhodophyceae [12, 13]. Due to its definitely eukaryotic cell organization [14, 15] only the Cryptophyceae and Rhodophyceae are likely to be considered as correct. However, there are also certain ecological and biochemical features which clearly point to the Cyanophyceae, and this is why even a transition status of this alga, between the blue-green and the red algae, has been discussed [16, 17].

The present contribution presents some further investigation of *Cyanidium caldarium* and compares the photosynthetic carbon metabolism of this unique species with the equivalent features of some representatives of the Cyanophyceae, Cryptophyceae, and Rhodophyceae. Particular attention will be given to the accumulated low-molecular weight assimilates as a possible parameter for further evaluation of the equivocal systematics of *Cyanidium caldarium.*

Materials and Methods

Organisms

The following species were provided as unialgal stock cultures by the culture collection of the Pflan-

zenphysiologisches Institut, D-3400 Göttingen: *Anabaena cylindrica* Lemmermann (1403-2), *Anacystis nidulans* (Richter) D. (1402-1), *Gloeotrichia echinulata* (Smith) Richter (1432-1) (Cyanophyceae), and *Porphyridium cruentum* (Ag.) Naegeli (1380-1 a) (Rhodophyceae). Cultures of *Chroomonas* spec. and *Hemiselmis rufescens* (Cryptophyceae) as well as of *Rhodella violacea* (Kornm.) Wehrmeyer were kindly supplied by Prof. Dr. W. Wehrmeyer (Marburg). *Cyanidium caldarium* Geitler, originating from a Yellowstone hot spring algal mat, was obtained from Prof. H. A. W. Schneider (Köln). *Rhodomonas* spec. (Cryptophyceae), isolated from a phytoplankton community of the North Sea, was kindly given by Dr. E. Hagmeier (Helgoland). Media used for the cultivation were those of Hemerick [18] for *Anabaena, Anacystis,* and *Gloeotrichia,* of McLachlan [19] for *Porphyridium,* and of Allen [6] for *Cyanidium.* The algae were cultured in aerated 1 l Erlen Erlenmeyer flasks at 20 °C (*Cyanidium:* 35 °C) and 3000 lx.

^{14}C-Incubation

Cell suspensions of the algal species investigated were harvested by centrifugation and diluted with culture medium so that 1 ml of suspension was equivalent to about 30 µg Chl a. Experiments on photosynthetic CO_2-fixation were performed with 1 ml – samples in closed polyethylene reaction vials (1.5 ml) at 20 °C (*Cyanidium* 20 °, 35 °, 45 °C) and 10000 lx with continuous shaking. After a 15 – 30 min pretreatment to achieve steady-state conditions, the experiments were started by application of 10 µCi $NaH^{14}CO_3$ (specific activity 59.7 mCi/mmol) to each sample. After the appropriate incubation period (3 – 180 min) the algae were harvested by rapid centrifugation, fixed by immediate deep-freezing in liquid N_2 and subsequently extracted in small volumes (0.6 ml) of methanol-chloroform-formic acid (MCF) = 12 : 5 : 2 and ethanol (EtOH) (50% 30%).

Analytical

Aliquots of the combined MCF-/EtOH-extracts were counted for ^{14}C-incorporation by liquid scintillation counting. Equivalent volumes were further analyzed by two-dimensional thin-layer chromatography on normal (MN 300) or microcrystalline cellulose according to ref. [20]. All further analytical procedures concerning characterization and identification of assimilates as well as of determination of insoluble ^{14}C-assimilates have been detailed in a recent survey [21].

Results

Time course of photosynthetic carbon assimilation

Preliminary experiments have shown that light-independent carbon incorporation by all phycobiliprotein-containing species included in this investigation is in the range of about 1% of the equivalent photosynthetic CO_2-fixation under comparable conditions. Thus, only photosynthetic carbon assimilation will be considered further. Fig. 1 presents the time courses of light dependent $^{14}CO_2$-uptake and -incorporation into soluble (low-molecular weight) and insoluble (polymeric) constituents for incubation periods ranging up to 3 h. Under steady-state conditions ^{14}C from $H^{14}CO_3^-$ is taken up very rapidly by all species over the entire time range regarded. The time-dependent increase in total ^{14}C taken up is approxima-

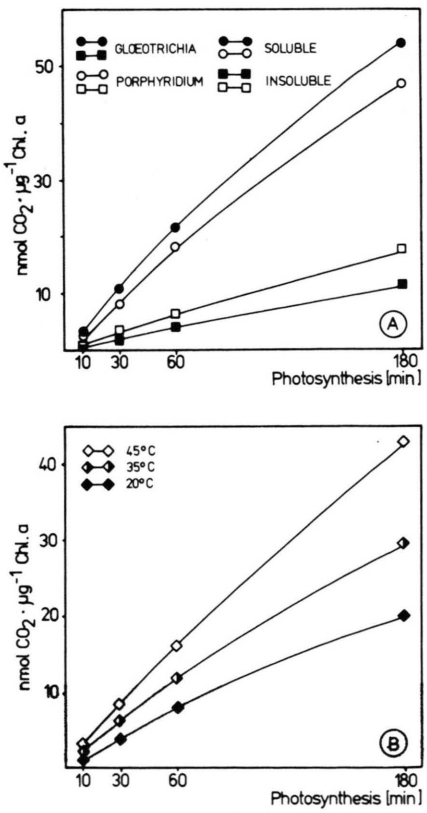

Fig. 1. Time course of photosynthetic carbon incorporation, A *Gloeotrichia echinulata,* B *Cyanidium caldarium.*

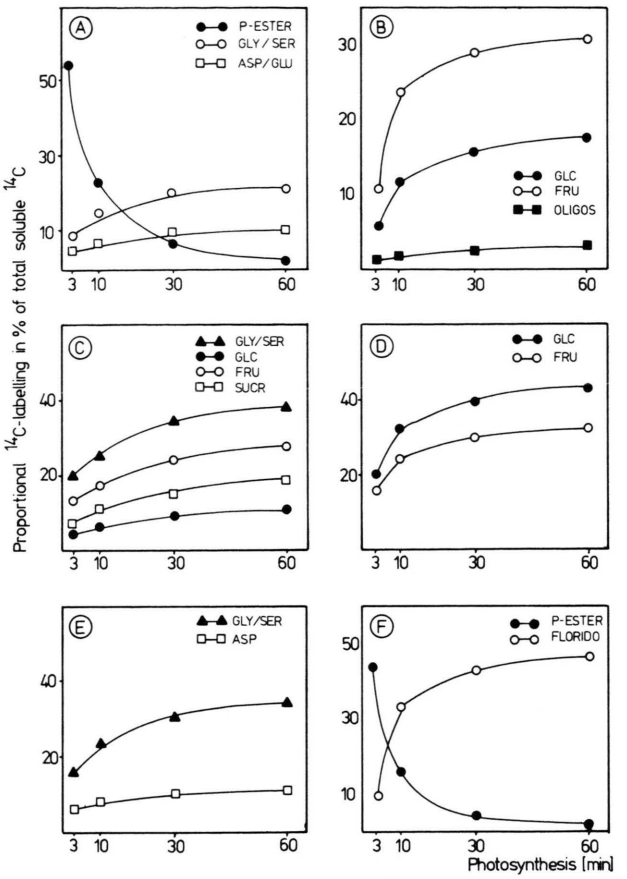

Fig. 2. Time dependent ^{14}C-labelling of selected photoassimilatory products, A, B *Cyanidium caldarium;* C *Gloeotrichia echinulata;* D *Hemiselmis rufescens;* E, F *Porphyridium cruentum.* P-Ester: phosphorylated compounds, Gly: glycine, Ser: serine, Asp: aspartate, Glu: glutamate, Glc: glucose, Fru: fructose, Sucr: sucrose, Oligos: oligosaccharides, Florido: floridoside (galactosylglycerol).

tely linear with incubation time and amounts to about 25 – 30 nmol $CO_2 \cdot mg^{-1}$ Chl a $\cdot h^{-1}$. Due to the different pH values of the appropriate incubation media used and thus differences in total alkalinity, the gross rates of ^{14}C-uptake obtained cannot be converted exactly into total carbon assimilation under the experimental conditions. They give only an approximate figure of the actual rates of photosynthetic carbon assimilation. Considerable enhancement of carbon uptake is achieved if cell suspensions of *Cyanidium caldarium* are allowed to photosynthesize at temperatures higher than 20 ° up to 45 °C (Fig. 1). This results is consistent with earlier findings [22].

In all species investigated more than 70% of ^{14}C taken up after 3 h photosynthesis is confined to the fraction of soluble (low-molecular-weight) compounds. At least 12% of the total assimilated radiocarbon (*Chroomonas*) and not more than 27% (*Porphyridium*) is recovered from MCF-/EtOH-insoluble material.

Pattern of ^{14}C-labelled photosynthates

Photosynthetic assimilation of ^{14}C from $H^{14}CO_3^-$-incubation media results in ^{14}C-labelling of a wide variety of assimilatory products which were visualized by contact autoradiography of two-dimensionally developed thin-layer chromatograms. In all species investigated the (MCF-/EtOH-) soluble fraction of assimilates shows, in general, the usual spectrum of diverse organic phosphate esters (phosphorylation products), several amino acids, organic acids, lipids and some neutral constituents. This neutral fraction (*i. e.* low-molecular weight carbohy-

Table I. Carbohydrates among the photoassimilatory ^{14}C-labelled products of various phycobiliprotein-containing algae (+ present; – lacking).

Genus Compounds	*Anabaena* *Anacystis* *Gloeotrichia*	*Cyanidium*	*Chroomonas* *Hemiselmis* *Rhodomonas*	*Porphyridium* *Rhodella* *
Hexose-/pentose mono-/bisphosphate	+	+	+	+
Monosaccharides	fructose **glucose**	fructose **glucose**	glucose **fructose**	–
Disaccharides	**sucrose**	–	–	–
Heterosides	–	–	–	floridoside
Glucan-type oligosaccharides	+	+	–	+

* *cf.* ref. [41].

drates) is represented by different compounds in different groups of phycobiliprotein-containing organisms (Table I). While the representatives of the cryptomonad algae (*Hemiselmis, Chroomonas, Rhodomonas*) as well as *Cyanidium caldarium* produce considerable amounts of [^{14}C]glucose and [^{14}C]fructose, the members of the Cyanophyceae (*Anabaena, Anacystis, Gloeotrichia*) additionally synthesize certain amounts of [^{14}C]sucrose. On the other hand, *Porphyridium* and *Rhodella* (Rhodophyceae) do not contain any free mono- or disaccharides, but show rather intense ^{14}C-labelling of a galactosidoglycerol-type heteroside, 2-O-D-glycerol-α-D-galactopyranoside (= floridoside). Glucan-type oligosaccharides yielding [^{14}C]glucose on cautious hydrolysis have been observed in *Anabaena, Anacystis, Gloeotrichia, Cyanidium* as well as in *Porphyridium* (Table I).

Kinetics of ^{14}C-labelling of some selected assimilates

Fig. 2 presents ^{14}C-labelling courses of some selected photoassimilatory products. From the characteristic labelling courses intermediary compounds and accumulated assimilates can easily be differentiated. While the percentage of ^{14}C-labelling of all phosphorylated compounds such as 3-phosphoglycerate or the diverse hexose/pentose mono- and bisphosphates distinctly decline (as exemplified by *Cyanidium* and *Porphyridium* in Fig. 2), the equivalent time dependent incorporation of radiocarbon into certain amino acids (such as aspartate, glycine, serine, alanine) as well as into soluble carbohydrates shows the typical convex upward curve suggesting that through time increasing amounts of ^{14}C are transferred into these compounds. The strongest ^{14}C-labelling is achieved in the carbohydrates, *i. e.* glucose and fructose in the blue-green algae, cryptomonads, and in *Cyanidium caldarium*, and floridoside in the representatives of the unicellular red algae, respectively. In general, these compounds account for at least 40 – 50% of the total ^{14}C recovered from the soluble fraction of assimilates. In *Cyanidium* notably more ^{14}C is confined to fructose than to glucose, while in *Hemiselmis* (and in the other cryptophycean species included) glucose is more strongly ^{14}C-labelled than the ketohexose. In this respect, *Cyanidium caldarium* resembles the species of the Cyanophyceae more.

When the algal species are allowed to photosynthesize $H^{14}CO_3^-$ during a 30 min pulse and are then further illuminated in a normal $H^{12}CO_3^-$-medium, the relatively high percentage of ^{14}C recovered from the accumulated soluble and insoluble carbohydrates is even enhanced, whereas the proportion of ^{14}C of all other compounds including the amino acids rapidly declines due to continuous interconversion within the intermediary metabolism (data not included in Fig. 2).

Discussion

The objective of the present investigation is to consider the systematic status of the enigmatic acidophilic and thermophilic alga *Cyanidium caldarium* as compared to some representatives of the phycobiliprotein-containing classes Cyanophyceae, Cryptophyceae, and Rhodophyeae. Though *Cyanidium caldarium* has previously been characterized as a member of the Chlorophyceae [6, 7, 23], it may be reasonable to consider only the representatives of the Cyanophyceae, Cryptophyceae, and Rhodophyceae as possible or probable relatives on the basis of the typical pigment composition (chlorophyll a, no chlorophyll b, phycocyanin, allophycocyanin).

There are certain physiological and biochemical characters which emphasize the rather close relationship between *Cyanidium* and members of the Cyanophyceae. The most striking feature, undoubtedly, is the extraordinary choice of habitat: *Cyanidium caldarium* is the sole photosynthesizing organism in volcanic hot springs or in hot soils with a pH less than 5 and average temperatures exceeding 40 °C [24, 25]. This alga shares its pronounced preference at least for unusual thermal habitats with a variety of blue-green algae [25], whereas all members of the Cryptophyceae and Rhodophyceae may be defined as mesophilic organisms with respect to their average autecological features. A remarkable similarity between *Cyanidium* and species of the Cyanophyceae is found when its sterols and the wide range of saturated and unsaturated fatty acids is regarded [15, 26]. The storage glucan of *Cyanidium* and of the blue-green Oscillatoria is strikingly similar, if not identical, and the structure and action of isozymes involved in polyglucan biosynthesis [16, 27] are also similar. Further evidence has been derived from immunodiffusion studies of the primer-independent phosphorylase isozyme [17, 18]. Döhler *et al.* [28] found the photosynthetic action spectra of *Cyanidium* and *Anacystis* to be identical. However, *Cyanidium calda-*

rium cannot simply be included in the Cyanophyceae, since it possesses a well-defined nucleus and mitochondria as well as other cell organelles and hence shows all essential structural criteria of a typical eukaryotic cell [14, 15, 29]. This mode of cellular organization suggests a closer relationship to either the Rhodophyceae or the Cryptophyceae. In fact, there are also some findings from investigations of branching isozymes and phosphorylase isozymes which point to a "transition status" between the Cyanophyceae and the Rhodophyceae [16, 17].

Porphyridium cruentum and *Rhodella violacea* are two species of the few known unicellular red algae. Free monosaccharides such as glucose or fructose are generally lacking in these species, while a special heteroside, galactosidoglycerol, is produced. According to the kinetics of its ^{14}C-labelling, this heteroside is the main accumulation product within the soluble assimilates. In this regard, *Porphyridium* as well as *Rhodella* resemble all red algae not belonging to the order Ceramiales [1, 2]. Since the occurrence of galactosidoglycerol as the single accumulated photosynthate in the majority of red algae may be regarded as a reliable criterion of chemotaxonomic significance ("marker function"), an assimilate pattern of a principally different composition might exclude an alga from the Rhodophyceae. *Cyanidium caldarium* does not produce any heteroside, but accumulates free monosaccharides such as fructose and glucose. At least in this respect, *Cyanidium* does not conceivably fit the criteria of a typical unicellular red alga.

Due to the pattern of accumulated photoassimilatory carbohydrates, *Cyanidium caldarium* admittedly shows certain affinities to the Cryptophyceae investigated. The cryptomonad nature of *Cyanidium* has already earlier been discussed [10, 11]. However, this systematic treatment of the *Cyanidium* problem seems not to be consistent either, because of several peculiar features of cell structure and morphology. Cryptophycean algae are generally organized dorsiventrally and bear two anterior flagella of different length, whereas *Cyanidium* completely lacks flagellate characters. Furthermore, the chromatophores of *Cyanidium* are not surrounded by four membranes as in the Cryptophyceae, but exhibit a different mode of thylakoid arrangement within the chromatophor. They are additionally equipped with interthylakoidal electron-dense particles probably identical with phycobilisomes [14, 15, 30], whereas members of the Cryptophyceae bear intrathylakoidal phycobilisomes [31]. Provided that not only the nature of the accumulated photosynthate, but also quantitative aspects of accumulation are significant for a taxonomic evaluation (fructose > glucose in *Cyanidium;* glucose > fructose in the cryptomonads; *cf.* Fig. 2 and Table I), the occurrence of glucose and fructose in *Cyanidium* and the cryptophycean algae must not be of the same significance, but could be weighed with the necessary caution. For these reasons, mostly based on micromorphological properties, we do not believe that *Cyanidium caldarium* is actually belonging to the Cryptophyceae.

The majority of the biochemical characteristics of *Cyanidium* suggest a closer relationship to the Cyanophyceae rather than to any other algal class. This view is consistent with the pattern of accumulated monosaccharides. A general compatibility of the biochemical properties of a blue-green alga and the structural organization of an undoubtedly eukaryotic cell is achieved, if *Cyanidium caldarium* is interpreted as an endocyanome consisting of a colourless host cell (perhaps an apoplastidal alga) and an integrated blue-green endosymbiont (endocyanella) which provides the metabolic functions of a normal chloroplast [32]. Symbioses of this type have already been documented for a variety of associations such as *Cyanophora paradoxa, Glaucocystis nostochinearum, Glaucosphaera vacuolata,* and *Gloeochaete wittrockiana* as well as for *Paulinella chromatophora* [33 – 36]. Structural properties which support this interpretation are the typical ring-shaped arrangement of thylakoids within the chromatophore reminiscent of a coccal blue-green alga and the observation that no chloroplast ER is present [14, 15, 30]. Though blue-green algae in the free-living state are able to synthesize sucrose [37] the blue-green endosymbionts (endocyanelles) of *Cyanophora* or *Glaucocystis* have been shown not to produce this or any other disaccharide [38]. In this particular regard, the well-documented endocyanelles symbiotic with colourless algae as well as those of the thecamoeba *Paulinella chromatophora* [39] are consistent with the potentials of the chloroplasts of *Cyanidium caldarium.*

Even though earlier workers were unable to trace diaminopimelic acid, muramic acid or amino sugars as cell wall constituents of *Cyanidium* – endosymbiotic algae must not necessarily possess a cell wall or even cell wall remnants [40] – there are actually no experimental results which are contradictory to this new interpretation of *Cyanidium caldarium* as an en-

docyanome. On the other hand, it might be more likely to classify *Cyanidium* as a symbiotic association like many others than to attribute it with the taxonomically undefined status of a transitory alga or "bridge alga" [17]. The evolutionary step from the prokaryotes to the eukaryotes is a distinct, abrupt change at the cellular organization level rather than a shifting transition.

Acknowledgements

The authors wish to thank Mrs. C. Zander for skillful technical assistance. Thanks are also due to Prof. Dr. L. Kies (Hamburg) and Prof. Dr. W. Wehrmeyer (Marburg) for critical suggestions and comments. The help of Dr. W. N. Wheeler with the English version of the manuscript is gratefully acknowledged.

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