Arch Microbiol (1983) 134:23–27

Archives of
Microbiology

Distribution of Plastocyanin and Soluble Plastidic Cytochrome c in Various Classes of Algae

Gerhard Sandmann[1], Hildegard Reck[1], Erich Kessler[2], and Peter Böger[1]

[1] Lehrstuhl für Physiologie und Biochemie der Pflanzen, Universität Konstanz, D-7750 Konstanz, Federal Republic of Germany
[2] Institut für Botanik and Pharmazeutische Biologie, Universität Erlangen, Schloßgarten 4, D-8520 Erlangen, Federal Republic of Germany

Abstract. Several eukaryotic algae belonging to the main taxonomic classes have been cultured autotrophically in liquid medium supplemented with or depleted of copper to assay their ability to form plastocyanin or exchange it against plastidic cytochrome c-553. Most Chlorophyceae are able to substitute cytochrome c-533 for plastocyanin with some exceptions like *Haematococcus* or *Dunaliella*, which can only synthesize plastocyanin. Also within the *Chlorella* group, about half of the 28 strains assayed cannot synthesize cytochrome c-553 under copper deficiency. Species of Chrysophyceae, Xanthophyceae, and Rhodophyceae, on the other hand, cannot synthesize plastocyanin even when a comparatively high copper concentration (10 µM) is available.

Serological cross-reactions of various plastocyanin-containing *Chlorella* homogenates against an antibody towards *Scenedesmus* plastocyanin exhibit a pattern which cannot be taxonomically used at the moment.

Including previous data on blue-green algae, it appears that, in the course of evolution, cytochrome c-553 dominates in the older species. In the Chlorophyceae, it is mutually exchangeable against plastocyanin which becomes the only electron donor to P700 in higher plants.

Key words: Plastocyanin – Cytochrome exchange – Algal taxonomy – *Chlorella*

In higher plants, plastocyanin functions as redox carrier between thylakoid-bound cytochrome f and P700 in photosynthetic electron transport. In some algae, however, plastocyanin can be replaced by cytochrome c. This means that the membrane-bound cytochrome present in all algal thylakoids is accompanied in some species by a soluble c-type cytochrome (Crofts and Wood 1978; Sandmann and Böger 1983). Depending on physiological conditions, the replacement of plastocyanin by cytochrome c-553 can either be permanent or temporary. Consequently, three types of algae with respect to their ability to form plastocyanin and/or plastidic cytochrome c-553 can be observed (Sandmann and Böger 1981). One type contains plastocyanin only, the other cytochrome c only, and a third one can mutually exchange plastocyanin and cytochrome c-553 depending on copper supply (Bohner and Böger 1978; Wood 1978).

In this paper, we report the occurrence of plastocyanin and cytochrome c-553 in various groups of algae. The affiliation of the investigated algae to the three types mentioned above can be regarded as an indicator for the phylogenetic evolution of the photosynthetic apparatus and is of interest for the taxonomy of the genus *Chlorella*.

Materials and Methods

Organisms and Cultivation. Several seawater algae including *Platymonas subcordiformis* (no. 161-1a, G), *Ectocarpus siliculosus* (stock Dr. G. Müller, Konstanz, FRG), *Phaeodactylum tricornutum* (no. 1090-1b, G), and *Skeletonema costatum* (no. LB 1077/1, C) were grown in an enriched seawater medium according to Starr (1978). (The letter at the end of the strain is added for information on the origin of the culture, it is not part of its designation. *G* stands for Algae Culture Collection, University of Göttingen, FRG, and *C* for Culture Collection Cambridge, England.) Published procedures were employed for cultivation of the 28 *Chlorella* strains (Kessler and Czygan 1970; Kessler and Zweier 1971) and for the other green and xanthophycean algae (Sandmann and Böger 1981b). The following additional strains were used: *Haematococcus pluvialis* (no. 34-1a, G), *Dunaliella parva* (stock of Dr. H. Gimmler, Würzburg, FRG), *Chlamydomonas reinhardii* (no. 11-32, G), *Gonium sociale* (no. 32-2a, G), *Eudorina elegans* (no. 24-1c, G), *Pandorina morum* (no. 60-1c, G), *Scenedesmus obliquus* (formerly *S. acutus*, no. 276-3a, G), *Scenedesmus armatus* (formerly *S. quadricauda*, no. 276-4a, G), *Pediastrum boryanum* (no. 261-2, G), *Monoraphidium braunii* (formerly *Ankistrodesmus braunii*, no. 202-7c, G), *Eremosphaera viridis* (no. 228-1, G), *Netrium digitus* (no. 652-1, G), *Micrasterias thomasiana* (no. 649-4a, G), *Bumilleriopsis filiformis* (from the Konstanz stock), *Vischeria stellata* (no. 887-2, G), *Tribonema aequale* (no. 880-1, G), and *Bumilleria sicula* (no. 808-1, G). Growth of *Porphyridium aerugineum* (no. 1380-2, G), and *Cyanidium caldarium* (no. RK-1 from Dr. J. Fukuda) was carried out as described by Hoogenhout and Amesz (1965), and Enami and Fukuda (1975), respectively. *Porphyridium cruentum* (no. 1380-1a, G) was grown in the following medium: KNO_3, 10 mM; $MgSO_4 \times 7 H_2O$, 2.6 mM; $MgCl_2 \times 6 H_2O$, 2.8 mM; KH_2PO_4, 28 µM; $K_2HPO_4 \times 3 H_2O$, 0.9 mM; $NaHCO_3$, 0.5 mM; $CaCl_2$, 0.9 mM; $Fe(NO_3)_3 \times 9 H_2O$, 72 µM; EDTA, 72 µM; NaCl, 0.23 M. Trace elements were given in a 3-fold concentration of the *Scenedesmus* medium reported by Sandmann and Böger (1981b). All cultures were grown either without copper present or with 1 or 2 µM $CuSO_4$ added when indicated.

Offprint requests to anyone of the authors

Determination of Redox Proteins. Plastocyanin, soluble cytochrome c-553, and bound cytochrome f-553/4 were measured by differential spectroscopy after chemical oxidation and reduction as previously described (Sandmann and Böger 1980a). With extracts of red algae, the procedure was modified as reported for blue-green algae (Sandmann and Böger 1980b). Data of Tables 1 and 3 are means of duplicate redox-protein determinations from 3 to 5 separate batches. Data of Table 2 are means from duplicates of one culture batch. Double-diffusion tests with an antibody against plastocyanin of *Scenedesmus obliquus* (276-3a) were carried out according to Böhme (1978). This assay used homogenates containing 10 pmol of plastocyanin from different *Chlorella* strains cultivated in the presence of 1 µM $CuSO_4$.

Results

As demonstrated in Table 1, all green algae investigated herein formed plastocyanin when the nutrient was supplemented with 1 µM $CuSO_4$. Cultures grown with copper did not contain soluble cytochrome c-553. Species from the orders Volvocales, Chlorococcales, and Desmidiales responded to copper depletion by substituting cytochrome c-553 for plastocyanin. However, three species of Table 1 did not react this way. *Haematococcus pluvialis*, *Dunaliella parva*, and *Platymonas subcordiformis*, a marine alga belonging to the Pyramimonadales, have virtually lost their ability to form cytochrome c-553 under copper starvation. They all showed a plastocyanin content decreased to about 50% or less. Much lower plastocyanin values were obtained when a copper-free medium was inoculated with cells from a previously copper-depleted culture, and not using cultures pregrown in the presence of Cu^{2+} as in the experiments for Table 1.

A comprehensive study of the genus *Chlorella* (Chlorococcales), represented by 28 strains belonging to 14 species (Kessler 1976, 1978), revealed a mixed pattern (Table 2). Whereas 13 strains produced cytochrome c-553 instead of plastocyanin under copper-deficient conditions; 15 strains were unable to do so. In some instances, even strains of the same species exhibited a different reaction towards copper deficiency. Noteworthy, all *Chlorella* strains (except for strain 211-8g) exhibit a ratio of about 1 nmol thylakoid-bound cytochrome f-553/4 to 1 µmol chlorophyll. This constancy apparently is a general rule and has been found previously for *Scenedesmus* or *Anabaena variabilis* (Bohner and Böger 1978; Sandmann and Böger 1980b).

The contents of cytochrome c-553 of various species of Chrysophyceae, Xanthophyceae, and Rhodophyceae are listed in Table 3. Common to all of them is their inability to form plastocyanin even when media are supplemented with 2 µM $CuSO_4$. Increasing the copper concentration to 10 µM in the media as used for *Bumilleriopsis filiformis* or *Porphyridium cruentum* did not result in the detection of plastocyanin in either species (data not shown). Concentrations exceeding about 40 µM were found to be toxic.

Table 4 demonstrates cross-reactions of homogenates from *Chlorella* strains grown in the presence of copper. It is seen that the antibody against plastocyanin from *Scenedesmus* exhibits quite a variable reactivity with different *Chlorella* strains.

Discussion

As can be seen from Tables 1 and 3, eukaryotic algae may contain either plastocyanin or cytochrome c-553. The latter cannot be replaced physiologically by plastocyanin in chry-

Table 1
Formation of plastocyanin and cytochrome c-553 in green algae grown in coper-deficient or supplemented media. Concentrations of redox proteins are in nmol/µmol chlorophyll

Species	Growth without copper		Growth with 1 µM Cu^{2+}	
	Plastocyanin	Plastidic cytochrome c-533	Plastocyanin	Plastidic cytochrome c-533
Volvocales				
Haematococcus pluvialis	0.9	–	1.9	–
Dunaliella parva	1.1	–	2.1	–
Chlamydomonas reinhardii	–	2.6	2.2	–
Gonium sociale	–	2.0	2.3	–
Eudorina elegans	–	2.2	1.9	–
Pandorina morum	–	1.6	1.2	–
Pyraminonadales				
Platymonas subcordiformis	0.9	–	2.9	–
Chlorococcales				
Scenedesmus obliquus (formerly acutus)	–	2.6	2.3	–
Scenedesmus armatus (formerly quadricauda)	–	2.4	2.0	–
Pediastrum boryanum	–	1.9	2.4	–
Monoraphidium braunii (formerly Ankistrodesmus)	–	2.8	2.2	–
Eremosphaera viridis	–	2.6	2.0	–
Desmidiales				
Netrium digitus	–	1.8	1.6	–
Micrasterias thomasiana	–	2.5	2.3	–

Table 2. Ability of *Chlorella* strains to form plastocyanin, soluble cytochrome c-553, and thylakoid-bound cytochrome f-553/4 in the presence or absence of added Cu^{2+}. Data are nmol/µmol chlorophyll

Strains	Growth without copper			Growth with 1 µM Cu^{2+}		
	Plastocyanin	Plastidic cytochrome		Plastocyanin	Plastidic cytochrome	
		c-533	f-553/4		c-533	f-553/4
C. sorokiniana						
211-8k	–	1.3	1.4	2.7	–	1.3
211-31	–	2.2	1.4	3.3	–	1.9
211-40a	0.3	–	0.7	3.5	–	0.6
1-9-30	0.4	–	0.9	1.8	–	1.4
C. vulgaris						
211-11b	0.5	–	0.8	2.2	–	0.7
211-11c	–	3.6	0.9	2.6	–	1.0
211-12	0.6	–	1.0	2.2	–	1.1
211-19	0.3	–	0.8	2.0	–	0.7
C. saccharophila						
211-1c	–	1.9	0.7	1.8	–	1.4
211-1d	–	2.8	1.6	2.3	–	1.2
211-9a	–	2.9	1.1	1.9	–	1.1
C. spec.						
211-30	0.7	–	1.1	4.1	–	1.4
C. fusca var. *vacuolata*						
211-8b	–	3.0	0.9	3.1	–	1.4
211-8g	–	1.0	2.4	1.9	–	2.9
211-15	–	3.2	1.2	2.6	–	1.1
C. fusca var. fusca						
343	–	4.5	1.1	4.8	–	1.1
C. fusca var. *rubescens*						
232/1	–	1.3	0.9	2.1	–	1.1
C. zofingiensis						
211-14a	–	1.0	0.8	1.6	–	1.1
C. minutissima						
C-1.1.9	0.3	–	1.6	3.1	–	1.1
C. spec.						
211-11r	0.6	–	0.9	4.1	–	1.2
C. homosphaera						
211-8e (Ca.)	–	1.2	0.8	3.4	–	0.9
C. kessleri						
211-11g	0.5	–	0.8	2.7	–	0.8
211-11h	0.5	–	0.8	2.5	–	0.9
C-1.1.1	0.3	–	1.6	3.1	–	1.1
C. luteoviridis						
211-2b	0.4	–	1.5	2.6	–	1.5
211-5b	0.5	–	0.8	2.8	–	1.1
C. protothecoides						
211-7a	1.2	–	1.0	2.2	–	0.8
211-10a	0.2	–	0.7	1.5	–	0.6

sophycean, xanthophycean, phaeophycean, and rhodophycean algae. This was reported previously for *Bumilleriopsis filiformis* (Xanthophycea; Kunert and Böger 1975). Most likely these algae have no biosynthetic pathway for plastocyanin, since even high copper concentrations do not induce formation of plastocyanin. Thus, we have not been able to confirm the presence of plastocyanin in *Porphyridium aerugineum* proposed by Visser et al. (1974) who had applied the epr technique which, in general, is equivalent to our method in order to detect plastocyanin (Bohner et al. 1980). Evidently, eukaryotic plastocyanin synthesis is restricted to green algae (Tables 1 and 2).

Table 3. Presence of plastocyanin and cytochrome c-553 in various classes of Chromophyta and Rhodophyta grown in a medium containing 2 μM Cu^{2+}. Data are nmol/μmol chlorophyll

	Plastocyanin	Plastidic cytochrome c-553
Chrysophyceae		
Phaeodactylum tricornutum	–	1.6
Skeletonema costatum	–	2.2
Xanthophyceae		
Bumilleriopsis filiformis	–	3.6
Vischeria stellata	–	2.4
Tribonema aequale	–	2.7
Bumilleria sicula	–	2.2
Phaeophyceae		
Ectocarpus siliculosus	–	2.3
Cutleria multifida	–	4.5[a]
Rhodophyceae		
Cyanidium caldarium	–	2.4
Porphyridium cruentum	–	3.3
Porphyridium aerugineum	–	2.3
Polysiphonia sp.	–	4.6[a]

[a] These species were collected at their natural marine habitat, the copper concentration there was determined as 0.08 μM

Table 4
Cross-reactions of plastocyanin-containing homogenates from Cu^{2+}-supplemented *Chlorella* strains with an antibody against *Scenedesmus* plastocyanin

Homogenates from	Cross-reaction
C. sorokiniana	
211-8k	–
211-40a	–
C. vulgaris	
211-11b	–
211-11c	+
211-19	–
C. saccharophila	
211-9a	–
C. fusca var. *vacuolata*	
211-8b	+
C. fusca var. *fusca*	
343	–
C. fusca var. *rubescens*	
232/1	+
C. minutissima	
C-1.1.9	–
C. homosphaera	
211-8e(Ca.)	–
C. kessleri	
C-1.1.1	+
C. luteoviridis	
211-2b	–
C. protothecoides	
211-7a	+
211-10a	+

About two thirds of all genera of green algae assayed can synthesize both plastocyanin *or* cytochrome c-553. This behavior was studied in detail for *Scenedesmus* (Bohner and Böger 1978) and *Chlamydomonas* (Wood 1978). Recently, the presence of both redox proteins was reported for *Enteromorpha prolifera* which had been collected from the sea (Yoshizaki et al. 1981). One third of the green algae investigated resemble higher plants by exclusively forming plastocyanin, indicating their inability to counteract copper deficiency by cytochrome c-553 formation. Consequently, their plastocyanin content is small when the copper supply is low, as was demonstrated for oat and spinach (Baszyński et al. 1978), as well as parsley (Sandmann, unpublished).

Some data on plastocyanin and cytochrome c-553 content of blue-green algae cultured in copper-deficient or copper-supplemented media are available (Sandmann and Böger 1983). In this group, the cytochrome c-553 containing type is predominant. However, some of the blue-green algae investigated so far, including *Anabaena variabilis* and *Plectonema boryanum* (Sandmann and Böger 1980b) are able to mutually exchange plastocyanin versus cytochrome c-553.

Based on the results mentioned, a line of evolution can be drawn starting from blue-green algae, with species containing cytochrome c-553 through green algae that show a plastocyanin-cytochrome c-553 exchange, to higher plants which form plastocyanin only. This relationship is further substantiated by the finding that some species of a group examined already show the properties of the next group in this progression, indicated by their ability of forming plastocyanin or cytochrome c-553. Consequently, the algal group of Table 3, which do not produce plastocyanin, should have evolved their chloroplast apparatus independently from the Cyanophyta-Chlorophyta-Spermatophyta line. The Euglenophyceae may be added to this group, since no plastocyanin was found in *Euglena gracilis* either (see Wildner and Hauska 1974; Sandmann and Böger 1983).

Chlamydomonas mundana described by Wood (1978) to be free of plastocyanin in copper-supplemented culture does not fit into this scheme. Therefore, it must be regarded as a species that lost its regulatory response to copper due to its natural sulfide-containing habitat where no soluble Cu^{2+} is available.

In addition, our results are of interest for the taxonomy of *Chlorella*. This genus was found to be very heterogeneous biochemically (Kessler 1976, 1978) and to comprise at least three more or less unrelated groups of species (Kerfin and Kessler 1978; Kümmel and Kessler 1980; Kessler 1982). Thus, it is not surprising to see that both types of response to copper-deficient growth conditions can be observed within this genus (Table 2). Fifteen strains produce only plastocyanin, and 13 strains are able to form cytochrome c-553. Usually, different strains of the same species react the same way. Within *C. sorokiniana* and *C. vulgaris*, however, both types appear, and it is interesting to note that especially the thermophilic species *C. sorokiniana* was found to be much more heterogeneous than the other *Chlorella* species (Kessler 1976). The species of the "*C. fusca* group", i.e., the three varieties of *C. fusca* and *C. zofingiensis*, are uniformly of the cytochrome c-553 forming type. Among these species, *C. fusca* var. *fusca* was shown to belong to the genus *Scenedesmus* (Fott et al. 1975; Hegewald and Schnepf 1979), and serological studies indicated that the same may be true also for the other taxa (Kümmel and Kessler 1980; Kessler 1982). The *Scenedesmus* strains studied, too, produce cytochrome c-553 in copper-deficient media (Table 1). Serological cross-reactions of plastocyanin-containing extracts from some *Chlorella* strains with an antibody against plastocyanin from *Scenedesmus* can, however, not be interpreted as results of immediate interest in this connection (Table 4).

Acknowledgements. This study was supported by the Deutsche Forschungsgemeinschaft. The authors are grateful to Drs. J. Fukuda, Tokyo, and D. G. Müller, Konstanz, for providing several algal strains. Due thanks are expressed to Mrs. Silvia Kuhn (Konstanz) and Ullrike Knoch (Erlangen) for expert technical assistance.

References

Baszyński T, Ruszkowkska M, Król M, Tukendorf A, Wolińska D (1978) The effect of copper deficiency on the photosynthetic apparatus of higher plants. Z Pflanzenphysiol 89:207–216

Böhme H (1978) Reactions of antibodies against ferredoxin, ferredoxin-NADP$^+$ reductase, and plastocyanin with spinach chloroplasts. Eur J Biochem 84:87–93

Bohner H, Böger P (1978) Reciprocal formation of cytochrome c-553 and plastocyanin in *Scenedesmus*. FEBS Lett 85:337–339

Bohner H, Merkle H, Kroneck P, Böger P (1980) High variability of the electron carrier plastocyanin in microalgae. Eur J Biochem 105:603–609

Crofts AR, Wood PN (1978) Photosynthetic electron transport chains of plants and bacteria and their role as proton pumps. In: Sanadi DR, Vernon LP (eds) Current topics in bioenergic, vol 7. Academic Press, New York San Francisco London, pp 175–224

Enami J, Fukuda J (1975) Mechanisms of the acido- and thermophily of *Cyanidium caldarium* Geitler. I. Effects of temperature, pH and light intensity on the photosynthetic oxygen evolution of intact and treated cells. Plant Cell Physiol 16:211–220

Fott B, Lochhead R, Clémençon H (1975) Taxonomie der Arten *Chlorella ultrasquamata* Clèm. et Fott and *Chlorella fusca* Shih. et Krauss. Arch Protistenk 117:288–296

Hegewald E, Schnepf E (1979) Geschichte und Stand der Systematik der Grünalgengattung *Scenedesmus*. Schweiz Z Hydrobiol 40:320–343

Hoogenhout H, Amesz J (1965) Growth rates of photosynthetic micro-organisms in laboratory cultures. Arch Mikrobiol 50:10–25

Kerfin W, Kessler E (1978) Physiological and biochemical contributions to the taxonomy of the genus *Chlorella*. XI. DNA hybridization. Arch Microbiol 116:97–103

Kessler E (1976) Comparative physiology, biochemistry, and the taxonomy of *Chlorella* (Chlorophyceae). Plant Syst Evol 125:129–138

Kessler E (1978) Physiological and biochemical contributions to the taxonomy of the genus *Chlorella*. XII. Starch hydrolysis and a key for the identification of 13 species. Arch Microbiol 119:13–16

Kessler E (1982) Chemotaxonomy in the Chlorococcales. In: Round FE, Chapman DJ (eds) Progress in physiological research, vol I. Elsevier Biomedical Press, Amsterdam New York Oxford, pp 111–135

Kessler E, Czygan FC (1970) Physiologische und biochemische Beiträge zur Taxonomie der Gattung *Chlorella*. IV. Verwertung organischer Stickstoffverbindungen. Arch Microbiol 70:211–216

Kessler E, Zweier I (1971) Physiologische und biochemische Beiträge zur Taxonomie der Gattung *Chlorella*. V. Die auxotrophen und mesotrophen Arten. Arch Microbiol 79:44–48

Kümmel H, Kessler E (1980) Physiological and biochemical contributions to the taxonomy of the genus *Chlorella*. XIII. Serological studies. Arch Microbiol 126:15–19

Kunert KJ, Böger P (1975) Absence of plastocyanin in the alga *Bumilleriopsis filiformis* and its replacement by cytochrome c-553. Z Naturforsch 30c:190–200

Sandmann G, Böger P (1980a) Physiological factors determining formation of plastocyanin and cytochrome c-553 in *Scenedesmus*. Planta (Berlin) 147:330–334

Sandmann G, Böger P (1980b) Copper-induced exchange of plastocyanin and cytochrome c-553 in cultures of *Anabaena variabilis* and *Plectonema boryanum*. Plant Sci Lett 17:417–424

Sandmann G, Böger P (1981a) Plastocyanin and cytochrome c-553, two different electron donors to photosystem I in algae. In: Akoyunoglou G (ed) Proc 5th Int Congr Photosynthesis, vol II. Balaban International Science Services, Philadelphia, pp 623–632

Sandmann G, Böger P (1981b) Influence of light on plastocyanin formation in the alga *Scenedesmus acutus*. Photosynth Res 2:281–289

Sandmann G, Böger P (1983) The enzymological function of heavy metals and their role in electron-transfer processes of plants. In: Läuchli A, Bielesky RL (eds) Encyclopedia of plant physiology: Inorganic plant nutrition, vol 12. Springer, Berlin Heidelberg New York (in press)

Starr RC (1978) The Culture Collection of Algae at the University of Texas at Austin. J Phycol 14:47–100

Visser JWM, Amesz J, van Gelder BF (1974) EPR signals of oxidized plastocyanin in intact algae. Biochim Biophys Acta 333:279–287

Wildner FG, Hauska G (1974) Localization of the reaction site of cytochrome c-553 in chloroplasts from *Euglena gracilis*. Arch Biochem Biophys 164:127–135

Wood PM (1978) Interchangeable copper and iron proteins in algal photosynthesis. Eur J Biochem 87:9–19

Yoshizaki F, Sugimura Y, Shimokoriyama M (1981) Purification, crystallization, and properties of plastocyanin from a green alga, *Enteromorpha prolifera*. J Biochem 89:1533–1539

Received October 27, 1982/Accepted December 23, 1982