RECIPROCAL FORMATION OF CYTOCHROME *c*-553 AND PLASTOCYANIN IN *SCENEDESMUS*

Herbert BOHNER and Peter BÖGER
Fachbereich Biologie, Universität Konstanz, D-7750 Konstanz, FRG

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1. Introduction

All algae so far examined contain a soluble plastidic *c*-type cytochrome. Some lack plastocyanin as was shown for *Bumilleriopsis filiformis*, using various preparative methods, EPR or antibody techniques [1]. Lack of plastocyanin was also shown for *Euglena* (see e.g. [2]) and for a mutant of *Chlamydomonas* [3]. With *Bumilleriopsis* and *Euglena* it could be demonstrated that the soluble *c*-type cytochrome takes over the role of electron donor during P700 reduction.

Algal chloroplasts contain a second *c*-type cytochrome firmly bound to the photosynthetic membranes [4]. This cytochrome has been isolated [5] from three species and its occurrence confirmed in this laboratory for *Bumilleriopsis* and *Scenedesmus* [6]. This heme protein resembles cytochrome *f* from the plastids of higher plants and will be so referred to here. In contrast to the soluble cytochrome *c* it exhibits a very asymmetrical β-band and a shift of the γ-band to longer wavelengths (421 nm).

Scenedesmus (or *Chlorella*) contain plastocyanin in addition to the soluble cytochrome *c* and cytochrome *f*. Since in these species the soluble cytochrome *c* is apparently the electron donor for the photosystem-I reaction center [7], the questions arise whether this cytochrome and plastocyanin are interchangeable in vivo and whether their formation is interrelated. Here we report that the two redox proteins can replace each other in *Scenedesmus* chloroplasts.

2. Materials and methods

Scenedesmus acutus (strain 276-3a, Algal Culture Collection, Göttingen) was grown autotrophically at varying copper concentrations in sterile liquid mineral medium at 22–23°C as described [7], each culture vessel contained 2.5 liter. The cultures had a density of 10^5 cells/ml at the start and were generally harvested after 62 h growth period when the density was $2.3–2.5 \times 10^7$ cells/ml. The inocula were from cultures with 0.21 μM copper in the medium.

Washed cells were suspended in 20 mM Tricine buffer, pH 8.0, [*N*-tris-(hydroxymethyl)-methyl glycine, adjusted with NaOH] and broken in a Vibrogen-Zellmühle (Bühler, Tübingen) for 5 min at 2–4°C. After removal of debris by low-speed centrifugation (3 min, $800 \times g$) the homogenate was centrifuged at $200\,000 \times g$ for 4–5 h. In the clear supernatant the soluble cytochrome *c*-553 (the α-peak was found at 553 nm) was determined by difference spectroscopy (oxidized versus reduced form; ferricyanide/ascorbate; ϵ [mM^{-1} cm^{-1}] = 17.3 at 553 nm). The pellet was resuspended in the buffer mentioned including 1% Triton X-100, adjusted to 100 μg chlorophyll/ml and the bound cytochrome *f*-553 (α-peak at 553 nm) determined from the ferricyanide/hydroquinone difference spectra (ϵ [mM^{-1} cm^{-1}] = 20; c.f. [8]).

Plastocyanin was extracted according to [9] with modifications. After removal of the green fragments by 35% saturated ammonium sulfate and centrifugation, more ammonium sulphate was added to the

resulting supernatant to 55% saturation. The supernatant obtained after centrifugation (25 000 × g, 15 min) was used for plastocyanin determination as noted in the legend to fig.1. An extinction coefficient of 4.9 [mM^{-1} cm^{-1}] for a 1-copper chromophore was used (c.f. [10]). It should be noted that due to some accompanying soluble cytochrome c-553 (fig.1, trace b) an enzymatic assay (e.g., with photosystem-I particles [7]) for quantitative plastocyanin determination could not be carried out.

3. Results and discussion

Employing the mineral medium of [12] supplemented with trace elements according to [13] the soluble cytochrome c-553 was found to be in the range 0.5–0.7 nmol/μmol total chlorophyll during the first hours of cultivation. It increased with cultivation time, approaching 3 nmol/μmol chlorophyll after 7–10 days. Changing the medium to that in [14], which generally has a higher concentration of all trace elements, the level of soluble cytochrome was 0.1 nmol/μmol cytochrome c-553 at the start and later attained a maximum of about 0.7 nmol/μmol chlorophyll only. This low level is, however, not due to the general increase of the mineral content. Increasing the iron in the first medium from 0.036–0.36 mM did not change the cytochrome level. Only an increase of copper content 'induced' a decrease of cytochrome c-553. The medium according to [12,13] contained 0.02 μM copper only and led to a high cytochrome c-553 content whereas that from [14] had 0.32 μM copper which resulted in low cytochrome levels. Figure 2A demonstrates this effect in more detail.

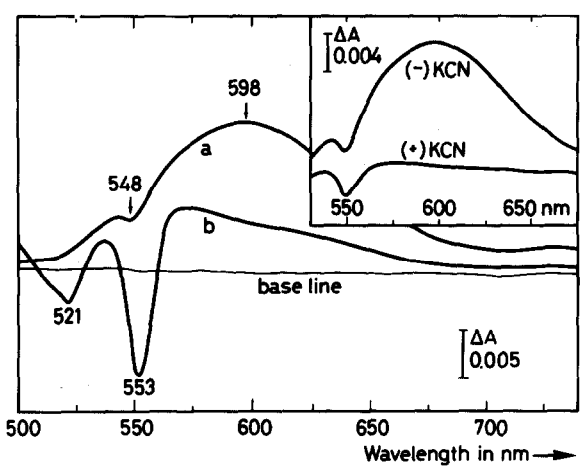

Fig.1. Determination of plastocyanin in extracts from broken *Scenedesmus* by difference redox spectroscopy (cuvettes containing some solid ferricyanide or ascorbate, respectively). Sample (a) is from cultures with the medium containing 0.14 μM copper at the start; sample (b) is from a culture with 0.01 μM copper. In (b) soluble cytochrome c-553 is present (β-band at 521 nm), whereas it is absent in (a). The small 548 nm trough is due to low amounts of an unknown, but consistently observed cytochrome. The inset indicates complete loss of the 598 nm absorption band, after treatment with KCN (30 mM, 1–2 min; c.f. [10]).

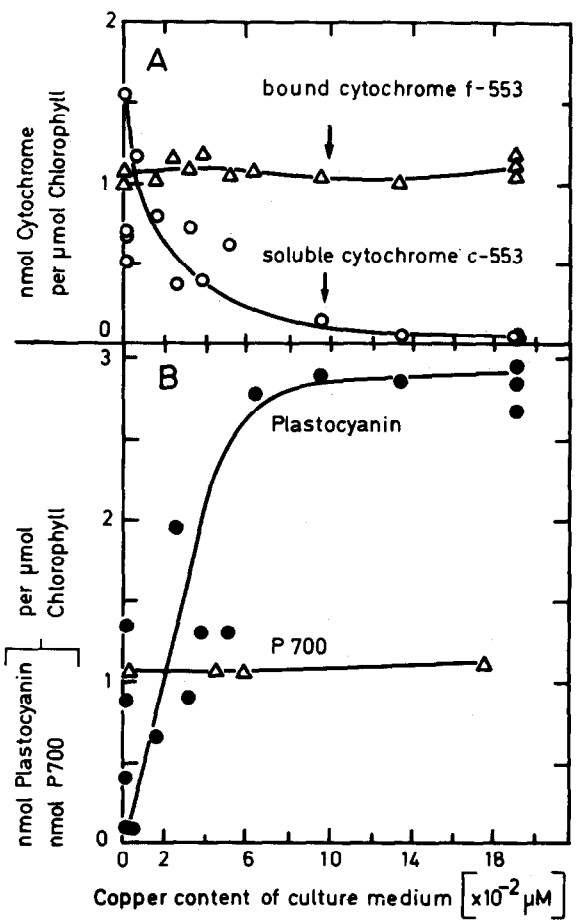

Fig.2. (A) Amounts of soluble cytochrome c-553 and bound cytochrome f-553 in *Scenedesmus* cells cultured with increasing copper content; medium according to [12]. (B) Amounts of plastocyanin and pigment 700 under the same conditions (see section 2).

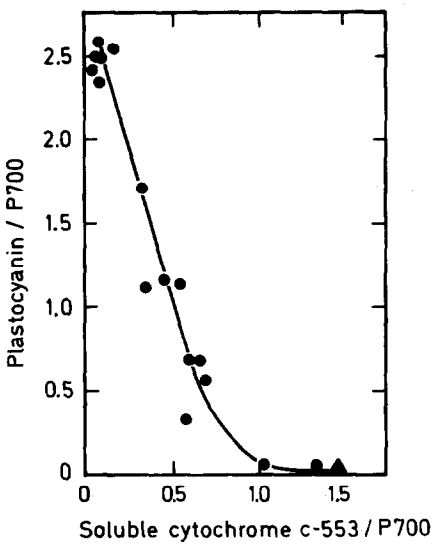

Fig.3. Quantitative molar relationship of plastocyanin to cytochrome *c*-553 in *Scenedesmus* under conditions of varying amounts of both redox proteins in the plastid.

While the soluble cytochrome *c*-553 decreases with increasing copper concentration and is almost undetectable after growth in a medium with about 0.2 μM copper, the amount of bound cytochrome *f*-553 remained unchanged, as did the level of P700 (fig.2B). It should be noted that the increase of copper in the medium as indicated influenced neither the growth rate nor the chlorophyll content of the cell ($1.5-2 \times 10^{-6}$ μg/cell).

Concurrently with the decrease of cytochrome *c*-553 the level of plastocyanin rose from zero to 3 nmol/μmol chlorophyll and remained constant with copper concentrations between 0.12 and 0.25 μM. There is a clear reciprocal relationship although, as shown in fig.3, the sum of both variable redox proteins is not constant at different copper concentrations in the medium but always exceeds the molar amount of P700.

Triton X-100 has been shown to be very effective in removing plastocyanin from thylakoids [15]. The spectroscopic assays for both cytochrome and plastocyanin are very reliable. The samples exhibit the typical sensitivity of the plastocyanin chromophore towards KCN (fig.1). The $A_{598\ nm}$ could be quantitatively related to EPR-signal heights and plastocyanin added to the samples proved to be stable in the extraction procedure used in its determination.

Since it was shown that soluble cytochrome *c*-553 acts as a direct electron donor to P700 in *Bumilleriopsis* which lacks plastocyanin, the experiments described here with *Scenedesmus* give strong evidence that plastocyanin and cytochrome *c*-553 have the same functional role as electron donor for P700.

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References

[1] Kunert, K.-J. and Böger, P. (1975) Z. Naturforsch. 30c, 190–200.
[2] Wildner, G. F. and Hauska, G. (1974) Arch. Biochem. Biophys. 164, 127–135.
[3] Wood, P. M. (1977) 4th Int. Congr. Photosynth., Reading, England, Abst., pp. 415–416.
[4] Grimme, L. H. and Boardman, N. K. (1974) in: Proc. 3rd Int. Congr. Photosynth. (Avron, M. ed) pp. 2115–2124, Elsevier, Amsterdam.
[5] Wood, P. M. (1977) Eur. J. Biochem. 72, 605–612.
[6] Böger, P. (1978) in: Proc. 4th Int. Congr. Photosynth. (Hall, D. O. et al. eds) pp. 755–764, The Biochemical Soc., London.
[7] Kunert, K.-J., Böhme, H. and Böger, P. (1976) Biochim. Biophys. Acta 449, 541–553.
[8] Lach, H.-J. and Böger, P. (1975) Z. Naturforsch. 30c, 628–633.
[9] Powls, R., Wong, J. and Bishop, N. J. (1969) Biochim. Biophys. Acta 180, 490–499.
[10] Siegelman, M. H., Rasched, I. R., Kunert, K.-J., Kroneck, P. and Böger, P. (1976) Eur. J. Biochem. 64, 131–140.
[11] Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 267, 160–171.
[12] Soeder, C. J., Schulze, G. and Thiele, D. (1967) Arch. Hydrobiol., Suppl. 33, 127–171.
[13] Payer, H.-D. and Trültsch, U. (1972) Arch. Mikrobiol. 84, 43–53.
[14] Miller, J. D. A. and Fogg, G. E. (1957) Arch. Mikrobiol. 28, 1–17.
[15] Sane, P. V. and Hauska, G. A. (1972) Z. Naturforsch. 27b, 932–938.