SHORT COMMUNICATIONS

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Nature of heme moiety and oxidation–reduction potential of cytochrome 558 in Euglena chloroplasts

Precise knowledge of the cytochromes in the chloroplasts is a requisite for the elucidation of the electron transport of photosynthesis.

Three cytochromes are known to occur in the chloroplasts of green plants, *i.e.* cytochrome *f*, cytochrome 558 and cytochrome b_6 (refs. 1–4). Among these, cytochrome *f* is well characterized, while the other two components remain largely unelucidated. In the present work, the redox potential of cytochrome 558 was determined in its chloroplast-bound state. It will also be shown from a study of its pyridine hemochrome that cytochrome 558 is another *b*-type cytochrome in the chloroplast.

The chloroplasts were prepared from autotrophically grown cells of *Euglena gracilis* as described previously, except that 0.05 M Tricine–KOH buffer (pH 6.5) was used in place of phosphate buffer[5].

The redox reactions of the cytochromes in the chloroplasts induced by the addition of redox reagents in the dark were followed with a Chance-type dual wavelength spectrophotometer. As shown in Fig. 1, addition of a minute amount (20 mμmoles) of ferricyanide to the chloroplast suspension caused a marked drop in absorbance. The final constant level of absorbance thus attained was not altered by

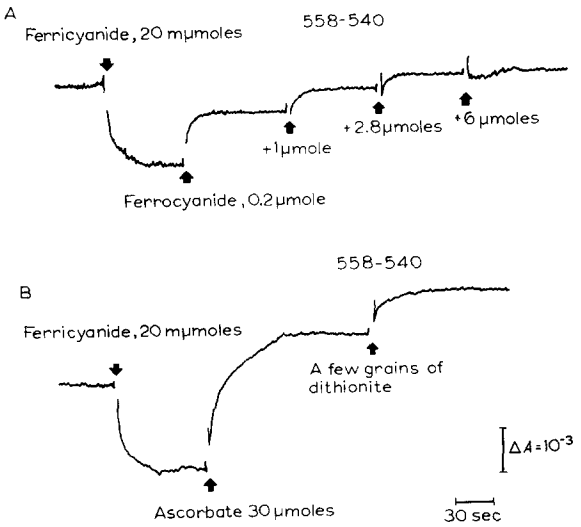

Fig. 1. Absorption changes at 558 mμ of cytochromes in Euglena chloroplasts induced by the addition of redox reagents. Reaction mixture contains, in a final volume of 2 ml, 0.4 M sucrose, 0.05 M Tricine–KOH buffer (pH 6.5), 0.01 M KCl and 80 μg chlorophyll *a*. Reference wavelength, 540 mμ.

further addition of ferricyanide. Subsequent addition of ferrocyanide, ascorbate or dithionite to the reaction mixture caused a rapid increase in absorbance to reach a new steady level (Fig. 1, A, B).

The reduced *minus* oxidized difference spectra obtained with the use of saturating amounts of these reductants are depicted in Fig. 2. An absorption peak at 558 mμ was obtained in the ferrocyanide- (or ascorbate-)reduced *minus* ferricyanide-oxidized difference spectrum. The spectrum obtained with dithionite showed an absorption band with a flattened peak, indicating the presence of another cytochrome component having an absorption peak at a longer wavelength. In fact, a peak at 563 mμ was discovered on the dithionite-reduced *minus* ascorbate-reduced difference spectrum. These two absorption bands observed in Euglena chloroplasts are similar to those observed previously in the chloroplasts of other green plants (*e.g.* Chlamydomonas, spinach), and correspond to the α band of cytochromes 558 and 563 (b_6)[2-4]. It will be noted that cytochrome *f* is readily released from the chloroplasts of Euglena on isolation in the ordinary medium, a circumstance which greatly facilitates the spectrophotometric determination under investigation.

The relative amounts of cytochromes 558 and 563 in Euglena chloroplasts were estimated from the difference spectra, assuming a value of $2.07 \cdot 10^4$ for the difference molar extinction coefficient ($\varepsilon_{M\ red-ox}$) for either cytochromes[6]. The amounts of the two cytochromes were the same, amounting to 1 mole of each cytochrome to 340 moles of chlorophyll *a*.

Cytochrome 558 in chloroplasts has generally been assumed to be of *b*-type, but without any substantial evidence. The absorption spectrum of the pyridine hemochrome prepared by incubating the Euglena chloroplasts with 20% pyridine and 0.2 M NaOH showed, when reduced, only one peak at 558 mμ and no band nor shoulder at 551 mμ, thus indicating the presence of protoheme, and absence of heme *c*. In addition, the total protoheme content of the isolated chloroplasts (estimated by assuming the difference molar extinction coefficient of pyridine hemochrome of protoheme as $2.07 \cdot 10^4$ ($\varepsilon_{M\ red-ox}$)[9]), amounted to 1 mole of protoheme to 170 moles of chlorophyll *a*; precisely accounting for the actually estimated amounts of cytochromes

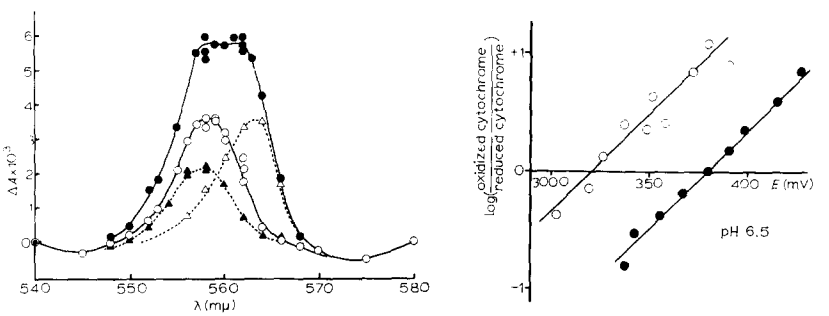

Fig. 2. Reduced *minus* oxidized difference spectra of cytochromes in Euglena chloroplasts. Chlorophyll *a* concentration, 50 μg/ml. Other experimental conditions are the same as in Fig. 1. ●—●, dithionite-reduced *minus* ferricyanide-oxidized; ○—○, ascorbate-reduced *minus* ferricyanide-oxidized; ▲---▲, ferrocyanide-reduced *minus* ferricyanide-oxidized; △---△, dithionite-reduced *minus* ascorbate-reduced.

Fig. 3. Normal redox potentials of Euglena cytochrome 558 (○—○) and cytochrome 552 (●—●). A value of 409 mV (ref. 10) was used for the normal redox potential of ferricyanide–ferrocyanide couple.

558 and 563 in the chloroplasts. This makes a decisive proof that cytochrome 558, as well as cytochrome 563, is a *b*-type cytochrome.

The magnitudes of absorbance changes at 558 mμ (Fig. 1, A) depended solely on the ratio of ferricyanide to ferrocyanide rather than the amounts of ferrocyanide added. Fig. 3 shows the linear relationship between the logarithms of the ratio of ferri- to ferrocytochrome 558 and the redox potential of the ferri- and ferrocyanide mixture in the reaction medium. The dip of the straight line corresponded to a one electron transfer involved. From Fig. 3, the normal redox potential of cytochrome 558 bound to the chloroplast was estimated to be 320 mV at pH 6.5 and at room temperature. The same value of normal potential was obtained between pH 6 and 9. Fig. 3 also shows that the normal redox potential of the isolated Euglena cytochrome 552 is 380 mV, which is in close agreement with the values reported previously[7].

These observations indicate that the chloroplast-bound Euglena cytochrome 558 under investigation is different from the "soluble" Euglena cytochrome 558 (556) which has been isolated from green and etiolated cells of Euglena, and reported to have a normal potential of 307 mV and have heme *c* as prosthetic group[7,8].

For comparison, cytochrome 558 in spinach (chloroplast-bound) was also studied. It was found to be reduced with ascorbate and ferrocyanide, and, therefore, must have a redox potential similar to that of Euglena, although the concomitant presence of cytochrome *f* in spinach chloroplasts makes an accurate determination difficult. In contrast, cytochrome 558 in the chloroplasts of *Porphyra yezoensis* was not reduced by ferrocyanide.

There is evidence that cytochrome 558 in the chloroplasts is functioning as electron carrier in the electron transport chain connecting the two photosystems of photosynthesis[2–4]. If this is the case, its relatively high redox potential makes it reasonable to assume that cytochrome 558 is functioning at a site close to cytochrome *f*. The small difference in redox potential of these two cytochromes disqualifies this step as a site of photosynthetic ATP synthesis.

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