LOCALIZATION OF LOW POTENTIAL CYTOCHROME *b*-559 IN PHOTOSYSTEM I

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1. Introduction

The earlier work of Hill and coworkers [1], showed that chloroplasts contain a *c*-type cytochrome with an α-band at 554 nm and a midpoint potential of + 0.36 V at pH 7 (cytochrome *f*) and an autoxidizable *b*-type cytochrome with an α-band at 563 nm and a potential of about zero (cytochrome b_6). Subsequent studies [2, 3] established the presence in chloroplasts of a nonautoxidizable *b*-type cytochrome with an α-band at 559 nm. This cytochrome has a midpoint potential close to that of cytochrome *f*, but it is sensitive to a variety of treatments which convert it to a form of lower potential [4, 5]. The situation is further complicated by the discovery [6] that chloroplasts also contain an autoxidizable *b*-type cytochrome with an α-band at 559 nm. Its redox potential is somewhat more positive than that of cytochrome b_6, but considerably more negative than the nonautoxidizable cytochrome *b*-559. It is designated cytochrome *b*-559 – low potential (LP) to distinguish it from the nonautoxidizable cytochrome, which is designated cytochrome *b*-559 high potential (HP).

Fractionation of the photochemical systems with digitonin [3, 7] showed that cytochromes *f* and b_6 are associated with Photosystem I (PS I) and cytochrome $b\text{-}559_{HP}$ with Photosystem 2 (PS II). Recently, Nelson and Neumann [8] reported the isolation from PS I subchloroplast fragments of a complex containing both cytochromes b_6 and *f*.

In the present studies we demonstrate that cytochrome $b\text{-}559_{LP}$ also is located in PS I, and it is present in the cytochrome b_6–*f* complex of Nelson and Neumann [8].

2. Materials and methods

2.1. Isolation of PS I fragments and the Nelson–Neumann complex

Spinach plants were grown in nutrient solution and chloroplasts isolated in 0.05 M phosphate buffer, pH 7.2, containing 0.3 M sucrose and 0.01 M KCl [7]. Chloroplasts (540 μg chl/ml) were incubated with 0.5% digitonin for 30 min at 0° [3]. The mixture then was diluted with 7 vol of 0.05 M phosphate buffer pH 7.2, and fractionated by differential centrifugation [3]. PS I fragments were collected by centrifugation of the 50,000 *g* supernatant for 1 hr at 144,000 *g* and suspended in 0.05 M phosphate buffer pH 7.2.

The cytochrome b_6–*f* particles were prepared by the procedure of Nelson and Neumann [8] except that the final steps of gel filtration with Bio-Gel P-200 and precipitation with ammonium sulphate were omitted. After DEAE chromatography, the cytochrome b_6–*f* particles were concentrated by pressure ultrafiltration.

2.2. Cytochrome difference spectra

Reduced *minus* oxidized difference spectra were recorded on a Cary Model 14R spectrophotometer, fitted with a scattered transmission accessory [3] and a modified Bonner cuvette assembly [9]; the optical pathlength was 4 mm. Hydroquinone, sodium ascorbate and potassium ferricyanide were added to a final conc. of 6.5 mM, and dithionite was added as the solid. Reduction of cytochromes with ferrooxalate was carried out by adding the sample to an equal volume of 1.0 M potassium oxalate in 0.1 M phosphate buffer, pH 7.2.

Ferrous sulphate, freshly prepared and kept anaerobically, was then added to a final conc. of 29 mM. To balance the yellow colour of ferrooxalate split cuvettes were used for measurements at 25°C, and a yellow filter of cellophane was used on the reference side for measurements at 77°K. For spectral measurements at 77°K, the medium contained 65% by volume of glycerol.

3. Results and discussion

Reduced *minus* oxidized difference spectra of the Nelson–Neumann complex at 77°K are shown in fig. 1. The hydroquinone reduced *minus* ferricyanide oxidized difference spectrum shows bands at 552 nm and 548 nm in the α-band region. These bands indicate the presence of cytochrome *f* [3], but there is no trace of cytochrome *b*-559$_{HP}$ (α-band at 557 nm at 77°K [3]). The dithionite reduced *minus* ferricyanide oxidized difference spectrum at 77°K has bands at 557 nm and 561 nm in addition to the bands of cytochrome *f*. The dithionite reduced *minus* hydroquinone reduced spectrum also shows bands at 557 nm and 561, but the bands of cytochrome *f* are small. The relative heights of the bands at 557 nm and 561 differ in figs. 1B and 1C, but this can be attributed to the slope of the baseline in 1B, and partly to the presence of the overlapping bands of cytochrome *f*. The difference spectra of the Nelson–Neumann complex closely resemble those obtained previously with PS I fragments (cf. fig. 6 [3]). The relative heights of the 557 and 561 nm bands in dithionite reduced *minus* hydroquinone reduced spectra are similar for chloroplasts, PS I fragments and the Nelson–Neumann complex. In our earlier paper [3], the bands at 557 nm and 561 nm in PS I fragments were tentatively attributed to a splitting of the α-band of cytochrome b_6 at liquid nitrogen temperature, but we mentioned the possibility that chloroplasts might contain a fourth cytochrome. Bendall [6, 10] distinguished two autoxidizable *b*-type cytochromes in chloroplasts (cytochromes b_6 and *b*-559$_{LP}$) because cytochrome *b*-559$_{LP}$ was reduced more rapidly by dithionite, compared with cytochrome b_6.

Fig. 2 shows a series of dithionite reduced difference spectra of PS I fragments at 77°K. The fragments in the sample cuvette were reacted with dithionite for at least 20 min at room temp., while the fragments in the reference cuvette were reacted for shorter periods.

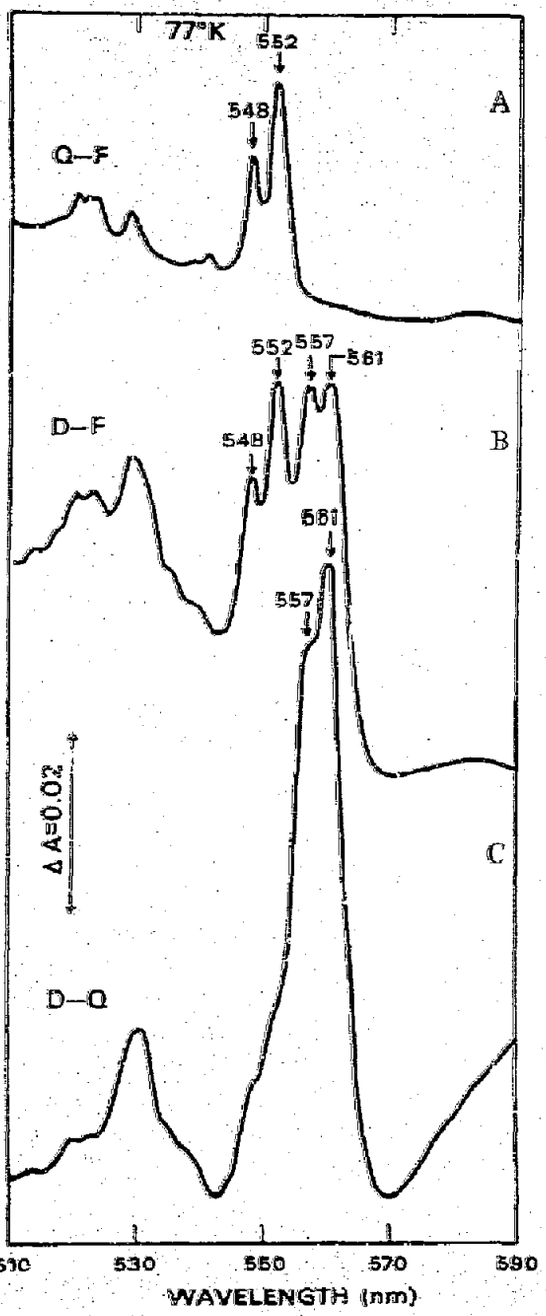

Fig. 1. Cytochrome difference spectra of Nelson–Neumann complex at 77°K. Hydroquinone reduced *minus* ferricyanide oxidized (Q–F). Dithionite reduced *minus* ferricyanide oxidized (D–F). Dithionite reduced *minus* hydroquinone reduced (D–Q). Optical pathlength, 4 mm.

The spectrum shown in fig. 2A was recorded immediately after addition of dithionite to the reference cuvette. The band at 557 nm is larger than the 561 nm band. With increasing time of reaction with dithionite, (fig. 2B and C) there is a decrease in the height of the

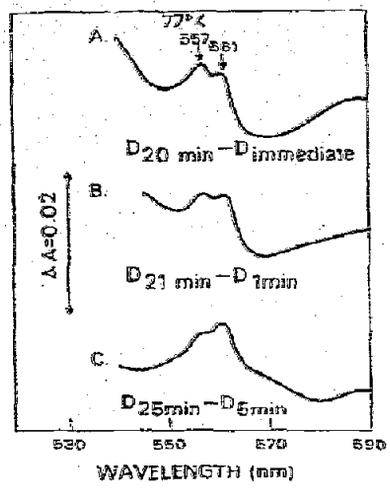

Fig. 2. "Long time" *minus* "short time" dithionite reduction of cytochromes of PS I fragments at 77°K. Chlorophyll concentration, 240 μg chl/ml; optical pathlength, 4 mm. A) $D_{20\,min}$ *minus* $D_{immediate}$; dithionite was added to the sample cuvette and allowed to react for 20 min at room temp. before freezing. The reference cuvette was cooled after the addition of dithionite. The cuvette assembly was then thawed and left for 1 min at room temp. prior to freezing to give spectrum B) $D_{21\,min}$ *minus* $D_{1\,min}$, and subsequently for an additional 4 min at room temp. to give C) $D_{25\,min}$ *minus* $D_{5\,min}$.

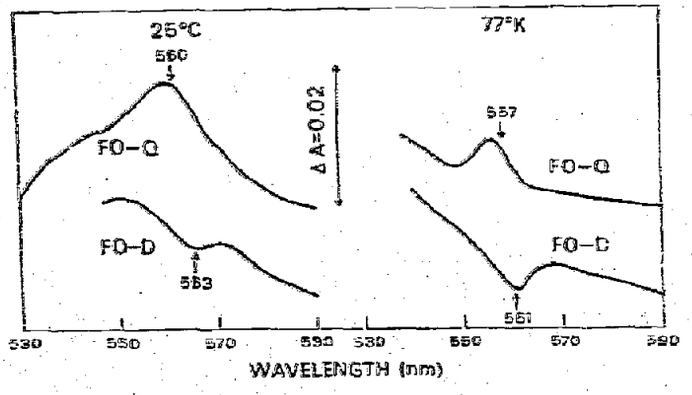

Fig. 3. Cytochrome difference spectra of PS I fragments at 25°C and 77°K. Ferrooxalate reduced *minus* hydroquinone reduced (FO−Q). Ferrooxalate reduced *minus* dithionite reduced (FO−D). The chlorophyll concentration of the sample was 200 μg chl/ml at 25°C and 112 μg chl/ml at 77°K; optical pathlength, 4 mm.

band at 557 nm, relative to that at 561 nm. Since cytochrome b-559_{LP} is reduced more rapidly than cytochrome b_6 [10], it would seem that the band at 557 nm at low temperature is due to cytochrome b-559_{LP} and the band at 561 nm to cytochrome b_6.

More convincing evidence for this conclusion is provided if ferrooxalate is used as the reducing agent. The midpoint potential of the ferri–ferrooxalate couple is zero at pH 7 [11]. Fig. 3 shows both room temp. and low temperature difference spectra of PS I fragments. The ferrooxalate *minus* hydroquinone difference spectra show maxima at 560 nm at 25°C and 557 nm at 77°K, while the ferrooxalate *minus* dithionite difference spectra show troughs at 564 nm at 25°C and 561 nm at 77°K. Under the incubation conditions with the PS I fragments, cytochrome b-559_{LP} is reduced by ferrooxalate, but cytochrome b_6 is only reduced by the stronger reducing agent, dithionite. The cytochrome b_6–f particles gave a similar result, except that there was some reduction of cytochrome b_6 by ferrooxalate under the conditions of incubation, and the separation of the bands at 557 nm and 561 nm was not as complete as with PS I fragments.

The present studies indicate that cytochrome b-559_{LP} is located in PS I, together with cytochromes f and b_6. There appears to be a close association of the three cytochromes, since they are retained together in the Nelson–Neumann complex which is devoid of chlorophyll. On the other hand, cytochrome b-599_{HP} is located in PS II. As mentioned earlier, cytochrome b-559_{HP} is convertible to forms of lower potential [4, 5]. It seems likely that these forms will remain associated with PS II and therefore they are distinguishable from cytochrome b-559_{LP}. Further evidence that cytochrome b-559_{LP} is associated with cytochromes f and b_6 is provided by the cytochrome composition of etioplasts from dark-grown seedlings [12, 13]. The three cytochromes are found in etioplasts, but cytochrome b-559_{HP} is absent.

The location of two b-cytochromes in photosystem I is intriguing in terms of the function of the cytochromes in the chloroplast.

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