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Cytochrome *b*-560, a new component of thylakoid membranes

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During a survey of a series of electron transport mutants of *Chlamydomonas reinhardtii*, we have observed a hitherto unrecognised component, cytochrome *b*-560, in a strain (F18) which entirely lacks the cytochrome *bf* complex. This component is present at approx. 1 nmol / mg chlorophyll, has a midpoint potential of −125 mV ($n = 1$) at pH 7.2 and is slowly reduced by dithionite. The cytochrome was also observed in thylakoid membranes prepared from wild-type *Chlamydomonas* and from higher plants after extraction of the cytochrome *bf* complex by detergent treatment. It could not be observed in thylakoid membrane fragments prepared from the cyanobacterium *Phormidium laminosum*.

Introduction

In higher-plant chloroplasts, four membrane bound cytochromes have been recognised [1,2], i.e., cytochrome *f* (*c*-type, $E_{m,7} = +365$ mV), cytochrome *b*-559$_{HP}$ ($E_{m,7} = +370$ mV), cytochrome *b*-559$_{LP}$ ($E_{m,7} = +20$ mV) and cytochrome *b*-563 ($E_{m,7} = -110$ mV). Cytochromes *f* and *b*-563 are associated with the cytochrome *bf* complex, and cytochrome *b*-559$_{HP}$ with photosystem II, but the location of cytochrome *b*-559$_{LP}$ is not clear. In addition to these, *Chlamydomonas* chloroplasts contain soluble cytochrome *c*-552 as a functional alternative to plastocyanin if conditions are such that plastocyanin cannot be formed [3,4]. However, under the conditions used for the experiments described below, chloroplast fragments isolated from *Chlamydomonas* do not contain detectable amounts of cytochrome *c*-552. The cyanobacterium, *Phormidium laminosum*, also contains cytochrome *c*-549 [5], the function of which is not known.

In the course of a survey of a series of electron transport mutants of *Chlamydomonas* isolated by Dr. P. Bennoun, we have observed a hitherto unrecognised component, cytochrome *b*-560, in a strain (F18) which completely lacks the cytochrome *bf* complex. Detection of cytochrome *b*-560 in the presence of cytochrome *b*-563 is difficult owing to the similarity of their properties. This paper describes the properties of cytochrome *b*-560 in *Chlamydomonas* and reports evidence for its occurrence in chloroplasts from higher plants and its absence in membrane fragments from the blue-green alga *Phormidium laminosum*.

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Abbreviations: E_h, ambient redox potential with respect to standard hydrogen electrode; E_m, midpoint potential; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; HP, high potential; LiDS, lithium dodecyl sulphate; LP, low potential; Mega-9, nonanoylmethylglucamide; Mes, 4-morpholineethanesulphonic acid; PS, Photosystem.

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Materials and Methods

Biological materials

Cultures of *Chlamydomonas reinhardtii* were obtained from Dr. P. Bennoun, Institut de Biologie Physico-chimique, Paris, France. Liquid cultures were grown in the heterotrophic medium of Gorman and Levine [6] in a Gallenkamp Orbital Incubator at a shaking speed of 110 rev/min and 25 °C with continuous illumination at 200 lux. *Phormidium laminosum* was grown as previously described [5]. Pea seedlings (var. Superb) were grown in damp horticultural Perlite (Silvaperl) at 16 °C in a greenhouse. Seedlings were harvested after 10–14 days. Spinach (var. Virtuosa) was grown under artificial light with a 10-h day on moist Levington compost with the nutrient medium described by Walker [7].

Chloroplast fragments of *Chlamydomonas* [8] and membrane fragments from *P. laminosum* [10] were prepared as previously described. Chloroplasts from pea and spinach were isolated by the method of Cerović et al. [9] with the inclusion of an osmotic shock (10 mM $MgCl_2$ for 20 s between centrifugations followed by a return to isotonic medium). Chloroplasts from lettuce were prepared in the same way but with the addition of 10 mM sodium ascorbate to the grinding medium.

Photosystem II particles from pea were prepared by the method of Berthold et al. [11]. Photosystem II particles from *P. laminosum* were prepared as previously described [12] except that 0.34% dodecyldimethylamine oxide was used to extract photosystem II from the thylakoids.

Digitonin and octyl glucoside/cholate extractions of *Chlamydomonas* membranes were performed as previously described [8]. Sodium bromide-washed membranes of pea, spinach and lettuce were extracted as described in Refs. 14 and 14. Thylakoid membranes of *P. laminosum* were depleted of cytochrome *bf* complex by the following procedure. Membranes were resuspended in 0.4 M sucrose/10 mM Hepes (pH 7.5) at a chlorophyll concentration of 0.2 mg/ml and centrifuged for 10 min at $12000 \times g$. The membranes were resuspended in the same buffer to give a concentration of 2 mg chlorophyll/ml and an equal volume of 4 M NaBr/0.4 M sucrose/10 mM Hepes (pH 7.5) was added. The suspension was stirred for 30 min at 4 °C in darkness, then diluted with an equivalent volume of cold water and centrifuged for 20 min at $48000 \times g$. The membranes were washed once with 0.2 M sucrose/20 mM Hepes (pH 7.5) and resuspended with a Dounce homogeniser in a medium containing 0.2 M sucrose/20 mM Hepes (pH 7.5)/0.4 M $(NH_4)_2SO_4$ to give a chlorophyll concentration of 1.5 mg/ml. A stock solution of 200 mM Mega-9/3.3% (w/v) potassium cholate/20 mM Hepes (pH 7.5) was added to give the desired detergent concentration. After stirring for 30 min, the sample was centrifuged for 90 min at $200000 \times g$ and the depleted membranes were resuspended in 50 mM Mes (pH 6.0). Cytochrome *bf* complex was purified from lettuce by the method of Hurt and Hauska [13].

Analytical procedures

Cytochrome analysis. Cytochromes were assayed by the methods of Bendall and Rolfe [1]. Difference spectra were measured with either a Johnson Foundation split-beam spectrophotometer or a microcomputer-linked single-beam spectrophotometer (Applied Photophysics Ltd., London). Cytochrome *b*-560 in the absence of cytochrome *b*-563 was quantitated by peak height at $560\frac{1}{2}$ nm above a baseline drawn between 548 nm and 570 nm assuming an absorption coefficient of 20 $mM^{-1} \cdot cm^{-1}$. Redox titrations were performed by a method similar to that described by Rich and Bendall [2] except that difference spectra were obtained with the single-beam instrument. The cuvette was kept anaerobic by a flow of water-saturated oxygen-free argon. The following redox mediators were used: 20 μM 2,6-dimethylhydroquinone; 25 μM duroquinone, 12.5 μM phenazine ethosulphate, 12.5 μM phenazine methosulphate, 5 μM 2-hydroxy-1,4-naphthoquinone, 20 μM anthraquinone 2,6-disulphonate, 30 μM 2-methyl-1,4-dihydroxynaphthoquinone, 30 μM 2,3-dimethyl-1,4-dihydroxynaphthoquinone and 10 μM 2-methyl-3-decyl-1,4-dihydroxynaphthoquinone.

During the course of the experiment, chlorophyll bleaching caused distortion of the baseline. To correct for this, a fully reduced spectrum ($E_h = -400$ mV) was taken at the beginning and end of the experiment to give a spectrum of the components which had bleached. The size of the cor-

rection to be applied was estimated in each case by assuming that isosbestic points of the cytochrome occurred at 520 and 548 nm.

Samples were treated with acid acetone as described by Basford et al. [19] to extract non-covalently bound haem. Pyridine haemochromogen derivatives of the supernatant and pellet were prepared as described by Appleby et al. [20].

Polypeptide analysis. Polyacrylamide gel electrophoresis was performed with the buffer system of Laemmli [17] and 10–20% (w/v) acrylamide gradient gels containing 4 M urea. The gels were run at 4°C and stained either by the method of Thomas et al. to reveal haem [18] or with 0.1% (w/v) Coomassie brilliant blue R-250.

Chlorophyll assays. Chlorophyll *a* from *Phormidium laminosum* was assayed by the method of Arnon et al. [15]. Chlorophyll *a* and *b* in samples from other organisms were determined by the method of Arnon [16].

Results

Mutants of Chlamydomonas reinhardtii

Chloroplast fragments from wild-type *Chlamydomonas* possess a group of *b*-type cytochromes similar to that of chloroplasts from higher plants. The difference spectrum of wild-type fragments obtained with hydroquinone minus ferricyanide showed a broad peak centred at 558 nm due to cytochrome b-559_{HP} and cytochrome *f* (Fig. 1). The menadiol-minus-methylhydroquinol spectrum gave a peak at 559 nm, due to cytochrome b-559_{LP}, and dithionite-minus-menadiol gave a peak at 563 nm, due mainly to cytochrome *b*-563. Similar measurements were performed on mutant F18, which lacks the cytochrome *bf* complex [8]. The absorption band obtained with hydroquinone had become narrower with a peak at 559 nm which represented cytochrome b-559_{HP} alone. The dithionite-minus-menadiol spectrum showed a smaller peak at $560\frac{1}{2}$ nm. We attribute this to a new low-potential component, cytochrome *b*-560, present at a concentration of 1.1 nmol/mg chlorophyll. Peroxidase was ruled out as the origin of this spectrum by the fact that bubbling CO through the sample for 2 min in the presence of dithionite induced no spectral change. Mitochondria-enriched fractions gave no spectrum attributable to cytochrome *b*-560; hence, it is not a mitochondrial contaminant.

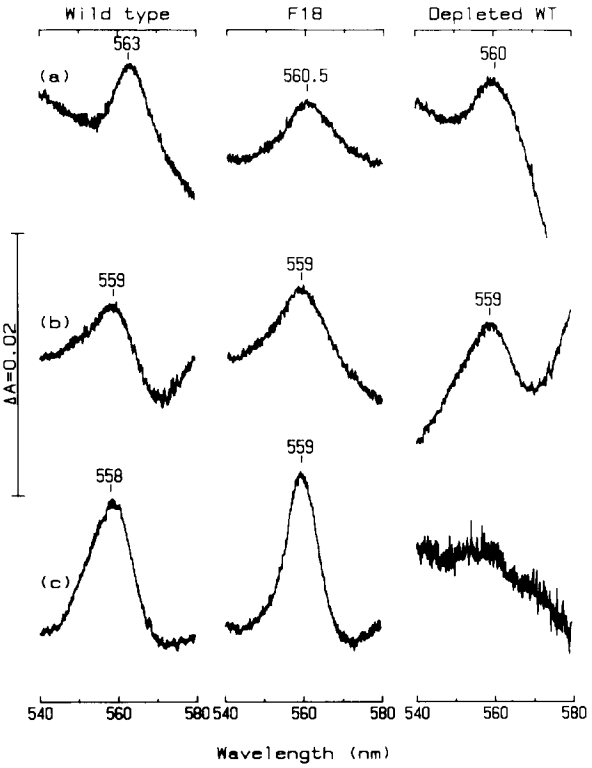

Fig. 1. Room-temperature difference spectra of membrane-bound cytochromes in *C. reinhardtii*. Chloroplast fragments from wild type, mutant (F18) or digitonin-depleted wild type (depleted WT) were suspended in 50 mM Mes-NaOH buffer at pH 6.0 (a, b) or pH 6.5 (c) at a concentration of 150 μg chlorophyll/ml. Depleted membranes were prepared as described in Materials and Methods. Difference spectra: (a), dithionite minus menadiol; (b), menadiol minus methylhydroquinol; (c), hydroquinone minus ferricyanide.

Estimation of the concentration of cytochrome *b*-560 in untreated thylakoid membranes from the wild-type was difficult, but was easier after extraction of the cytochrome *bf* complex. Fig. 1 shows that after treatment with 1.25% digitonin, a dithionite-minus-menadiol spectrum of the depleted membranes showed a peak at $560\frac{1}{2}$ nm similar to that of untreated F18 membranes. The concentration of cytochrome *b*-560 in the depleted membranes was measured as 2.2 nmol/mg chlorophyll, which corresponded to 1.1 nmol/mg chlorophyll in the original membranes after allowance for extraction of 50% of the chlorophyll by digitonin. During the course of the experiment, cytochrome b-559_{HP} was converted to a low potential form.

A mixture of 20 mM octyl glucoside and 0.33% cholate was also used to extract the cytochrome *bf* complex and spectra (dithionite minus menadiol) obtained with membranes from wild-type, and some further mutants treated by this method are shown in Fig. 2. Membranes from mutant F34, which lacks the entire photosystem II complex [21], showed traces of residual cytochromes *b*-563 and *f* after detergent treatment but no cytochrome *b*-560. However, mutants BF25 (Fig. 2), FuD39 and FuD44 (spectra not shown), which still contain the photosystem II reaction-centre core [22,23], all contained cytochrome *b*-560 at a concentration comparable to that in F18. This was supported by the shift in peak position from 563½ nm in F34 to 563 nm in untreated membranes from wild type, BF25 and FuD44 chloroplast fragments. Mutant F54-14, which lacks both the membrane ATPase activity and the whole of the photosystem I complex [24], was also found to contain cytochrome *b*-560. Extraction with octyl glucoside/cholate removed 90–100% of the cytochrome *bf* complex and cytochrome *f* was normally undetectable in the depleted membranes. In the case of mutant F18, treatment with octyl glucoside/cholate extracted 10–15% of cytochrome *b*-560 and 5–10% of the chlorophyll. Table I summarises cytochrome analyses of several *Chlamydomonas* mutants.

Depleted BF25 and FuD44 membranes, which should only contain cytochromes *b*-559$_{LP}$ and *b*-560, showed no polypeptide staining with tetramethylbenzidine/H_2O_2 after being electrophoresed in 4 M urea/LiDS gels at 4°C. This

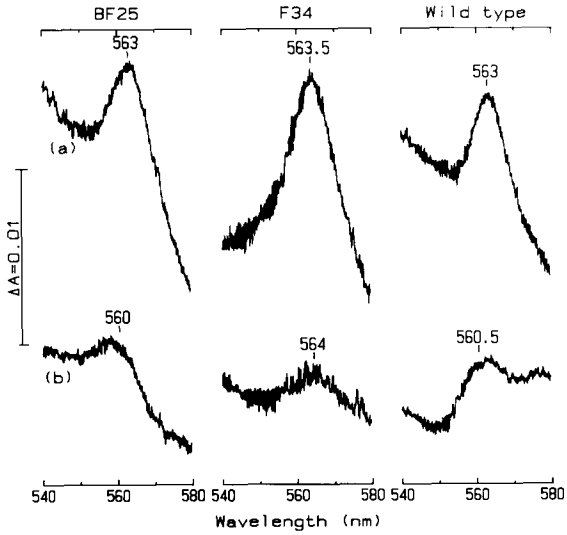

Fig. 2. Difference spectra of membrane-bound cytochromes in *Chlamydomonas* Photosystem II mutants before and after detergent treatment. Chloroplast fragments of wild type or mutants of Photosystem II (BF25 and F34) were depleted of the cytochrome *bf* complex using 20 mM octyl glucoside/0.33% cholate treatment as described in Materials and Methods. Samples were resuspended at 150 μg chlorophyll/ml in 50 mM Mes-NaOH (pH 6.0) and dithionite-minus-menadiol difference spectra were made. (a), untreated membranes; (b), detergent depleted membranes.

suggested that cytochrome *b*-560 (and also cytochrome *b*-559$_{LP}$) was indeed a *b*-type cytochrome. This was confirmed by treatment of the depleted membranes with acid acetone to extract any non-covalently bound haem. Difference spectra of pyridine haemochromogen derivatives (Fig. 3) showed a protohaem peak at 556 nm with the supernatant and no detectable haem in the pellet.

TABLE I

CYTOCHROME CONTENT OF *CHLAMYDOMONAS* THYLAKOID MEMBRANES

All concentrations are given as nmol cytochrome/mg chlorophyll. PS, photosystem; Cyt, cytochrome; WT, wild type.

Mutant	Complexes lacking	Untreated chloroplast fragments				*bf*-depleted membranes
		b-559$_{HP}$	*f*	*b*-559$_{LP}$	*b*-563/560	*b*-560
WT	wild type	3.1	1.2	1.9	1.9	0.8
F34	all of PS II	none	1.3	1.3	2.8	none
BF25	part of PS II	none	1.0	1.0	2.1	1.0
FuD44	part of PS II	none	1.2	1.1	2.9	1.0
FuD39	part of PS II	none	1.2	1.3	2.0	0.6
F18	Cyt *bf* complex	3.9	none	1.2	1.2	1.2
F54-14	PS I/ATPase	2.3	1.0	1.0	2.9	0.7

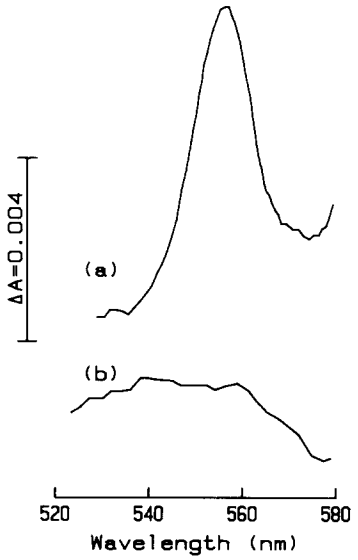

Fig. 3. Difference spectra of pyridine haemochromogen derivatives of *Chlamydomonas* FuD44 membranes depleted of the cytochrome *bf* complex. FuD44 membranes were treated with 20 mM octyl glucoside/0.33% cholate and then extracted with acid acetone to remove *b*-type cytochromes. The extract and pellet were evaporated to dryness under vacuum before conversion to the pyridine haemochromogen derivative. Dithionite-minus-ferricyanide difference spectra were then measured. (a), acid acetone extract containing *b*-type cytochromes; (b), pellet containing *c*-type cytochromes.

No polypeptide staining for haem that could be associated with cytochrome *b*-560 was detectable in F18 membranes.

Properties of cytochrome b-560 in Chlamydomonas mutant F18

Reduction of cytochrome *b*-560 by dithionite, like cytochrome *b*-563, was slow. A first-order plot of the increase of absorbance at 559 nm and $560\frac{1}{2}$ nm at intervals after the addition of dithionite to F18 membranes (Fig. 4) showed a rapid reduction of cytochrome *b*-559_{LP} and a slow reduction of cytochrome *b*-560 with $t_{1/2} = 230$ s. By extrapolation to zero time the concentration of cytochrome *b*-560 in F18 was found to be 1.2 nmol/mg chlorophyll.

Redox titration of cytochrome *b*-560 was performed with membranes from mutant F18. The main wave of reduction occurred around −100 mV (Fig. 5), but the titration curve needed to be corrected for the presence of cytochrome *b*-559_{LP}. The midpoint potential of this component could

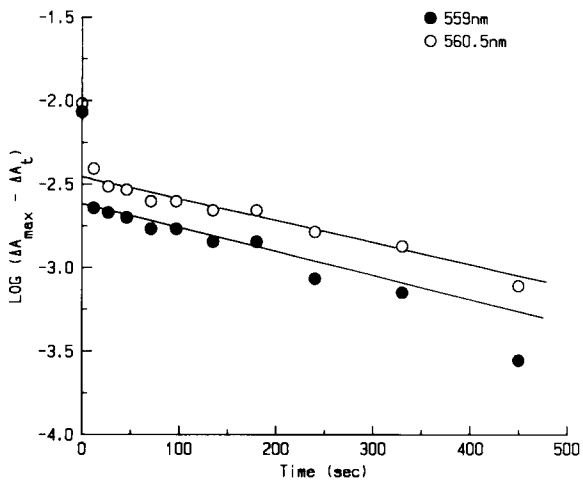

Fig. 4. Time course of cytochrome reduction by dithionite in *Chlamydomonas* mutant F18. Chloroplast fragments (150 μg chlorophyll/ml) were reduced by dithionite as described by Bendall et al. [1]. Absorption at 559 nm and $560\frac{1}{2}$ nm was measured above a baseline drawn between 548 nm and 570 nm.

not be determined with F18 membranes because of conversion of cytochrome *b*-559_{HP} to a low-potential form during the course of the experi-

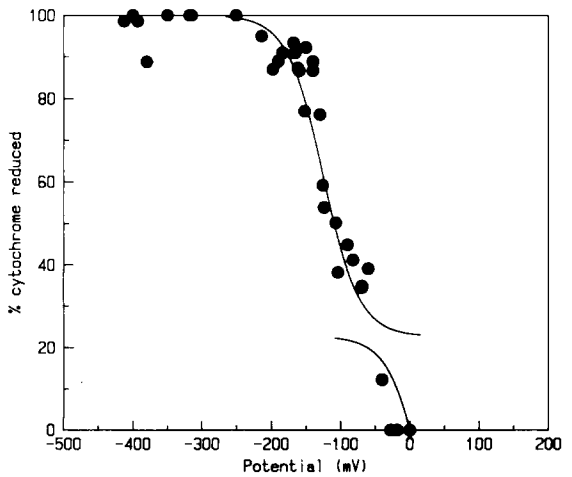

Fig. 5. Redox titration of cytochrome *b*-560 in chloroplast fragments from *Chlamydomonas* mutant F18. Chloroplast fragments were suspended at a concentration of 150 μg chlorophyll/ml in 50 mM potassium phosphate buffer (pH 7.2) to which redox mediators and catalase had been added. A theoretical line is shown for a Nernst curve with $E_m = -125$ mV and $n = 1$. The contribution of cytochrome *b*-559_{LP} is also shown ($E_m = +32$ mV, $n = 1$).

ment. (Rich and Bendall gave the midpoint of this form as +110 mV in lettuce [2].) A titration was therefore carried out with membranes from mutant F34, which contained cytochromes b-559$_{LP}$ and b-563 but lacked both b-560 and b-559$_{HP}$. The spectra were analysed for the contribution of each component as described in Materials and Methods and the resultant titration curves are shown in Fig. 6. The midpoint potential of cytochrome b-559$_{LP}$ was found to be +32 mV. From this we calculated that 20% of the overall absorption change observed during the titration of F18 membranes was contributed by cytochrome b-559$_{LP}$. The corrected midpoint potential of cytochrome b-560 was thus −125 mV at pH 7.2 (Fig. 5). The results for cytochrome b-563 (Fig. 6) could best be described by a theoretical line composed of two equal components ($n = 1$) with midpoints of −23 mV and −123 mV, respectively.

Cytochrome b-560 in other organisms

Extraction of the cytochrome *bf* complex with octyl glucoside/cholate allowed the occurrence of cytochrome b-560 in other oxygenic plants to be determined. Membrane fragments depleted of the cytochrome *bf* complex were prepared from chloroplasts of pea, spinach and lettuce (Fig. 7). An absorption band at 560½ nm in the dithionite-minus-menadiol difference spectrum of the depleted membranes corresponded to a concentration of cytochrome b-560 of 0.9 nmol/mg chlorophyll for pea and 1.3 nmol/mg chlorophyll for spinach. Depleted lettuce membranes showed an absorption band at 561 nm which corresponded to 1.2 nmol/mg chlorophyll. Cytochrome f could not be detected spectrophotometrically in the depleted membranes.

Membranes depleted of the cytochrome *bf*

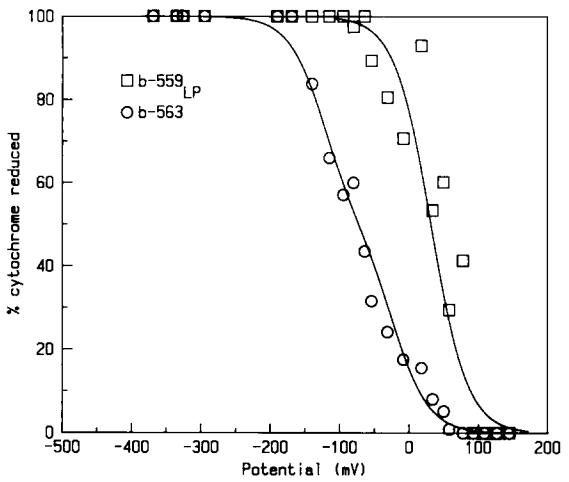

Fig. 6. Redox titration of cytochrome b-563 and cytochrome b-559$_{LP}$ in chloroplast fragments from *Chlamydomonas* mutant F34. The titration was performed in the same way as that described in Fig. 5. The contributions of cytochrome b-559$_{LP}$ and cytochrome b-563 were deconvoluted as described in Materials and Methods. A line corresponding to a Nernst curve with $E_m = +32$ mV, $n = 1$, has been drawn through the points corresponding to cytochrome b-559$_{LP}$. A line corresponding to two combined Nernst curves with $E_{m,1} = -23$ mV, $n = 1$, and $E_{m,2} = -123$ mV, $n = 1$, has been drawn through the cytochrome b-563 points. It was assumed that equal contributions from each component were present at 563 nm.

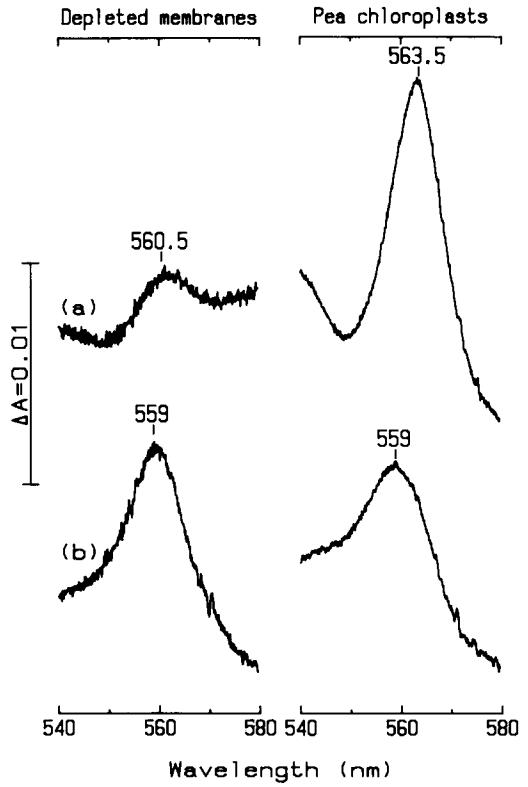

Fig. 7. Difference spectra of pea chloroplasts before and after detergent treatment. Pea chloroplasts were depleted of the cytochrome *bf* complex using 20 mM octyl glucoside/0.33% cholate treatment as described in Materials and Methods. Samples were resuspended at 150 μg chlorophyll/ml chlorophyll in 50 mM Mes-NaOH (pH 6.0) and difference spectra were taken. (a), dithionite minus menadiol; (b), menadiol minus methylhydroquinol.

complex from the cyanobacterium, *P. laminosum*, were prepared by treatment with mixtures of Mega-9 and potassium cholate. Analysis of dithionite-reduced difference spectra was complicated by the presence of cytochrome *c*-549. However, cytochrome *c*-549 was rapidly reduced by dithionite, hence subtraction of a spectrum taken 15 s after the addition of dithionite from spectra taken at later intervals gave spectra which contained only slowly reducing components, i.e., *b*-563 and *b*-560 (Fig. 8). Approx. 1 nmol cytochrome *b*-563/mg chlorophyll remained in the thylakoid membranes after treatment with 20 mM Mega-9/0.33% cholate. The peak positions of both extract and membrane residues were identical to that of a highly purified cytochrome *bf* preparation from *P. laminosum* (Fig. 9), and the width at half peak height was approx. $9\frac{1}{2}$ nm in each case. This strongly suggested that cytochrome *b*-560 was absent from *P. laminosum*.

Since there appeared to be an association of cytochrome *b*-560 with Photosystem II, dithionite-minus-menadiol difference spectra were taken of

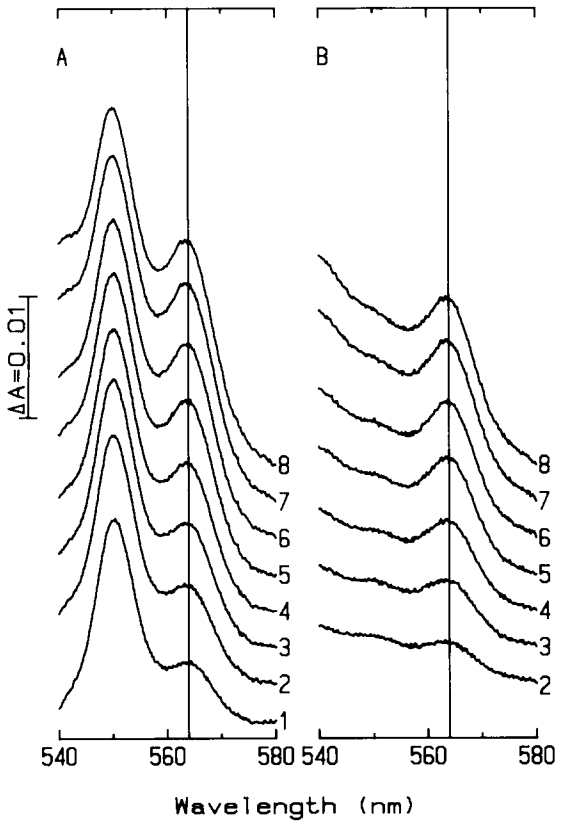

Fig. 8. Time course of low-potential cytochrome reduction by dithionite in a detergent extract from *Phormidium* membranes. 1 ml of a 20 mM Mega-9/0.33% cholate extract from *P. laminosum* thylakoid membranes was added to 4 ml of 10% glycerol/50 mM Mes (pH 6.0)/400 units per ml catalase and the sample was divided equally between two cuvettes. A baseline was scanned after the addition of menadiol to one cuvette. (A) 5 μl of 50 mg/ml dithionite in the same buffer was added to the second cuvette and difference spectra were scanned at the following times: 1, 15 s; 2, 28.6 s; 3, 43.2 s; 4, 57.8 s; 5, 87.4 s; 6, 117 s; 7, 236.6 s; 8, 356.2 s. (B) Spectra of components slowly reduced by dithionite were obtained by subtraction of trace 1 in (A) from all other traces. The scale line was drawn at $563\frac{1}{2}$ nm.

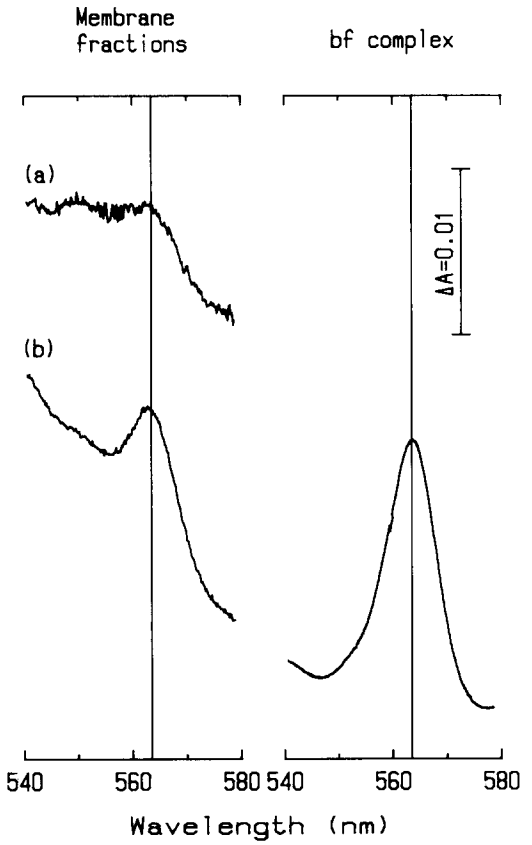

Fig. 9. Low-potential cytochromes slowly reduced by dithionite in detergent extracts and depleted membranes of *P. laminosum*. Spectra of low-potential cytochromes slowly reduced by dithionite in Mega-9/cholate extracts and depleted membranes of *P. laminosum* were obtained by subtraction as in Fig. 8. (a) Detergent treated membranes resuspended at 150 μg/ml chlorophyll. (b) Detergent extracts diluted five times. A spectrum of purified cytochrome *bf* complex from *P. laminosum* is shown for comparison. The scale line was drawn at 563.5 nm.

Photosystem II preparations from pea and *P. laminosum*. No absorption band attributable to cytochrome *b*-560 could be observed in either case. We therefore conclude that cytochrome *b*-560 was not required for rapid electron flow from water to artificial electron acceptors in these preparations.

Discussion

The data presented here show strong evidence for the presence in chloroplasts of green algae and higher plants of cytochrome *b*-560, a hitherto unrecognised component.

The failure to distinguish this component in earlier studies of chloroplast cytochromes can be explained at least in part by the similarity of some of its key properties (redox potential, rate of reduction by dithionite) to cytochrome *b*-563, which is present at twice the concentration. Assuming a ratio of cytochrome *b*-563/*b*-560 of 2 : 1, a combined spectrum would be expected to have a peak position $\frac{1}{2}$ nm lower than that of cytochrome *b*-563 alone. This difference was observed between dithionite-minus-menadiol difference spectra of chloroplast fragments from wild-type *Chlamydomonas* and mutant F34. However, the asymmetry of the cytochrome *b*-563 spectrum and the uncertainty over the isosbestic points in a combined cytochrome *b*-563 and *b*-560 spectrum prevent the width at half peak height being used as an indication of the presence of cytochrome *b*-560. Cytochrome *b*-560 can be observed in *Chlamydomonas* mutants lacking the cytochrome *bf* complex or in wild-type thylakoids after detergent depletion of the cytochrome *bf* complex. Observation of cytochrome *b*-560 in higher plants without detergent treatment will require the analysis of mutants deficient in the cytochrome *bf* complex such as those reported from maize [26] or *Lemna* [27].

The presence of cytochrome *b*-560 in chloroplast fragments from *Chlamydomonas* is not due to mitochondrial contamination, since it could not be detected in a mitochondria-enriched fraction and also is absent from mutant F34.

The origin of cytochrome *b*-560 is not clear. It is possible that cytochrome *b*-560 is a distinct gene product fulfilling an as yet unknown role, or that it is derived from another chloroplast component. Lemaire et al. [25] found that membranes from mutant F18 did not cross-react with antibodies against cytochrome *b*-563 and lacked all the polypeptides of the cytochrome *bf* complex. This shows that cytochrome *b*-560 is not a precursor or product of cytochrome *b*-563. It has been reported that cytochrome *f* can accumulate in thylakoid membranes before incorporation into the cytochrome *bf* complex [29] but if cytochrome *b*-560 were such precursor of cytochrome *f*, then it would be expected to be present in mutant F34 (and probably *P. laminosum*). The midpoint redox potential of cytochrome *b*-559_{HP} is lowered by a variety of treatments, and so it would appear that cytochrome *b*-560 could be a modified form of cytochrome *b*-559_{HP}. However, Rich and Bendall found that the midpoint redox potential of the low-potential form of cytochrome *b*-559_{HP} is +110 mV after heating, or +85 mV in a detergent extract [2]. Very severe treatment such as ethanol washing is required before the midpoint of cytochrome *b*-559_{HP} falls beneath 0 mV. The presence of cytochrome *b*-560 in freshly prepared F18 membranes suggests that cytochrome *b*-560 is not such a modified form. This is supported by its absence in higher-plant photosystem II preparations and its presence in *Chlamydomonas* mutants which lack cytochrome *b*-559_{HP}.

The absence of cytochrome *b*-560 from the cyanobacterium, *P. laminosum*, implies that cytochrome *b*-560 is involved in a function limited to higher plants. It has been reported that cyclic phosphorylation in cyanobacteria, if present, is insignificant compared to dark respiration rates [28]. If a cytochrome is involved in cyclic phosphorylation, cytochrome *b*-560 could be a candidate.

Many other functions for cytochrome *b*-560 can be proposed, such as an involvement in chlororespiration [30] or as an alternative acceptor from photosystem II. However, much more work is required before the origin or function of cytochrome *b*-560 can be determined.

Acknowledgments

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