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INVOLVEMENT OF PLASTOQUINONE BOUND WITHIN THE ISOLATED CYTOCHROME b_6-f COMPLEX FROM CHLOROPLASTS IN OXIDANT-INDUCED REDUCTION OF CYTOCHROME b_6

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(1) Oxidant-induced reduction of cytochrome b_6 is completely dependent on a reduced component within the isolated cytochrome b_6-f complex. This component can be reduced by dithionite or by NADH/ *N*-methylphenazonium methosulfate. It is a $2H^+/2e^-$ carrier with a midpoint potential of 100 mV at pH 7.0, which is very similar to the midpoint potential of the plastoquinone pool in chloroplasts. (2) Oxidant-induced reduction of cytochrome b_6 is stimulated by plastoquinol-1 as well as by plastoquinol-9. The midpoint potential of the transient reduction of cytochrome b_6, however, was not shifted by added plastoquinol. (3) Quinone analysis of the purified cytochrome b_6-f complex revealed about one plastoquinone per cytochrome f. The endogenous quinone is heterogeneous, a form more polar than plastoquinone-A, probably plastoquinone-C, dominating, This is different from the thylakoid membrane where plastoquinone-A is the main quinone. (4) The endogenous quinone can be extracted from the lyophilized cytochrome b_6-f complex by acetone, but not by hydrocarbon solvents. Oxidant-induced reduction of cytochrome b_6 was observed in the lyophilized and hexane-extracted complex, but was lost in the acetone-extracted complex. Reconstitution was achieved either with plastoquinol-1 or plastoquinol-9, suggesting that a plastoquinol molecule is involved in oxidant-induced reduction of cytochrome b_6.

Introduction

Oxidant-induced reduction of cytochrome *b* seems to be a common characteristics of cytochrome *b*-*c* complexes. This phenomenon has long been known for the mitochondrial cytochrome *b*-c_1 complex [1–3], and it was recently demonstrated also for the isolated cytochrome *b*-c_1 complex from the photosynthetic bacterium *Rhodopseudomonas sphaeroides* [4], for the purified cytochrome b_6-f complexes from the blue-green algae *Anabaena variabilis* [5] and from spinach chloroplasts [6], and for a digitonin-solubilized, Photosystem I-containing cytochrome b_6-f preparation from lettuce chloroplasts [7]. The Rieske Fe-S protein as a component of these cytochrome *b*-*c* complexes is involved in the reduction/oxidation reactions of cytochrome *b* [3,6,8–11], and it has been suggested that the Rieske Fe-S center oxidizes a special quinol molecule in the complex to the semiquinone which in turn reduces cytochrome *b*.

A specialized ubiquinone molecule which determines the rate of electron flow through the ubiquinol-cytochrome c_2 oxidoreductase was kinet-

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Abbreviations: Tricine, *N*-tris(hydroxymethyl)methylglycine; Mops, 3-(*N*-morphilino)propanesulfonic acid; Mes, 2-(*N*-morphilino)ethanesulfonic acid; PQ, plastoquinone; PQH_2 plastoquinol.

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ically identified in chromatophores of *Rps. sphaeroides* exhibiting an $E_{m,7.0}$ of 155 mV [11,12]. This specialized quinone, called Q_z, can be extracted from lyophilized chromatophores by isooctane and can be reconstituted [10,13]. Recently, Matsuura et al. [9] recognized and characterized a quinone molecule within the mitochondrial cytochrome b-c_1 complex (midpoint potential at pH 7.0 of 115 mV), which determines the electron-transfer rate in the ubiquinol-cytochrome c oxidoreductase, and it was speculated [14] that also in chloroplasts a specialized plastoquinone exists in the region of the cytochrome b_6-f complex.

In this paper, we demonstrate that a component within the isolated cytochrome b_6-f complex, which is a $2H^+/2e^-$ carrier, exhibiting a midpoint potential of 100 mV at pH 7.0, is involved in the oxidant-induced reduction of cytochrome b_6. This component is probably a plastoquinone which can be extracted by acetone and can be reconstituted either by PQ-1 or by PQ-9.

Methods

Preparations

Isolation of the cytochrome b_6-f complex and the test for plastoquinol-plastocyanin oxidoreductase activity were performed as previously described [15,16].

Lyophilization of the cytochrome b_6-f complex was carried out according to the method of Baccarini-Melandri et al. [17]. 2 ml of the cytochrome b_6-f complex, 30 μM in cytochrome f, in 30 mM octylglucoside/0.5% cholate/30 mM Tris-succinate buffer, pH 6.5, were dialyzed against water for 1 h to remove most of the sucrose. Then the sample was quickly frozen and lyophilized at -10°C for 15 h.

Extraction of the lyophilized cytochrome b_6-f complex with organic solvents was performed in a closed, stirred flask under a nitrogen atmosphere at room temperature. This was important, otherwise the powder became sticky during extraction, probably because of H_2O in the atmosphere. Extraction was carried out either with dried hexane, with hexane plus 10% acetone [18] or with acetone [19]. The lyophilized powder was extracted three times, in each case for 10 min with 20 ml organic solvent. After the last extraction the centrifuged pellet was dried under a stream of nitrogen and finally under vacuum. The powder was resuspended in water to a cytochrome f concentration of about 20 μM.

For reconstitution experiments, PQ-1 or PQ-9 was dissolved in 10% Triton X-100 by sonicating for 2 min in a water-bath sonicator. The plastoquinone solution (about 2 μl) was added to the cytochrome b_6-f complex which was already diluted in the cuvette.

Quinone extraction from aqueous cytochrome b_6-f complex was performed as described by Kröger and Klingenberg [20]. 2 ml of methanol plus a grain of solid ferricyanide were added to 0.5 ml of the cytochrome b_6-f complex (about 30 μM in cytochrome f), the methanol phase was extracted three times with 5 ml petroleum ether (40–60°C) in each case. The hydrocarbon phases were combined, dried with solid sodium sulfate, filtered and evaporated. The extract was finally dissolved in 1 ml ethanol and $NaBH_4$-induced difference spectra were recorded between 240 and 300 nm. An extinction coefficient of 15 mM^{-1} at 256 nm was taken for the estimation of the plastoquinone concentration [21].

Quinone analysis of the petroleum ether extract was made by thin-layer chromatography on silica gels [21]. The solvent system is given in the figure legends. Quinones were visualized by spraying the developed plate with leucomethylene blue solution. Quinone spots were scraped off, extracted with ethanol and $NaBH_4$-induced difference spectra were recorded. Chloroplasts were isolated as described in Ref. 22.

Assays

Oxidant-induced reduction of cytochrome b_6 in the isolated cytochrome b_6-f complex with ferricyanide was performed as described by Hurt and Hauska [6] in 60 mM KCl, 5 mM $MgCl_2$, 20 mM Tricine buffer, pH 8.0. Anaerobic experiments at fixed redox potential were carried out in the presence of 0.1 M glucose, glucose oxidase (10 units/ml; from Sigma) and catalase (10 μg/ml; from Boehringer). In addition, nitrogen was flushed through the cuvette. The reaction was measured in a continuously stirred cuvette, placed in the holder of an Aminco DW 2 spectrophotometer, with a

redox electrode installed. Addition of ferricyanide was made by a syringe. The mixing time was less than 1 s. The redox potential in the cuvette was adjusted by addition of 10 mM NADH or ferricyanide in the presence of 2 μM *N*-methylphenazonium methosulfate. *N*-Methylphenazonium methosulfate catalyzes the reaction with the redox electrode. The ambient redox potential was rather stable between 100 and 0 mV, although a slow drift towards negative potentials was always observed. To start the reaction, 100 μM ferricyanide was added to the stirred cuvette leading immediately to very positive potentials of about 400 mV. The following transient cytochrome b_6 reduction was monitored at the wavelength pair 563–575 nm. The amplitude of the transient cytochrome b_6 reduction was plotted vs. the ambient redox potential measured just before the ferricyanide pulse. Theoretical lines with a Nernst slope $n = 2$ were drawn through the experimental points, assuming that the highest observed amplitude of reduced cytochrome b_6 after the ferricyanide pulse reflects 100% of the reactive form of the responsible redox compound. The midpoint potential was obtained from half-maximal reduction of cytochrome b_6. Further details are given in the figure legends.

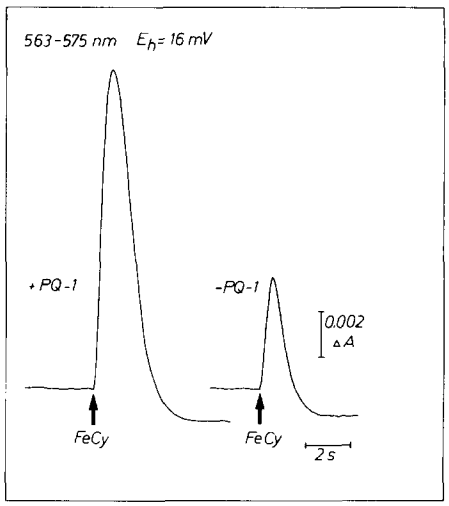

Fig. 1. Oxidant-induced reduction of cytochrome b_6 at a fixed redox potential. The experiment was carried out with lyophilized cytochrome b_6-f complex as described in Methods. The cytochrome f concentration in the cuvette was 1.66 μM. The volume was 2.4 ml. Where indicated, 100 μM ferricyanide (FeCy) was added to the permanently stirred cuvette. The redox potential adjusted by the NADH/*N*-methylphenazonium methosulfate system was 16 mV measured just before the ferricyanide pulse. The right-hand part shows absorption changes at 563–575 nm in the absence of added plastoquinone, the left-hand part the corresponding one in the presence of 8 equiv. plastoquinone-1 per cytochrome f. In the absence of PQ-1 about 5%, and in its presence about 20% of the total cytochrome b_6 were reduced.

Results and Discussion

Redox properties of the endogenous component involved in the oxidant-induced reduction of cytochrome b_6

Fig. 1 shows the ferricyanide-induced reduction of cytochrome b_6 with the isolated cytochrome b_6-f complex. The experiment was performed similarly to that described by Hurt and Hauska [6] except that the ambient redox potential was adjusted to 16 mV prior to the ferricyanide pulse. Under these conditions cytochrome b_6 is completely oxidized. This is explained by the fact that the two midpoint potentials of cytochrome b_6 found for the cytochrome b_6-f complex [15] are rather negative ($E_{m(1),8.3} = -112$ mV; $E_{m(2),8.3} = -215$ mV) so it cannot be directly reduced by plastoquinol at equilibrium. This is in clear contrast to the mitochondrial cytochrome b-c_1 complex where addition of ubiquinol leads to a partial and stable reduction of cytochrome b due to its more positive midpoint potential. The right-hand part of Fig. 1 demonstrates the transient cytochrome b_6 reduction in the absence of added plastoquinol-1, the left-hand part in the presence of 8 equiv. plastoquinol-1/cytochrome f. Since also without added plastoquinol the phenomenon of oxidant-induced cytochrome b reduction could be observed, we looked for the endogenous component(s) within the cytochrome b_6-f complex participating in this reaction, in addition to the Rieske Fe-S protein [6,8]. The redox properties of this component were analyzed by titrating the amplitude of reduced cytochrome b_6 vs. the ambient redox potential measured just before the addition of ferricyanide. Fig. 2 shows the corresponding titration curve. The E_h was adjusted using the NADH/*N*-methylphenazonium methosulfate system, giving more reliable results than the dithionite/*N*-methylphenazonium methosulfate system. In the latter system the ambient redox potential was rather

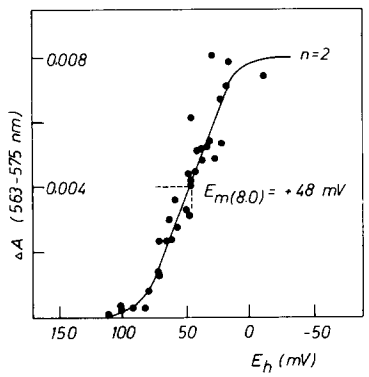

Fig. 2. Cytochrome b_6 reduction induced by ferricyanide as a function of the redox potential. The assay is described in Methods; the cytochrome *f* concentration in the cuvette was 1.83 μM. The cytochrome b_6-*f* complex contained one endogenous plastoquinone per cytochrome *f*. The amplitude of the ferricyanide-induced reduction of cytochrome b_6 was plotted vs. the redox potential measured just before addition of ferricyanide. The highest observed cytochrome b_6 reduction corresponds to 10% of the total cytochrome b_6 and was defined as 100%. A theoretical line with a Nernst slope $n = 2$ is drawn through the experimental points and the midpoint potential is given.

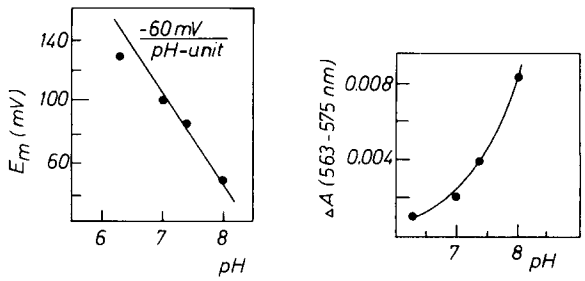

Fig. 3. pH dependence of the midpoint potential of the oxidant-induced reduction of cytochrome b_6. The experiments at different pH values were performed as described in Fig. 1. At pH 8.0 and 7.4 Tricine, at pH 7.0 Mops and at pH 6.3 Mes was the buffer in a concentration of 20 mM. A theoretical line with a slope of −60 mV/pH unit is drawn through the points.

Fig. 4. pH dependence of the oxidant-induced reduction of cytochrome b_6. The highest observed cytochrome b_6 reduction at the indicated pH values of Fig. 3 was plotted vs. the pH.

unstable and equilibrium with the redox electrode took a long time. It was necessary to use very low concentrations of *N*-methylphenazonium methosulfate as redox mediator, otherwise redox changes of cytochrome b_6 were small and fast. This indicates that *N*-methylphenazonium methosulfate can interact with cytochrome b_6. The height of the ferricyanide-induced transient cytochrome b_6 reduction plotted vs. the redox potential follows the Nernst equation with a slope for $n = 2$ and a midpoint potential of 48 mV at pH 8.0. A pH dependence of −60 mV/pH unit was found between pH 6.3 and 8.0 (Fig. 3). Therefore, the component involved in the oxidant-induced reduction of cytochrome b_6 is a $2H^+/2e^-$ carrier exhibiting an $E_{m,7.0}$ of about 100 mV. Golbeck and Kok [23] demonstrated that the plastoquinone pool in spinach chloroplasts titrated as a single component with a midpoint of 106 mV at pH 7.2, with $n = 2$ and a pH dependence of −60 mV/pH unit. This makes it very likely that the component involved in the oxidant-induced reduction of cytochrome b_6 is a plastoquinone molecule.

Similar results were found for specialized ubiquinone molecules within the mitochondrial cytochrome b-c_1 complex [9] ($E_{m,7.0} = 115$ mV) and in the cytochrome b-c_1 region of chromatophores from *Rps. sphaeroides* [11,12] ($E_{m,7.0} = 155$ mV).

We did the titration also with dithionite instead of NADH. The results were similar to those obtained in Fig. 2, but the E_m was shifted towards a more positive redox potential ($E_{m,7.5} = 125$ mV), indicating that equilibrium conditions were not fully achieved.

Fig. 4 shows that transient cytochrome b_6 reduction increases at higher pH. It was previously demonstrated [16] that plastoquinol-plastocyanin oxidoreductase activity has its pH optimum around pH 8.0. Optimal electron flow through the cytochrome b_6-*f* complex and a high extent of transiently reduced cytochrome b_6 thus seem to be connected.

Influence of added PQ-1 or PQ-9 on the oxidant-induced reduction of cytochrome b_6

Fig. 1 shows the ferricyanide-induced reduction of cytochrome b_6 at a fixed redox potential of 16 mV in the absence of added plastoquinone (right-hand part) and in the presence of 8 equiv. PQH_2-1/cytochrome *f* (left-hand part). It is obvious that the extent of reduced cytochrome b_6 depends on the amount of added quinone. This observation was studied in more detail in Fig. 5 where redox titrations for different plastoquinone/cytochrome

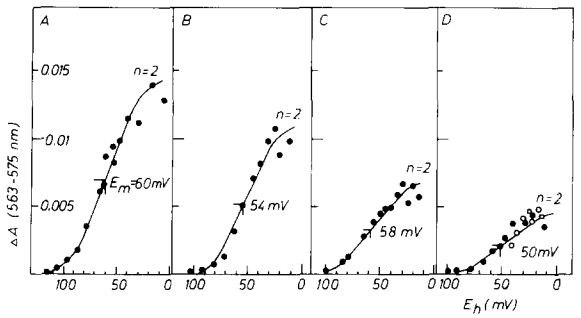

Fig. 5. Oxidant-induced reduction of cytochrome b_6 as a function of the redox potential in the absence and in the presence of added PQ-1 or PQ-9. The experiments were performed as described in Methods with a lyophilized cytochrome b_6-f complex. (A) 8 equiv. PQ-1/cytochrome f were added; (B) 2 equiv. PQ-1/cytochrome f were added; (C) 2 equiv. PQ-9/cytochrome f were added; (D) no plastoquinone was added; one endogenous plastoquinone per cytochrome f was found in the lyophilized cytochrome b_6-f complex; (●) lyophilized b_6-f complex; (○) hexane-extracted cytochrome b_6-f complex; (□) hexane plus 10% acetone-extracted cytochrome b_6-f complex. In every titration experiment the highest observed cytochrome b_6 reduction was defined as 100%. Theoretical lines with a Nernst slope $n = 2$ were drawn through the points, the midpoint potentials are given. In Fig. 5D, the theoretical line was obtained considering only the experimental points for the lyophilized complex.

f ratios are shown similar to that performed in Fig. 2. However, a lyophilized cytochrome b_6-f preparation was used which contained about one PQ/cytochrome f (see Fig. 6). A midpoint potential of 50 mV was found at pH 8.0. Addition of PQ-1 increased the ferricyanide-induced reduction of cytochrome b_6 (Fig. 5A and B), but the slope of the titration curve and the midpoint potential were not significantly changed. This demonstrates that the redox properties of PQ-1 and the endogenous component are similar. Therefore, it is difficult to distinguish between a direct reaction of added plastoquinol and a reaction via the endogenous component. Fig. 5C shows that also added PQ-9 can stimulate the cytochrome b_6 reduction, but compared with PQH-1 it was less efficient (in Fig. 5B, 2 equiv. PQH_2-1, in Fig. 5C 2 equiv. PQH_2-9 were additionally present). The midpoint potential of the reaction again was not shifted. In the micellar system, PQH_2-1 can probably exchange electrons faster with the cytochrome b_6-f complex than PQH_2-9 with its long side chain in the presence of detergent [16].

Quinone analysis of the cytochrome b_6-f complex

The previously purified cytochrome b_6-f complex contained 0.2–0.4 plastoquinone-9/cytochrome f detectable by thin-layer chromatography [16]. The omission of Triton X-100 from the preparation procedure for the cytochrome b_6-f complex [15] yielded a higher activity and allowed direct $NaBH_4$-induced difference spectroscopy of the petroleum ether extract performed according to the method of Kröger and Klingenberg [20], because the strong ultraviolet absorption of Triton X-100 was not interfering.

Fig. 6A shows the $NaBH_4$-induced difference spectrum of the petroleum extract from the cytochrome b_6f complex between 245 and 300 nm exhibiting an absorption maximum at 256–262 nm. The spectrum is similar to that obtained with

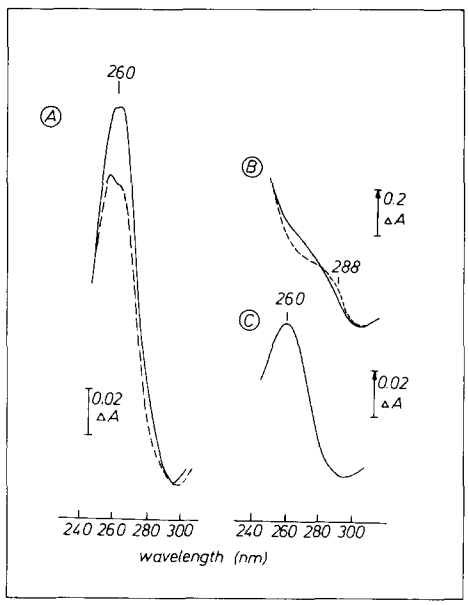

Fig. 6. Quinone analysis of the cytochrome b_6-f complex. Quinone extraction from the cytochrome b_6-f complex and $NaBH_4$-induced difference spectroscopy were performed as described in Methods. $NaBH_4$ was added to the reference cuvette. Therefore, an absorption increase is observed in the difference spectrum (oxidized minus reduced spectrum). (A) $NaBH_4$-induced difference spectra of the petroleum ether extract; the dashed curve represents the $NaBH_4$-induced difference spectrum of pure PQ-9 (PQ-A). (B) Absolute spectrum of a component not migrating in the benzene/hexane (85 : 15) TLC system. The spot was scraped off and extracted with ethanol for ultraviolet spectroscopy; (———) absolute spectrum, (— — —) spectrum after addition of $NaBH_4$; (C) $NaBH_4$-induced difference spectrum corresponding to B.

pure PQ-9 (dashed line), but its shape resembles the spectrum of PQ-C rather than that of PQ-A [24]. Using an extinction coefficient of 15 mM^{-1} at 256 nm [21], we calculated a plastoquinone/cytochrome *f* ratio of 1.15; the quinone analysis of four different cytochrome b_6-*f* preparations always gave a ratio of PQ/cytochrome *f* of about one (1.15, 0.9, 0.96, 1.1). This makes a specific binding site for plastoquinone within the cytochrome b_6-*f* complex very likely, which is permanently occupied by a plastoquinone molecule during the isolation procedure.

The tightly bound quinone seems to be heterogeneous. Thin-layer chromatography of the petroleum ether extract on silica gel as described by Barr and Crane [21] in chloroform/hexane (80 : 20, v/v), revealed at least three spots by spraying with leucomethylene blue solution. One spot was identical with PQ-A (for nomenclature, see Ref. 21), the next spot migrated like PQ-C and the last was found at the origin. In benzene/hexane (85 : 15, v/v) only PQ-A migrates, while PQ-C stays at the origin with the other more hydrophilic component [21], which could be a degradation product of PQ-C and PQ-D [25]. Based on the extinction coefficient above, recovery of total quinone in the pretroleum ether extract on thin-layer chromatograms was only about 60%, with a distribution of 1 : 2 for PQ-A and PQ-C plus other more hydrophilic components. Fig. 6B demonstrates that the fraction not migrating in the benzene/hexane system resembles a quinone, because in the absolute spectrum addition of $NaBH_4$ induced an absorption decrease at 260 nm and an absorption increase between 285 and 290 nm. Fig. 6C shows the corresponding difference spectrum exhibiting an absorption maximum at 260 nm, which, however, is more similar in shape to that described for the aerobic degradation product of PQ-C and PQ-D [25] than to those of the plastoquinones. It is interesting that in contrast to the thylakoid membrane [21], PQ-A seems not to be the predominant, bound quinone in the cytochrome b_6-*f* complex. It is possible, however, that the detergent mixture octylglucoside/cholate selectively solubilizes the more hydrophilic plastoquinones together with the cytochrome complex.

Extraction of the endogenous quinone and its reconstitution

We extracted the endogenous plastoquinone also under nondenaturating conditions to study its role in the oxidant-induced reduction of cytochrome b_6 and in plastoquinol-plastocyanin oxidoreductase activity. Successful ubiquinone extraction and reconstitution of lyophilized chromatophores of *Rps. sphaeroides* was demonstrated by Baccarini-Melandri et al. [13,17] and by Takamiya et al. [10]. An absolute requirement of an isooctane-extractable quinone, called Q_z, for electron flow through the ubiquinol-cytochrome c_2 oxidoreductase was discovered. Lyophilization of the cytochrome b_6-*f* complex under temperature control at $-10°C$ [17] did not affect the plastoquinol-plastocyanin oxidoreductase activity. The lyophilized powder was extracted with different organic solvents, either with hexane or with hexane plus 10% acetone or

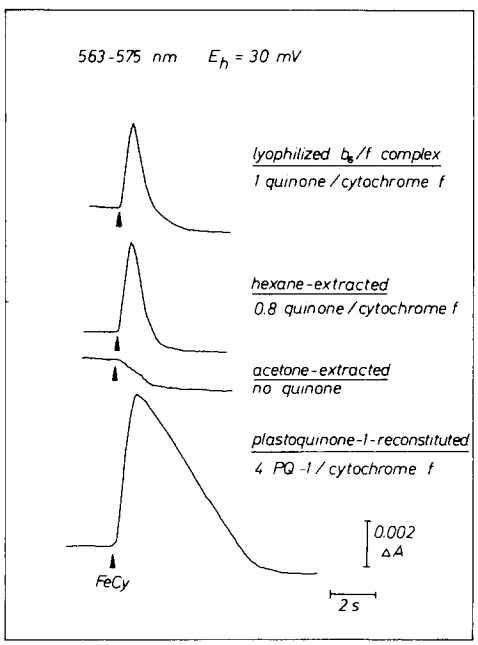

Fig. 7. Oxidant-induced reduction of cytochrome b_6 in the lyophilized, hexane-extracted, acetone-extracted and PQ-1-reconstituted cytochrome b_6-*f* complex. The experimental conditions are given in Methods. The cytochrome *f* concentration in the cuvette was 1.6 μM. 100 μM potassium ferricyanide (FeCy) was added where indicated. The redox potential just before the potassium ferricyanide pulse was 30 mV. Plastoquinone-1 reconstitution was performed with the acetone-extracted cytochrome b_6-*f* complex (4 equiv. PQ-1/cytochrome *f*) as described in Methods.

with acetone alone (see Methods). Only acetone extracts the endogenous quinone (and also chlorophyll) efficiently – no quinone could be detected in the residue. Hexane or hexane plus 10% acetone was ineffective. The hexane-extracted cytochrome b_6-f complex still contained 0.8 PQ/cytochrome f. One could speculate that the intrinsic quinone is protein bound and therefore not easily removed. But it could also be that resistance to extraction with apolar solvents just reflects its more hydrophilic character. Henninger and Crane [24] demonstrated that in contrast to PQ-A and PQ-B, PQ-C and PQ-D are not easily released from chloroplasts by hydrocarbon solvents. For complete extraction or PQ-C and PQ-D acetone was necessary. Fig. 7 shows the oxidant-induced reduction of cytochrome b_6 in lyophilized, hexane-extracted, acetone-extracted and PQ-1-reconstituted cytochrome b_6-f complex at an ambient redox potential of 30 mV. Cytochrome b_6 reduction was observed in the lyophilized complex (1 PQ/cytochrome f), in the hexane-extracted complex (0.8 PQ/cytochrome f) and the hexane plus 10% acetone-extracted complex (see Fig. 5D), but was completely lost in the acetone-extracted cytochrome b_6-f complex. Addition of PQ-1 (4 equiv. PQ-1/cytochrome f) reconstituted oxidant-induced reduction of cytochrome b_6. Further addition of 1 equiv. PQ-9 to the PQ-1-reconstituted system had no significant effect on the cytochrome b_6 signal, but plastoquinol-plastocyanin oxidoreductase activity was stimulated 2-fold. However, the activity in this case was still only about 20% of that in the lyophilized cytochrome b_6-f complex (20 μmol/h per nmol cytochrome f). The lower activity is also reflected by the kinetics of the reoxidation of cytochrome b_6 (Fig. 7), and is probably caused by partial damage of the cytochrome b_6-f complex during acetone extraction. Since the extent of cytochrome b_6 reduction in the reconstituted system exceeds that with the lyophilized preparation, this part of the oxidoreductase reaction seems to be less damaged.

It is tempting to conclude that the plastoquinone molecule carried along with the preparation plays a specific role in oxidoreduction within the cytochrome b_6-f complex. But is this conclusion really justified? The supporting evidence we see in the facts that there is a constant stoichiometry of one PQ per cytochrome f in various preparations tested, and that they are enriched in a more hydrophilic PQ, probably PQ-C. As already mentioned this enrichment could be caused by a coincident selectivity of the extracting detergent mixture, but it is unlikely that the quinone/detergent micelles would have the same density as the protein/detergent micelles and therefore would not separate on sucrose density gradients. (It has been found that the peak of PQ coincides with the peak of cytochrome f on the gradient.) The evidence against the above conclusion are the facts that, in spite of an apparently fixed stoichiometry, the PQ complement is heterogeneous, and that oxidant-induced reduction of cytochrome b_6 can be stimulated or even reconstituted by PQ-A, either PQ-1 or PQ-9. This inspecificity is in contrast to results obtained with ubiquinone-extracted chromatophores [13,17], but corresponds to a similar inspecificity reported for reconstitution of ubiquinol oxidation in extracted mitochondrial membranes [25].

Visualizing oxidant-induced reduction of cytochrome b in the frame of the Q-cycle of Mitchell [26], two binding sites for PQ would be required on the cytochrome b_6-f complex, one for reduction and one for oxidation of cytochrome b_6, both via the semiquinone. Recently, a higher specificity for the cytochrome b-oxidizing site has been reported for the mitochondrial cytochrome b-c_1 complex [27], which is feasible since this site, according to the Q-cycle, would require a more stable semiquinone form [26,28]. On this basis one might interpret our results as follows: PQ-C is bound to the cytochrome b_6-oxidizing site in the cytochrome b_6-f complex in a rather tight way, so that it almost completely remains with the preparation. Residual PQ-A in the preparation might mainly be responsible for residual oxidant-induced reduction of cytochrome b_6 (right-hand part of Fig. 1), which can be stimulated by addition of PQ-1 (left-hand part of Fig. 1). In line with this speculation is that reduction of cytochrome b_6 is reconstituted with PQ-1 much better than oxidation (Fig. 7). If oxidation of cytochrome b_6 by PQ-C is required for the turnover of the complex, the inefficiency of PQ-1 and also of PQ-9 in reconstituting oxidoreductase activity in the acetone-extracted preparation is also understood. Reconstitution experi-

ments with PQ-C would be required to support this interpretation.

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