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CYTOCHROME b-559 AS AN ENERGY-TRANSDUCER IN THE CHLOROPLAST ELECTRON TRANSPORT CHAIN BETWEEN THE TWO PHOTOSYSTEMS

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Summary

Cytochrome b-559 is, beyond doubt, the most controversial component of the photosynthetic electron transport chain. Whereas the location and role of the other components of the electron transport chain are well established, the site and function of cytochrome b-559 are still under discussion. On our hands, and using isolated spinach chloroplasts, red light (650 nm) causes an increase (reduction) in absorption at 559 nm, whereas subsequent illumination with far red light (720 nm) causes a dicrease (oxidation) in absorption at 559 nm. DCMU completely blocks cytochrome b-559 reduction by red light, while its oxidation by far red light is greatly stimulated and enhanced in the presence of the uncoupler CCCP at very low concentration (1 µM). It seems thus obvious that cytochrome b-559 is in the coupling site between both photosystems. Cytochrome b-559 is further characterized by exhibiting a pH-independent labile high-potential form and a pH--dependent stable low-potential one. Both forms are interconvertible, the proportion between them depending on the energization state of the chloroplast. In addition, there is a close correlation between light-induced proton uptake by thylakoid vesicles -coupled to noncyclic electron flow with $NADP^+$ or ferricyanide as Hill reagents- and the percentage of cytochrome b-559 in its high-potential form. We propose, in agreement with our general approach to energy transduction by bioelectrochemical systems, that cytochrome b-559 acts as a transducer of redox energy into acid-base energy between plastoquinone and cytochrome f in noncyclic photophosphorylation.

1. INTRODUCTION

Photosynthesis consists essentially in the conversion of light energy into electronic energy and subsequently into redox energy and phosphate-bond energy, which are then used for the reduction and further assimilation of the oxidized primordial bioelements, namely, carbon, nitrogen and sulfur (1,2).

We have recently proposed that electronic energy (resulting from either electron excitation or localization) seems to be the primary obligatory link between any two of the different kinds of energy (light, redox, acid-base, phosphate-metaphosphate) transducible by bioelectrochemical systems. The key point in energy coupling between any two systems, e.g., redox and acid-base systems, lies apparently in the

fact that both of them share a common intermediate that cyclically participates in the overall process by alternating between its electronically energized state and its unenergized ground state (2,3-5).

With regard specifically to chloroplast cytochrome b-559, its location and function in the photosynthetic electron transport chain(s), although widely and intensely investigated, remain yet very controversial and enigmatic (2,6-12). The results presented in this paper reinforce our previous proposal that cytochrome b-559 is located between the reducing side of photosystem II (plastoquinone) and the oxidizing side of photosystem I (cytochrome f), precisely one of the sites of the photosynthetic electron transport chain(s) coupled to photophosphorylation (2,13-17), where it seems to act as a proton translocator.

2. MATERIALS AND METHODS

Thylakoid suspensions were prepared from spinach leaves by the method of Arnon and Chain (18). Cytochrome absorbance measurements were made in an AMINCO DW-2a TM dual wavelength spectrophotometer at 20°C.

Cytochromes b-559 and f redox changes in thylakoid suspensions (65 µg Chl ml^{-1}) after ilumination with light absorbed either by photosystem II or I were determined by absorbance difference at 559-570 nm or 554-570 nm, respectively. A monochromator ORIEL 7240, adjusted at 565 nm, was inserted between the spectrophotometer photomultiplier and the sample compartment to allow the passage of reference and measurement lights, but not of actinic light (see below). Selective illumination either of PS II or PS I was attained with BAIRD-ATOMIC short-band filters of maximal transmitance at 650 nm and 720 nm, respectively.

Redox titrations were carried out under argon, using thylakoid suspensions containing 50 µg Chl ml^{-1} in 3 ml-cells thermostated at 20°C, by following the absorbance changes at 559-570 nm after oxidation with ferricyanide and reduction with dithionite. Redox potentials were simultaneously determined, in the presence of suitable redox mediators, with a BECKMAN-4500 potentiometer provided with a combined Pt-Ag/AgCl INGOLD electrode previously calibrated against a saturated solution of quinhydrone (E'_o, pH 7, +280 mV at 25°C).

Proton translocation measurements were carried out at 25°C in a 5 ml--vessel containing an unbuffered thylakoid suspension (100 µg Chl ml^{-1}) and either 0.6 mM ferricyanide or 1 mM $NADP^+$ (plus 10 µM Fd and 5 mM $MgCl_2$) as Hill reagents. The pH was determined by using an INGOLD (60-150 M Ω impedance) electrode attached to a BECKMAN-4500 pH-meter with a SERVOSCRIBE-S20 register that allowed a sensitivity of 20 mV. Starting from an initial pH value of 6.0, the maximum pH shift occurring upon irradiation of the cell with 150 W m^{-2} white light was estimated. In the experiments with ferricyanide, a red BALZERS filter was used to avoid interferences. Calibration and initial pH adjustment of the system were achieved by adding known amounts of either NaOH or HCl.

3. RESULTS AND DISCUSSION

Fresh spinach chloroplasts contain cytochrome b-559 in both its high-potential (HP) and low-potential (LP) redox forms, the first one being reducible by hydroquinone or dithionite and the second one being reducible only by dithionite (3,4). The hydroquinone-reducible high-potential (HP) couple -the oxidized form of which is energized and labile- is pH-independent and exhibits a constant midpoint redox potential value (E'_o, +340 mV) in the pH range between 6.5 and 8.5. The dithionite-reducible low-potential (LP) couple -the oxidized form of which is unenergized and stable- is pH-dependent (E'_o,pH 7, +115 mV) and undergoes a decrease in its midpoint potential of about 60 mV per pH unit below a pK_a of 7.6 (fig. 1).

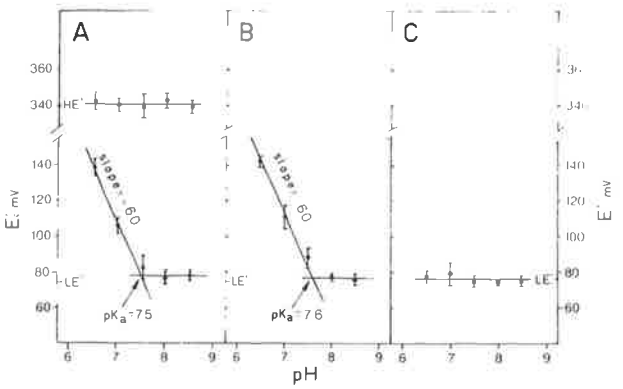

Figure 1. Dependence on pH of the midpoint redox potential of the HP and LP cytochrome b-559 couples in fresh (A), heated (B) and CCCP--treated (C) thylakoids. Redox potentials were determined at different pH, as described in Materials and Methods, in the presence of the following redox mediators: 20 μM 2,3,5,6--tetramethyl-p-phenylendiamine; 20 μM 1,2-naphtoquinone; 2.5 μM PMS; 10 μM phenazine ethosulfate; 20 μM duroquinone. The solution buffers were 50 mM potassium phosphate (pH 6.5 and 7) and 50 mM Tricine-KOH (pH 7.5, 8.0 and 8.5). In case B, the thylakoid suspension was heated for 5 min at 55°C. Final concentration of CCCP was 33 μM. The reported values, with their corresponding standard deviations, are averages of five independent experiments.

Figure 1 shows also that after mild heating of the chloroplast preparation, the HP couple is converted into the LP couple and becomes then pH-dependent. Treatment of the chloroplast preparation with Triton X-100 brings about similar modifications in redox potential and in pH-dependence. In contrast, in the presence of 33 μM CCCP, although the HP couple is also transformed into the LP couple, the uncoupler causes the loss of the pH-dependence characteristic of the latter. Cytochrome b-559 is, however, totally indifferent toward ammonia -a very effective uncoupler of photophosphorylation- even at 5 mM concentration (results not shown).

Figure 2 shows that red light (650 nm) absorbed by photosystem II induces an increase (reduction) -which can be inhibited by DCMU- in absorbance at 559 nm (relative to 570 nm), whereas subsequent ilumination of the fresh chloroplast preparation with far red light (720 nm) absorbed by photosystem I causes a decrease (oxidation) in absorbance at 559 nm. This oxidation by far red light is greatly accelerated and enhanced in the presence of 1 μM CCCP, the difference spectrum for the change corresponding unequivocally to cytochrome b-559.

Broken chloroplast suspensions were treated with Triton X-100 at different concentrations in order to correlate the effect of such a treatment on redox-linked proton translocation by thylakoid vesicles with their content in HP cytochrome b-559. As shown in figure 3, a close relationship exists (correlation coefficient = 0.98) between light--induced proton translocation coupled to noncyclic electron flow with either $NADP^+$ or ferricyanide as Hill reagents and the percentage of cytochrome b-559 in its HP form. Similar results were obtained in the

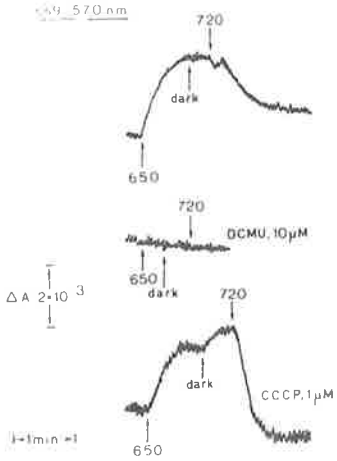

Figure 2. Effect of the inhibitor DCMU and of the uncoupler CCCP on absorbance changes at 559 nm induced in a thylakoid suspension by light absorbed either by PS II (650 nm) or PS I (720 nm). The absorbance changes were determined as described in Materials and Methods. Where indicated, 10 µM DCMU or 1 µM CCCP was added to the experimental cuvette immediately before the assay.

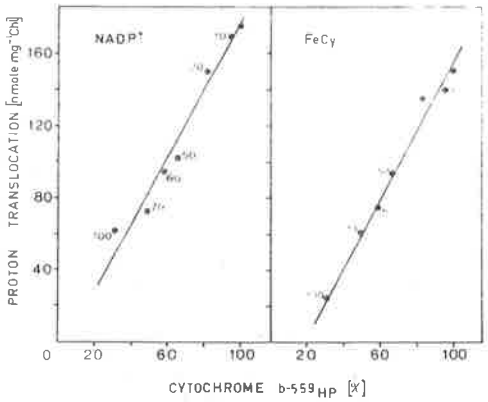

Figure 3. Correlation between proton translocation coupled to electron flow with $NADP^+$ or ferricyanide as Hill reagents and percentage of HP cytochrome b-559 in thylakoid vesicles treated with Triton X-100. After 5 min incubation with Triton X-100 at the indicated concentrations (ppm), proton translocation and the amount of HP cytochrome b-559 were measured as described in Materials and Methods.

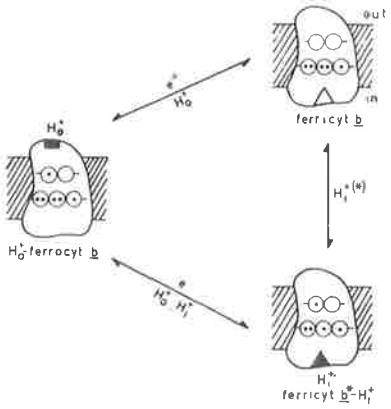

Figure 4. Diagrammatic representation of the reversible transduction of redox energy into acid-base energy through electronic energy by the cytochrome b-559 system. The (3 + 2) d orbitals of the heme iron ions at the two energy levels determined by ligand-field splitting energy and the 5 or 6 electrons corresponding to the ferric or ferrous states are depicted in the scheme. The pK_a of the reduced and oxidized (either in its ground or energized state) forms are represented by solid (high pK_a values) or empty (low pK_a value) rectangle or triangles, respectively. Energized electrons and protons are labelled with an asterisk. Out and in denote the extra- and intra-thylakoid spaces, respectively. Other details in the text.

presence of CCCP at very low concentration or when, instead of a Hill reagent, PMS was used as a cofactor of cyclic electron flow (data not shown).

It is concluded from a congruent interpretation of all the above mentioned separate facts (fig. 4) that, upon reduction at low potential by PS II, via plastoquinone, of the unenergized oxidized form (ferricyt. b), a high-pK_a group appears in the reduced form that determines fixation of one proton at high pH from the extrathylakoid space. Subsequently, oxidation at high potential by PS I, via cytochrome f, of the protonated reduced form (H_o^+-ferrocyt. b) is accompanied by proton translocation from the high-pK_a group of the reduced form to the high-pK_a* group of the resulting energized oxidized form. Finally, the energized oxidized and protonated form (ferricyt. b^*-H_i^+) can dissociate its proton toward the intrathylakoid space at the low pK_a of the unenergized oxidized form (ferricyt. b), thus closing a cycle that results in vectorial transduction of redox energy into acid-base energy, according to the following sequence of reactions:

$$\text{ferricyt. } \underline{b} + \underline{e}^* + H_o^+ \xleftrightarrow{LE'_o} H_o^+\text{-ferrocyt. } \underline{b}$$

$$H_o^+\text{-ferrocyt. } \underline{b} \xleftrightarrow[Hp\underline{K}_a(red)\,;\ Hp\underline{K}_a^*(ox)]{HE'^*_o} \text{ferricyt. } \underline{b}^*\text{-}H_i^+ + \underline{e}$$

$$\text{ferricyt. } \underline{b}^*\text{-}H_i^+ \xleftrightarrow[Lp\underline{K}_a(ox)]{} \text{ferricyt. } \underline{b} + H_i^{+(*)}$$

$$\underline{e}^* + H_o^+ \xleftrightarrow{} \underline{e} + H_i^{+(*)}$$

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