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Analysis of the transformation effect in cytochrome *b*559 of photosystem II in terms of the model of the heme-quinone redox interaction

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ABSTRACT

Transformation of three-component redox pattern of cytochrome (Cyt) b559 in PS II membrane fragments upon various treatments is manifested in decrease of the relative content (R) of the high potential (HP) redox form of Cyt b559 and concomitant increase in the fractions of the two lower potential forms. Redox titration of Cyt b559 in different types of PS II membrane preparations was performed and revealed that (1) alteration of redox titration curve of Cyt b559 upon treatment of a sample is not specific to the type of treatment; (2) each value of R_{HP} defines the individual shape of the redox titration curve; (3) population of Cyt b559 may exist in several stable forms with multicomponent redox pattern: three types of three-component redox pattern and one type of two-component redox pattern as well as in the form with a single E_m; (4) transformation of Cyt b559 proceeds as successive conversion between the stable forms with multicomponent redox pattern; (5) upon harsh treatments, Cyt b559 abruptly converts into the state with a single E_m which value is intermediate between the E_m values of the two lower potential forms. Analysis of the data using the model of Cyt b559-quinone redox interaction revealed that diminution of R_{HP} in a range from 80 to 10% reflects a shift in redox equilibrium between the heme group of Cyt b559 and the interacting quinone, due to a gradual decrease of 90 mV in E_m of the heme group at the virtually unchanged E_m of the quinone component.

1. Introduction

Cyt b559 is an integral component of the PS II complex of higher plants and cyanobacteria (see Refs. [1–4] for reviews). It is a part of the minimal pigment-protein unit of PS II capable of charge separation and electron transfer – D1-D2-Cyt b559 complex. Cyt b559 is composed of two small transmembrane subunits - psbE and psbF, each of which contains a single histidine (His) that serves as a ligand for the heme group. The molecular organization of Cyt b559 is revealed in the crystal structures of the PS II complexes from cyanobacteria *Thermosynechococcus (T) elongatus* and *T. vulvanus* [5–10], red alga *Cyanidium caldarium* [11] and spinach [12]. It is considered that Cyt b559 operates to protect the PS II complex from photoinhibition in unfavorable environmental conditions by providing a pathway of backflow of electrons to the oxidizing equivalents at the donor side of PS II - cycling around PS II [13–25] (reviewed in Refs. [1–4, 26]). Detailed mechanism of the Cyt b559 functioning remains however unclear. It is also suggested that Cyt b559 may operate as a plastoquinol oxidase, an oxygen reductase, or a superoxide oxidase/reductase (see Ref. [27] for a review).

Redox properties of Cyt b559 are peculiar. It has long been discovered that in intact higher plant chloroplast preparations Cyt b559 is redox heterogeneous and the redox transitions of its three forms cover a range of > 400 mV [28]. By different evaluations, the midpoint potentials of the individual redox forms of Cyt b559 in membrane preparations of PS II consist of 360–440, 200–280 and 20–130 mV for the high potential (HP), intermediate potential (IP) and low potential (LP) forms, respectively [29–38].

The HP form dominates in fresh preparations of thylakoids and PS II membrane fragments comprising 70–80% but various treatments (such

Abbreviations: Car, carotenoid; CHES, 2-[*N*-cyclohexylamino]ethanesulfonic acid; Chl, chlorophyll; Cyt, cytochrome; D1, D2, subunits of the PS II complex; β-DM, *n*-dodecyl-β-D-maltoside; DSC, differential scanning calorimetry; ε_{eff}, effective local dielectric constant; E, microscopic midpoint potential; E_m, macroscopic midpoint potential; E_h, ambient redox potential; EDTA, ethylenediaminetetraacetic acid; F, Faraday constant; FeCy, $K_3Fe(CN)_6$; HEPES, 4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid; His, histidine; HP, high potential; I, interaction potential; IP, intermediate potential; K_{eq}, equilibrium constant; LP, low potential; MES, 2-[*N*-morpholino]ethanesulfonic acid; P, redox microstate; PQH_2, plastoquinol; PS, photosystem; P680, a special pair of reaction center Chls; Q, quinone; QH, singly protonated quinone; R, gas constant; R_{HP}, R_{IP}, R_{LP}, relative contributions of the HP, IP, LP forms; T, temperature

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as mild heating, ageing, sonication, incubation at high concentrations of some inhibitors etc.) irreversibly diminish its content at the expense of the forms with lower E_m (the relative amount of HP Cyt b559 decreases along with increase in the sum percentage of the lower potential forms) [28,39]. This conversion is known as a transformation effect (reviewed in Refs. [2, 4, 40]). In samples of lower integrity - PS II core complexes and D1-D2-Cyt b559 complexes from higher plants [36,41], some preparation types of PS II core complexes from *T. elongatus* [42] and *Synechocystis* sp. *PCC 6803* [43], treated preparations of PS II membrane fragments [35] and in an isolated form [44] Cyt b559 exhibits a single E_m varying from 240 to 60 mV.

It was early shown that treatments of thylakoid membranes and PS II membrane fragments do not considerably change the redox potentials of the HP, IP and LP forms of Cyt b559 [29,30,32,33]. Therefore, the relative fractions of the Cyt b559 redox forms in PS II samples are often estimated by the rapid three-point registration method where the amounts of reduced Cyt b559 are determined in the presence of the successively added reductants - hydroquinone, ascorbate and dithionite relative to the initially ferricyanide (FeCy)-oxidized heme protein.

A type of modification at which the relative contributions of the redox forms of Cyt b559 are mainly affected allowed to consider transformation of Cyt b559 as irreversible conversion between the conformational forms with different E_m [28,39]. However, structural changes which may cause such large shifts in E_m of the heme group in the membrane cytochrome (about 150 and 300 mV during presumed conversions HP → IP and HP → LP, respectively) are unknown. Also, the E_m value of the HP form is anomalously positive for bis-His ligated heme (for a review, see Ref. [45]). It was suggested that difference between the HP and LP forms is due to a change in mutual orientation of the planes of the His heme ligands [46], however, later estimations showed that the effect would account for only a 50 mV E_m shift [47]. Other factors which may affect the redox potential of the heme group in Cyt b559 have been considered such as changes in dielectric properties of the heme environment [48] and different modes of protonation and H-bonding near the heme [32,37,49–51]. Recent data also suggest that molecular interactions of the Cyt b559 protein subunits psbE and psbF with the neighbor subunits psbY [52] and psbP [53] contribute to the high redox potential of the heme group of Cyt b559.

Recently we offered a new interpretation of redox heterogeneity of Cyt 559 in the native PS II complex [54]. It is proposed that the heme protein itself is redox homogeneous but its E_m may shift, as a result of redox interaction with the neighbor plastoquinone Q_C. The quinone Q_C was revealed, besides quinones Q_A and Q_B, in the crystal structure of PS II from cyanobacterium *T. elongatus* [5]. It is located at 20 Å from the Cyt b559 heme iron in the intraprotein hydrophobic cavity far from the protolytic groups and protein aminoacids. In the later published PS II structures of cyanobacterial PS II complexes having even higher resolution the Q_C site was found to be unoccupied [6–10]; the loss of quinone at the Q_C was ascribed to differences in purification and crystallization procedures, samples pretreatments as well as to the lack of a well-defined binding pocket [55–57]. The function of Q_C is presently unknown; several possibilities have been suggested such as a docking domain for a spare plastoquinone to accelerate an access of quinone to the Q_B site [5], a site of plastoquinol (PQH_2) binding and oxidation [58] and a role in the photoprotection mechanism in cyanobacteria [59].

Based on the suggested model of Cyt b559-quinone redox interaction, the microscopic redox potentials of the heme group and the interacting quinone were calculated using the parameters of the Cyt b559 redox titration curves [54]. Unusually positive E_m values of one-electron reduction of quinone were obtained as well as a high stability constant of the semiquinone form. We suggested that such peculiar redox properties can be manifested by a quinone present in the unusual singly protonated state (QH). Since the QH will be charged in the fully oxidized and fully reduced forms, the mechanism of the heme-quinone redox interaction can be an electrostatic effect between charged redox centers embedded in a low dielectric matrix. Under normal conditions, the singly protonated redox forms of quinone are thermodynamically unstable; therefore, their persistence in the PS II complex should be under kinetic control. It is proposed that the molecules of Car that surround the Q_C, including the closest Car_{D2}, as being easy polarizable, ensure partial stabilization of the energized charged forms of the Q_CH by formation of the delocalized dipoles.

The idea on the functional complex Cyt b559-Q_CH provides also a clear mechanism of cyclic electron flow around PS II. In the suggested mechanism [54], the anionic doubly reduced Q_CH^- induces a negative shift in the redox potential of an accessory chlorophyll Chl_{D2}, promoting its oxidation by the primary donor of PS II, P680. This reaction launches cascade of oxidation of the alternative electron donors of PS II.

Cyt b559 is a terminal reductant for oxidized P680 [13–16,18,21,22,25,60] and gets rapidly rereduced from PQH_2 of the pool [61–66]. It is proposed that fast redox equilibrium between Cyt b559 and plastoquinol of the pool is established via, yet unidentified in the structure, quinone binding site located near Cyt b559 called Q_D [66]. Initially, before the discovery of the third quinone in the PS II complex [5], this polar quinone site engaged in photoreduction of Cyt b559 mediated by short-chain plastoquinones [67] and influencing the redox potential of Cyt b559 [68,69] was named Q_C. Interaction of Cyt b559 with the quinone binding sites Q_C and Q_D probably lies at the heart of the complex machinery of cyclic electron transport in PS II, with its redox regulation by the level of reduction of the plastoquinone pool and by the rate of the photosynthetic electron flow.

In the new interpretation of the redox properties of Cyt b559, the redox transitions HP, IP and LP are viewed as stages of successive reduction of the three-electron acceptor, the Cyt b559-Q_CH cluster, and not as the individual conformational states of Cyt b559. Therefore, reconsideration of the Cyt b559 transformation effect in terms of the new model is required. This work presents detailed study on modification of redox pattern of Cyt b559 in PS II membrane fragments caused by various treatments. Redox titration of Cyt b559 in preparations of PS II membrane fragments was carried out and analysis of the titration curves was performed using the model of the Cyt b559-Q_CH redox interaction. The results obtained provide new understanding of the transformation effect in Cyt b559.

2. Materials and methods

PS II membrane fragments were obtained from sugar beet leaves according to the method of Berthold et al. [70] with some modifications [71]. Chlorophyll (Chl) concentration was determined according to Ref. [72]. The treatment procedures – ageing, heating, incubation at high pH and in the presence of hydroxylamine - were performed at a concentration of Chl in sample suspensions equivalent to 0.5 mg/ml.

Heat treatment of PS II membrane fragments was carried out by incubation of samples in a standard medium containing 100 mM MES, pH 6.5, 0.3 M sucrose, 10 mM $CaCl_2$, 10 mM NaCl and 10% (v/v) glycerol in a water bath at 40–57 °C in the dark. If indicated, 0.5 mM FeCy was added to the incubation medium. Heating was terminated by rapid cooling on ice.

PS II membrane samples were aged at 6 °C in the dark for different periods of time in the standard medium supplemented, if indicated, with 1–2 mM FeCy. Ageing of samples at pH 7.5 was achieved using incubation medium that contained 25 mM HEPES (pH 7.5) instead of MES.

Hydroxylamine treated PS II membranes were obtained by using the procedure described in Ref. [73] with modifications [74]. A sample was incubated at 4 °C in a buffer containing 50 mM MES (pH 6.5), 0.4 M sucrose, 15 mM NaCl, 1 mM $CaCl_2$, 2 mM sodium ascorbate and 5 mM hydroxylamine hydrochloride for 1 h in the dark. After the treatment, the samples were washed twice in the standard buffer medium.

For high pH treatment, suspensions of PS II membrane fragments were incubated at 20 °C for indicated periods of time in the standard