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The evidence that Cyt *b*-559 can act as an electron donor or protector to PSII reaction center

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Introduction

Cytochrome *b-559* is a *b*-type heme-binding protein and an integral component of the PS II reaction center. It has been proposed to be a heme-bridged protein heterodimer with two subunits, α and β, encoded by *psb*E and *psb*F genes, respectively. Each polypeptide chain forms a transmembrane α-helix, the heme being perpendicular to the thylakoid membrane plane and close to the stromal side. A peculiar property of this cytochrome is that it exhibits several redox potential forms, usually a labile high-potential (HP) form and a stable low-potential (LP) form. Although considerable efforts have been directed towards the elucidation of its physiological role in PS II, its function still remains controversial. Over the past two decades, the participation of Cyt *b*-559 in redox mechanisms to protect PSII against donor- and acceptor-side photoinhibition has been proposed. In the present report, by using a simplified isolation procedure, large quantities of Cyt *b*-559 from two higher plants were purified. From comparatively analysis of structure and function of purified Cyt *b*-559 and isolated PSII reaction center, it is shown that the stability of Cyt *b*-559 makes for exert its protective function. Moreover, the structure and function of this protein from different higher plants might be similar, i.e. Cyt *b*-559 act as an electron donor or protector to PSII reaction center under different conditions.

Methods and Meterials

Spinach (*Spinacia oleracea* L.) was obtained from a local market. Rice (*Oryza sativa* L.) leaves were harvested from 4 week-old seedlings. Cytochrome *b*-559 was purified according to Xin et al. (2000). Cytochrome concentrations in samples were determined by chemically induced difference spectra using potassium ferricyanide as an oxidant and a few crystals of sodium dithionite as reductant. For estimation of the ratio of HP to LP forms of cytochrome *b*-559 hydroquinone were used as reductant.

Fluorescence spectra were measured at 77K in visible light region with Hitachi F4500 fluorescence spectrophotometer.

Photoinhibition treatments were according to Kuang et al (1993). The specific chemical modification of histidine with DEPC (diethylpyrocarbonate) was followed Li et al (2000).

Results and Discussion

Elucidation of the native molecular properties of Cyt *b*-559 may be essential to the interpretation of its function. By our isolation procedure, Cyt *b*-559 was purified from two kinds of higher plants. This procedure is different from that of Garewal and Wasserman [10]. From the starting material containing 200 mg chlorophyll, 6 mg of purified cytochrome *b*-559 could be obtained. The high yield and purity of Cyt *b*-559 preparation are essential in studies of its structure and function.

The absorption spectrum of isolated Cyt *b*-559 shows that the peaks were at 559, 530 and 429nm in the reduced form. This form is virtually identical to the low potential cytochrome *b*-559 described by Stewart *et al* (1998). Addition of dithionite to the sample of this cytochrome did not alter the shape of the spectrum. Both the absolute spectrum and the difference spectrum of the rice Cyt *b*-559 were very similar to those of spinach.

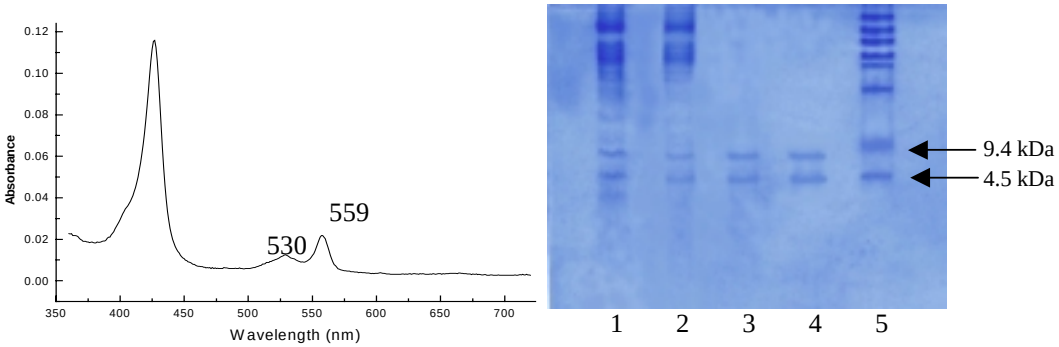

Fig.1. Absorption spectra (left) and Tricine-SDS-PAGE (right) analysis of cytochrome *b*-559 isolated from spinach and rice.
Lane 1, spinach OEC PSII; lane 2, rice OEC PSII; lane 3, purified spinach cytochrome *b*-559; lane 4, purified rice cytochrome *b*-559; lane 5, protein marker from Sigma M3913.

Analysis of the purified protein using novel Tricine-SDS-PAGE revealed two polypeptide bands with apparent molecular weight of 9.4 kD and 4.6 kD, respectively. This is consistent with the results of proposed amino acid distribution in α and β subunits transmembrane α-helix of cytochrome *b*-559. Polypeptide bands with mobility identical to that of both bands of the purified cytochrome could be observed in samples of spinach PS II membranes and rice PS II membranes (Fig. 1, lanes 1, 2). No distinct differences in the polypeptide patterns could be detected between these two samples.

Cytochrome *b*-559 is a heme-protein, in which one tetrapyrrole ring is bound. The tetrapyrrole is very similar to the chromophores of chlorophyll, except the central

metal Mg in the chlorophyll is replaced by Fe in cytochrome. Fig. 2 shows the low temperature (77 K) fluorescence excitation and emission spectra in visible light region of the purified cytochrome *b*-559 form spinach. The two excitation peaks are located at 413 nm and 439 nm. As for fluorescence emission, one major peak was located at 668 nm and another at 563 nm. All these peaks were not found in excitation and emission spectra of PS II. Therefore it could be excluded that they are originated from chlorophyll in these particles. These results are the first indication that Cyt *b*-559 is able to emit fluorescence, although very much smaller compared with chlorophyll in PS II reaction center, and transfer excited electrons to chlorophyll *a* contained within the complex.

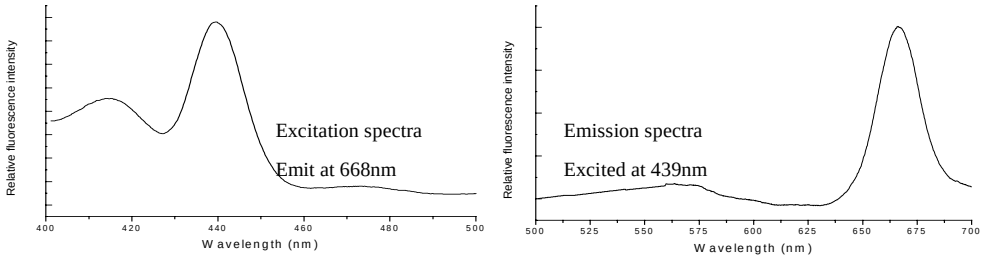

Fig. 2. Low temperature (77 K) fluorescence spectra of cytochrome *b*-559 purified from spinach.

The above results indicated that the biochemical and biophysical properties of the purified protein could provide useful data for the elucidation of its role in the photosynthetic process. Using the isolated PSII reaction center and purified Cyt *b*-559 and under same photoinhibition treatment condition, we investigated the stability of Cyt *b*-559 and relationship with its protective function.

We have first reported the photodamage of histidine residues of PSII reaction center under illumination by analyzing the amino acid composition (1993). The level of histidine residues declined about 26%, the positions and mechanism for photodamage of histidine residues, however, remains unclear. From the analysis of photoinhibition of purified Cyt *b*-559, it is shown that two histidine of Cyt *b*-559 are stable during treatment and not belong to the damaged histidine in PSII reaction center photoinhibition. So we speculated that histidine residues binding with P680 were damaged. The study in which preparations were damaged by active oxygen showed the same response (date not shown).

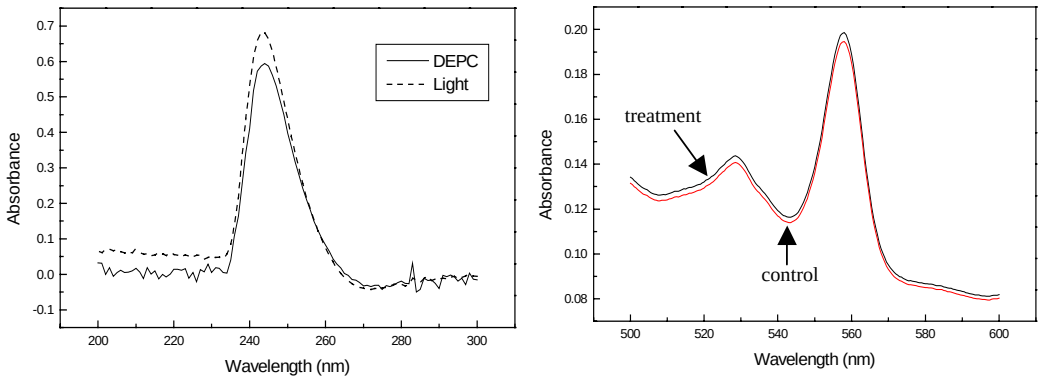

Fig. 3. Effect of modification with DEPC and photoinhibition on different absorption spectra of PSII reaction center (left) and purified Cyt *b*-559 (right).

Li et al (2000) suggested that the mechanism of photoinhibition is similar to the chemical modification with DEPC i.e. DEPC can react with imidazole ring of His and thus lead to the changes of binding state between pigments and proteins, so that spectra are affected by the changed conformation. Fig. 3 shows the absorption difference spectra of PSII reaction center after DEPC modification. The result of photoinhibition of PSII RC showed absorbance increase at 240nm, same as modification result. Although the results clearly showed that some His were modified by DEPC, it cannot decide the position of His. In order to clarify whether two His of Cyt *b*-559 were modified by DEPC, we modified purified Cyt *b*-559 and find that there is no change in spectra (Fig.3 right). The results showed again that two His of Cyt *b*-559 are not belong to the damaged His. The reasons of stability may due to heme-bridge to protein. These above stability make for exert the protective function of Cyt *b*-559.

Photoinduced changes in Cyt *b*-559 under low light photoinhibition have also been investigated using the same preparations. Under acceptor side photoinhibition, the absorbance of Cyt *b*-559 increased in the beginning several minutes of treatment then decreased with progressive illumination. However, the absorbance change under donor side photoinhibition showed a contrary tendency. These results suggested that Cyt *b*-559 is very sensitive to occurrence of photoinhibition in PSII and may be a protector in this case. Our results presented above are consistent with our previous proposal that under normal conditions Cyt *b*-559 functions as a electron donor to PSII reaction center and under stress condition, Cyt *b*-559 prevents PSII reaction center from photodamage and functions as a “molecular switch” between the two forms of Cyt *b*-559.

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