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RESTORATION OF HIGH-POTENTIAL CYTOCHROME *b*-559 IN PHOTOSYSTEM II PARTICLES IN LIPOSOMES

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The effect of a liposome environment on cytochrome *b*-559 was examined in two types of Photosystem II particles. A substantial fraction of the low-potential cytochrome *b*-559 of Photosystem II core particles, which do not evolve oxygen, was restored to a high-potential form in the liposome preparation. A preparation of oxygen-evolving Photosystem II particles, which was selected on the basis of having a relatively low rate of oxygen evolution (68 μmol oxygen/mg chlorophyll per h), showed very little high-potential cytochrome *b*-559 and a less-than-normal amount of variable-yield fluorescence. In the liposome preparation, however, these particles showed considerably more high-potential cytochrome *b*-559, an almost-normal amount of variable-yield fluorescence and a substantially greater rate of oxygen evolution (183 μmol oxygen/mg chlorophyll per h).

Introduction

We recently reported that the midpoint potential of purified cytochrome *b*-559 which was present in a low-potential, ascorbate-reducible form could be increased to give a high-potential, hydroquinone-reducible form by incorporating the cytochrome into liposomes [1]. The purpose of the present work was to examine the effect of the liposome environment on the cytochrome *b*-559 in PS II particles. The F_{II} particles, prepared by the method of Satoh and Butler [2], which contain no Chl *b* and are inactive in oxygen evolution and the oxygen-evolving PS II particles prepared by the method of Berthold et al. [3], which are enriched in Chl *b* and probably represent fragments of grana, were examined. We cannot state unequivocally whether these particles were incorporated into liposomes or whether the lipids were incorporated into the particles. In either case the environment around the cytochrome *b*-559 appears to have been altered, since substantial fraction of the low-potential cytochrome *b*-559 present in the particles was converted back to a high-potential form in the liposome preparation and, in the case of the oxygen-evolving PS II particles, this was accompanied by a substantial increase in the rate of oxygen evolution.

Materials and Methods

F_{II} particles, which presumably represent the core of PS II, were purified through the electrofocusing step by the method of Satoh and Butler [2]. These particles retain the primary photochemical activity of PS II but are not capable of oxygen evolution. Oxygen-evolving PS II particles were prepared by the method of Berthold et al. [3].

Liposomes were prepared from DGDG which

Abbreviations: DGDG, digalactosyldiacylglycerol; PC, phosphatidylcholine; Mes, 2-(*N*-morpholino)ethanesulfonic acid; PS, photosystem; Chl, chlorophyll.

had been purified from spinach [4] and PC purified from egg yolks [5]. 20 μl of 100 mM DGDG and 5 μl of 100 mM PC were flushed with nitrogen and kept in a vacuum desiccator overnight at 4°C to remove the organic solvents. 300 μl of the F_{II} particles (50 μg Chl/ml) in 10 mM Tris-HCl buffer, pH 7.2, containing 15% glycerol and 1 mM dithiothreitol or 300 μl of the oxygen-evolving PS II particles (200 μg Chl/ml) in 20 mM Mes buffer, pH 6.5, with 0.4 M sucrose, 15 mM NaCl, 5 mM $MgCl_2$ and 1 mM dithiothreitol were added and the mixture was stirred for 2 h at 4°C under nitrogen. Finally, 300 μl of the suspending buffer were added to give 600 μl of the liposome preparations.

Absorption spectra were measured on 300-μl samples with our computer-linked single-beam spectrophotometer [6] in a cuvette having a 1 cm vertical path. Redox difference spectra were plotted by the computer as differences between the single-beam spectra measured at room temperature after sequential additions of 0.2 mM potassium ferricyanide, 20 mM recrystallized hydroquinone, 20 mM sodium ascorbate and a few grains of sodium dithionite. Low-temperature light-induced difference spectra were plotted from single-beam spectra measured at −196°C before and after a saturating irradiation.

Fluorescence induction curves at −196°C were measured at 690 nm during irradiation at 633 nm on samples containing 10 μg Chl/ml. Oxygen evolution was measured with Clark-type oxygen electrode in 20 mM Mes buffer, pH 6.5, containing 0.4 M sucrose, 15 mM NaCl, 5 mM $MgCl_2$, 0.5 mM benzoquinone and 2.5 mM potassium ferricyanide.

Results

Difference spectra of the F_{II} particles in buffer and in the liposome preparation measured after sequential additions of 0.2 mM ferricyanide, 20 mM hydroquinone, 20 mM ascorbate and a few grains of dithionite are shown in Fig. 1. The cytochrome *b*-559 in the F_{II} particles in buffer was entirely in a low-potential form which was reducible by dithionite but not by 20 mM ascorbate. In the liposome preparation, however, the no addition-minus-ferricyanide difference spectrum shows

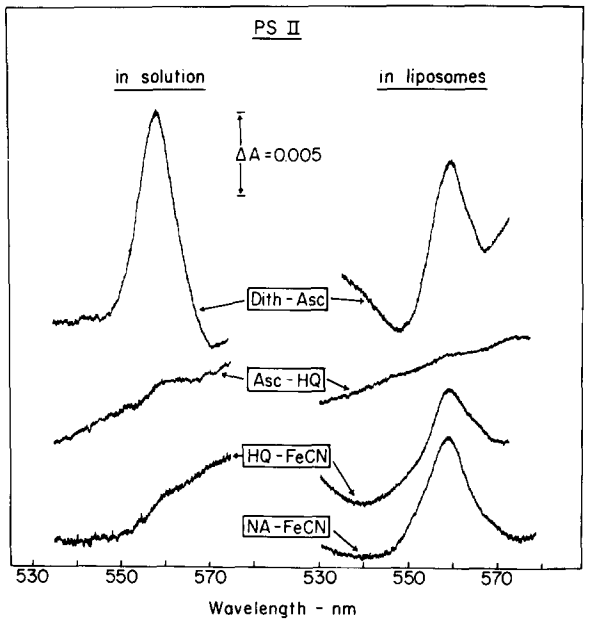

Fig. 1. Redox difference spectra of F_{II} particles (25 μg Chl/ml) in buffer and in liposomes. Single-beam absorption spectra were measured with no addition (NA) and after sequential addition of 0.2 mM potassium ferricyanide (FeCN), 20 mM hydroquinone (HQ), 20 mM sodium ascorbate (Asc) and a few grains of sodium dithionite (Dith) and difference spectra were plotted as indicated.

that a part of the cytochrome *b*-559 was present initially in the reduced state. This fraction appears to be stable in the reduced state and to resist autooxidation after the dithiothreitol has been exhausted. Most of this fraction, which was oxidized on addition of 0.2 mM ferricyanide, could be rereduced by 20 mM hydroquinone (see the hydroquinone-minus-ferricyanide difference spectrum of the liposomes in Fig. 1). While it could be argued that the resistance against autooxidation might be a better physiological criterion of 'high potential', we will use reducibility by 20 mM hydroquinone as our operational criterion, since it is more convenient. The ascorbate-minus-hydroquinone difference spectrum revealed no ascorbate-reducible cytochome *b*-559 in the liposomes while the dithionite-minus-ascorbate difference spectrum showed that a substantial fraction of the cytochrome *b*-559 remained in a dithionite-reducible form. We believe that the latter fraction repre-

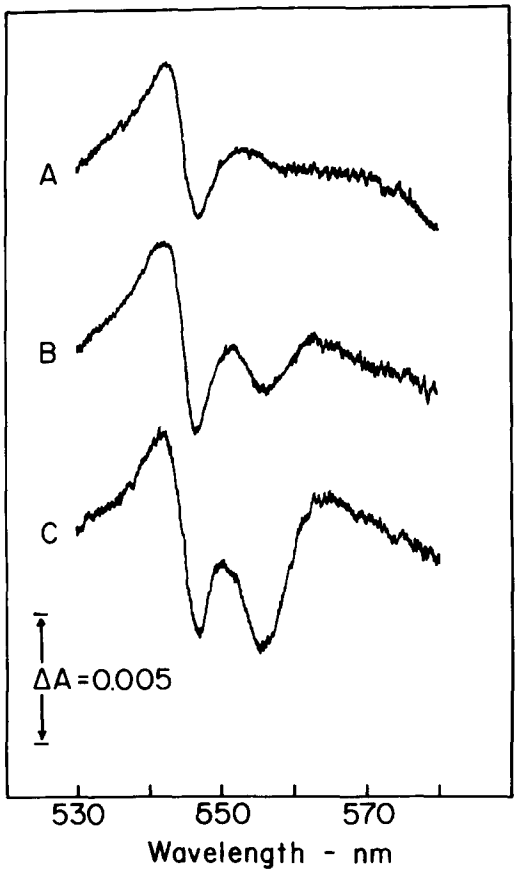

Fig. 2. Light-induced difference spectra of F_{II} particles (25 μg Chl/ml) in buffer and in liposomes at −196°C. The samples were all treated with 0.2 mM ferricyanide followed by 20 mM hydroquinone prior to freezing. Single-beam spectra were measured before and after a saturating irradiation at −196°C and light-minus-dark difference spectra were plotted. (A) F_{II} particles in buffer. (B) F_{II} particles in the normal DGDG liposomes. (C) F_{II} particles in liposomes which also incorporated plastoquinone in a molecular ratio DGDG : PQ of 50 : 1.

sents F_{II} particles which were not incorporated into liposomes.

Light-induced difference spectra of the F_{II} particles at −196°C are shown in Fig. 2. The samples were treated with 0.2 mM ferricyanide and then with 20 mM hydroquinone prior to freezing to ensure that only the hydroquinone-reducible cytochrome *b*-559 was present in the reduced state (this precaution was taken to preclude the possibility that some low-potential cytochrome *b*-559 might be in the reduced state due to the presence of some residual dithiothreitol). Irradiation of the F_{II} particles in buffer (curve A) shows only the photoreduction of C-550, since all of the cytochrome *b*-559 was in the oxidized state and none was available for photooxidation. With the F_{II} particles in liposomes (curve B) a part of the cytochrome *b*-559 was restored to a high-potential, hydroquinone-reducible form and was photooxidized by the photoreaction which reduced C-550. The fact that this photoreaction still occurs at −196°C shows that the cytochrome *b*-559 maintains its close proximity to the PS II reaction centers in the F_{II} particles in liposomes. For curve C in Fig. 2, plastoquinone A was incorporated into the liposomes in a molecular ratio of 1 plastoquinone per 50 DGDG. As was found previously with the incorporation of purified cytochrome *b*-559 into liposomes [1], the presence of plastoquinone A in the liposomes increased the amount of high-potential cytochrome *b*-559 in the liposome fraction. Also, in agreement with the previous study, liposomes made from DGDG and PC gave the grea-

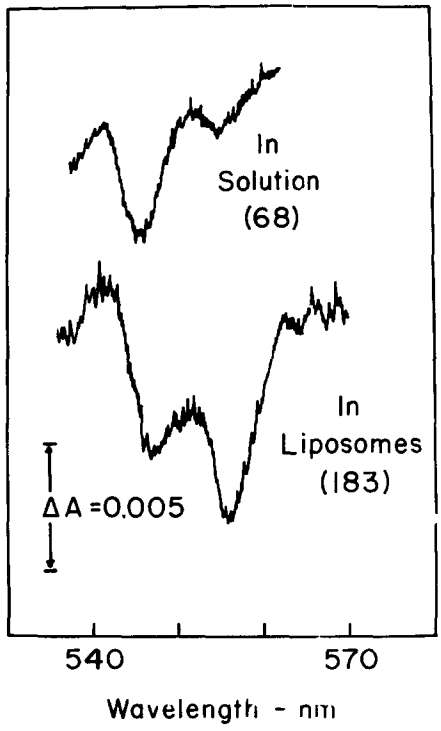

Fig. 3. Light-induced difference spectra of oxygen-evolving PS II particles (100 μg Chl/ml) in buffer and in liposomes at −196°C using the procedures described for Fig. 2.

test amount of high-potential cytochrome *b*-559. Much less was found if other detergents were substituted for PC and significantly less was found if other galactolipids purified from spinach were used in place of DGDG.

We also examined cytochrome *b*-559 in the oxygen-evolving PS II particles prepared by the method of Berthold et al. [3]. We chose to work with a preparation which had a relatively low rate of oxygen evolution. We expected that such a preparation would have relatively little high-potential cytochrome *b*-559 and we wished to determine if the amount of the high-potential form would be increased in the presence of liposomes and the effect that that might have on oxygen evolution. The results are shown in Fig. 3 where the high-potential cytochrome *b*-559 was assayed by the light-induced difference spectrum on samples which were frozen to −196°C after sequential additions of 0.2 mM ferricyanide and 20 mM hydroquinone (as in Fig. 2). The figures in parentheses indicate the rate of oxygen evolution in μmol oxygen/mg Chl per h. As expected there was rather little high-potential cytochrome *b*-559 in the original preparation . However, in the liposome preparation the amount of high-potential cytochrome *b*-559 was considerably greater and the rate of of oxygen evolution was increased 2.7-fold. We do not know if these particles, which presumably represent pieces of grana, were actually incorporated into liposomes or whether the lipids used in the liposome recipe was incorporated into the particles. In either case the environment around the cytochrome *b*-559 appears to have been modified in a way that caused both the midpoint potential of cytochrome *b*-559 and the capacity for oxygen evolution to increase.

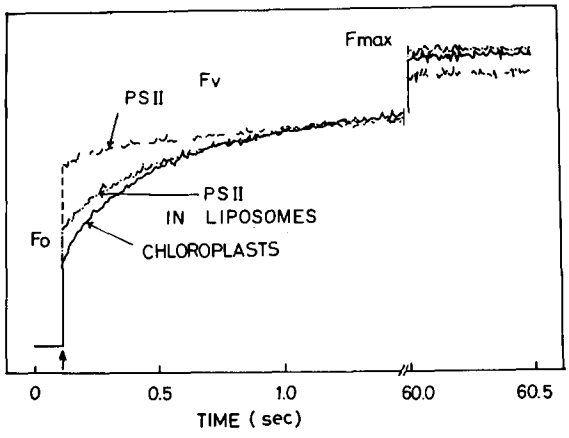

Fig. 4. Fluorescence induction curves at −196°C for chloroplasts and for the same PS II preparation that was used in Fig. 3 (10 μg Chl/ml). 1 mM dithiothreitol was present in the buffer and liposome preparations of the PS II particles.

Light-induced fluorescence yield changes at −196°C are shown in Fig. 4 for chloroplasts and for the oxygen-evolving PS II particles in buffer and in liposomes with 1 mM dithiothreitol. This was the same PS II preparation that was used to obtain the data in Fig. 3. It is apparent that the PS II particles in buffer had considerably less fluorescence of variable yield. F_V, than the chloroplasts, primarily because of the greater initial F_0 level of fluorescence. However, most of the F_V was regained in the liposome preparation.

Discussion

Our underlying thesis is that high-potential cytochrome *b*-559 is involved in oxygen evolution according to a scheme which has been presented previously [7]. The hypothesis assumes that the reduced form of cytochrome *b*-559 binds a proton more strongly than the oxidized form and that the proton bound is one released in the splitting of water. According to the scheme the energy difference between the high- and low-potential forms of cytochrome *b*-559, which is derived from electron transport on the reducing side of PS II, is available to bind the water proton and thereby pull the water-splitting reaction in the direction of oxygen evolution. The binding of the proton also stabilizes the S-state which released the proton. The theory predicts that the high-potential form of the cytochrome is the protonated form and that it should assume its high-potential character only when the proton-binding site is in a hydrophobic environment. Our previous work showed that the high-potential form could be restored from a low-potential form by incorporating purified cytochrome *b*-559 into liposomes. The present work shows that the restoration of high-potential cytochrome *b*-559 also occurs in the PS II particles and, in the case of the oxygen-evolving PS II particles, the restoration is accompanied by a greater capacity for oxygen evolution.

The results with the F_{II} particles encourage us to believe that it might be possible to reconstitute and oxygen-evolving system in liposomes using F_{II} particles as the photochemical driving force and such other components as are needed for oxygen evolution. Such a reconstitution would permit a detailed examination of the oxygen-evolving machinery. Our results indicate that liposomes made from DGDG and PC are suitable for such attempts at reconstitution.

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