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Rapid report

First photosystem II crystals capable of water oxidation

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Abstract

Oxygen evolution and proton release of crystallised photosystem II core complexes isolated from *Synechococcus elongatus* were measured. The yields show that the crystals themselves are capable of highly active water oxidation. This opens the possibility for the structural analysis of the outstanding water-oxidising apparatus. © 2000 Elsevier Science B.V. All rights reserved.

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In water-oxidising photosynthesis, two photosystems (PS I and PS II) operate in series. These two large pigment–protein complexes are integrated in the photosynthetic membrane. In the primary act of light energy conversion, a transmembrane charge separation takes place in PS I as well as in PS II (see [1], and literature cited therein). In PS II, this process starts with an electron transfer from the excited primary electron donor, chlorophyll P680 [2], located at the lumenal membrane side to the electron-stabilising acceptor, plastoquinone Q_A [3,4] on the stromal side. The cationic radical, $P680^{+\bullet}$, has the highest oxidation potential found in nature sufficient for the oxidation of water. For the oxidation of $2H_2O$, the univalent radical $P680^{+\bullet}$ abstracts four electrons from the water-oxidising complex in four successive turnovers of $P680\text{–}Q_A \rightarrow P680^{+\bullet}\text{–}Q_A^{-\bullet}$ step by step via a tyrosine Y_Z [5–8], ending in the last step with the evolution of one O_2 [9,10]. This process is coupled with the release of four protons from the water oxidising complex in the sequence 1:0:1:2 (see [11] and the literature cited therein). A cluster of probably four manganeses (Mn) catalyses this reaction. To understand this process, structural information started by electron microscopy of the isolated PS II complex. PS II showed a monomeric and dimeric structure [12–14]. Through the electron crystallography of two-dimensional crystals of PS II from spinach which, however, have lost their water-oxidising apparatus, a structural model at the level of 8 Å resolution was derived [15,16].

Recently, we obtained three-dimensional crystals of monomeric PS II core complexes [17] isolated from the thermophilic cyanobacterium *Synechococcus elongatus* [13,17]. The crystals have a size up to 1 mm and are suitable for X-ray structure analysis (see Fig. 1). X-ray diffraction data collected at 100 K indicate that the PS II crystals belong to the orthorhombic space group $P2_12_12_1$ with unit cell constants $a = 134$, $b = 227$, $c = 310$ Å. The quality of the crystals was characterised by a complete native data set with a resolution of 5 Å [17]. It is the sophisticated struc-

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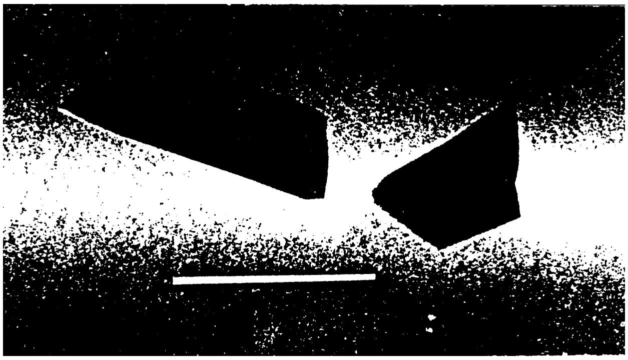

Fig. 1. Dark green crystals of PS II core complexes isolated from *Synechococcus elongatus*. Bar = 0.5 mm.

tural organisation of the manganeses, their ligands, bonded water derivatives, and cofactors such as Ca^{2+} and Cl^-, which makes water oxidation possible. This includes the special arrangement of the primary electron donor P680 as well as the structure of the immediate polypeptide environment of P680. Both of these unknown structural peculiarities of P680 are certainly responsible for the extreme positive oxidation potential of $P680^+/P680$, which drives the water-oxidising cycle. For a successful structural analysis of this cycle, it must therefore be established that this sensitive molecular apparatus has not changed structurally through its crystallisation procedure to an inactive conformation. This is by no means self-evident. In the present study, evidence is given that our crystals themselves are capable of water oxidation. The water-oxidising activity of these PS II complexes was estimated by the number of oxygen molecules and protons released per chlorophyll (Chl) and single turnover flash.

Flash-induced oxygen evolution was measured with a Clark-type electrode (MS 1 PO, Biolytik). The electrode signal was amplified (RE K1-1, Biolytik), transferred to a PC via an A/D-converter. The medium contained 100 mM PIPES pH 7.0, 200 μM 2.5-dichloro-*p*-benzoquinones, 100 μM $K_3(Fe(CN)_6)$, and the PS II microcrystals. Flash-induced changes of the pH value due to proton release were measured with a light-insensitive glass electrode of the flat membrane type (SA 9218/2.N, Schott), a reference electrode (B 2830, Schott), and a pH meter (Model 645, Knick) [11]. The medium contained 1.0 mM HEPES pH 7.0 and, as electron acceptor, 100 μM 2.5-dichloro-*p*-benzoquinones, 1 mM $K_3(Fe(CN)_6)$, and PS II crystals smaller than 100 μm. The stirred samples were illuminated with saturating Xenon flashes (pulse duration 15 μs, frequency 1 and 2 Hz, respectively).

The PS II complex in solution was characterised by an activity of 1/4 O_2/(66 Chl·flash) and 1 H^+/(67 Chl·flash). For activity measurement of PS II in the crystallised state, crystals sized smaller than 100 μm were used, because their lower optical density permits measurements under saturating flash-light intensities. Fig. 2 shows the relative activity of O_2 evolution of PS II in the crystallised state as a function of the number of preflashes at 20°C. The decrease after

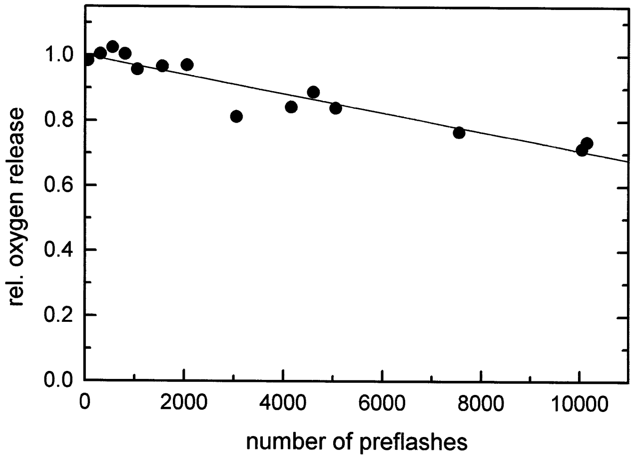

Fig. 2. Stability of the PS II crystals at 20°C characterised by the relative O_2 evolution in dependence on the number of preflashes up to 10 000.

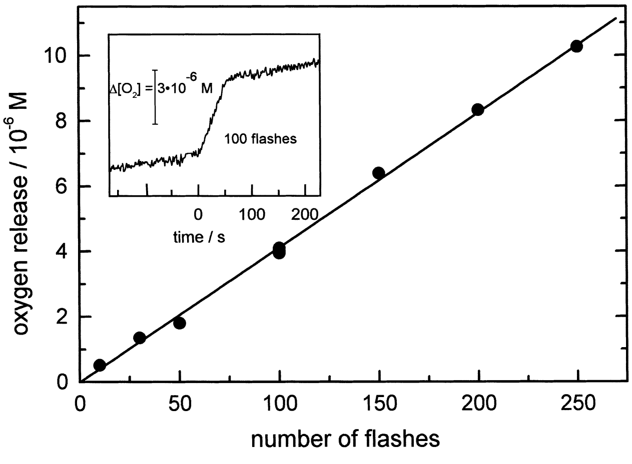

Fig. 3. O_2 evolution of PS II crystals at 20°C as a function of the number of exciting light flashes. The slope and chlorophyll concentration (11.4 μM) results in an activity of 1/4 O_2/(69 Chl·flash). The insert shows the signal/noise ratio of the O_2 evolution per 100 flashes.

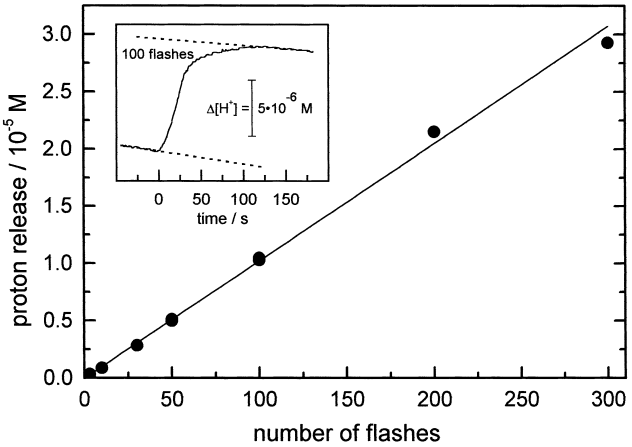

Fig. 4. Proton release of PS II crystals at 20°C as a function of the number of exciting light flashes. The slope and chlorophyll concentration (7.2 μM) results in an activity of 1 H^+/(71 Chl·flash). The insert shows the signal/noise ratio of the proton release per 100 flashes.

10 000 flashes is 30%. This indicates the great stability of the crystal. The crystals are even more stable than the PS II complex in solution ($\approx$70% decrease, data not shown). Fig. 3 shows the O_2 evolution as a function of the number of single turnover flashes up to 100. The slope and chlorophyll concentration yields in an activity of 1/4 O_2/(69 Chl·flash). In Fig. 4, the proton release is depicted as a function of the flash number. An activity of 1 H^+/(71 Chl·flash) was determined. Both activities are practically the same as those of PS II complexes in solution (see above). The ratio of the activity of O_2 evolution and proton release obtained with the crystals is $H^+/O_2 \approx 4$, which corresponds to the expected stoichiometry of water oxidation. These results provide evidence that the crystals described here retain an unrestricted, highly active water-oxidising capacity and are therefore suitable for the determination of the functionally intact organisation of the water-oxidising complex. For the elucidation of this crystal structure, multiple isomorphous replacements with heavy-atom compounds (MIR) have been initiated to obtain the phase angles of the X-ray diffraction data for calculation of the electron density map. A comparison of results, based on X-ray diffraction measurements on crystals with and without manganeses will support the elucidation of the structure of the Mn cluster. Crystals without the Mn cluster were obtained by soaking them in hydroxylamine (NH_2OH). This agent reduces the manganeses from higher to lower oxidised states [18]. In state Mn^{2+}, the manganeses are no longer bound and are released into the medium. Thus, at the level of ~5 Å resolution, we can expect to obtain the organisation of the helices of the PS II core and the structure of the water-oxidising apparatus as well as the construction of the driving electron transport chain.

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