Total recovery of $O_2$ evolution and nanosecond reduction kinetics of chlorophyll-a$_{II}^+$ (P-680$^+$) after inhibition of water cleavage with acetate

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Abstract. Oxygen evolution and reduction kinetics of the photooxidized Chl-a$_{II}^+$ have been measured in oxygen-evolving complexes from the thermophilic cyanobacterium Synechococcus sp.

1. Incubation of PS II particles with acetate resulted in an inhibition of oxygen evolution and a retardation of the Chl-a$_{II}^+$-reduction kinetics from the nanosecond range to the microsecond range, indicating a modification of the donor side of photosystem II (PS II).

2. After the first two flashes given to a dark-adapted, acetate treated sample, Chl-a$_{II}^+$ was re-reduced with a half-life time of 160 $\mu$s by a component of the donor side of PS II. Under repetitive excitation Chl-a$_{II}^+$ was re-reduced in 500 $\mu$s by electron back reaction from the primary acceptor Q$_A$ (X-320$^-$). Obviously, in the presence of acetate only two electrons are available from the donor side.

3. Both oxygen evolution and nanosecond reduction kinetics of Chl-a$_{II}^+$ were restored to the control level when acetate was removed.

4. The results indicate a tight coupling between $O_2$ evolution and nanosecond reduction kinetics of Chl-a$_{II}^+$.

5. The reversible inhibition is probably due to a replacement of Cl$^-$ by acetate within the water splitting enzyme.

6. Due to its strongly retarded kinetics, the reversibly modified system may facilitate investigations of the mechanism of the donor side.

Abbreviations

Chl, chlorophyll; PpBQ, phenyl-p-benzoquinone; PS, photosystem

Introduction

The fundamental reaction for water cleavage in photosynthesis is the photo-oxidation of Chl-a$_{II}^+$ (P-680$^+$) [7,8]. Light excitation of Chl-a$_{II}^+$ leads to the transfer of one electron to the first stable acceptor, a special plastoquinone, Q$_A$ (X-320$^-$) [25, 26]. The photooxidized Chl-a$_{II}^+$ extracts, via electron carriers, an electron from the $O_2$-evolving complex. In four single turnover flashes the complex runs through four oxidation states, $S_0$ to $S_3$, ultimately ending in the cleavage of 2 H$_2$O into 4 H$^+$ and one $O_2$. The kinetics of the
Chl-a\textsuperscript{+} reduction are a function of the S-states: the Chl-a\textsuperscript{+} reduction correlated with the $S_0$ and $S_1$ states occurs within 23 ns; whereas, in states $S_2$ and $S_3$ a biphasic reduction with 50 ns and 260 ns is observed [2]. The retardation of the electron transfer times in states $S_2$ and $S_3$ was explained by Coulombic attraction due to an excess positive charge in states $S_2$ and $S_3$ [2]. The existence of a positive surplus charge in states $S_2$ and $S_3$ has been shown independently by corresponding electrochromic signals [17, 18]. Under repetitive flash excitation a multiphasic Chl-a\textsuperscript{+} reduction is observed [3, 9, 20]. This has been explained quantitatively by a superposition of the individual kinetics correlated with the $S_0-S_3$ states [2, 21].

The electron transport from H\textsubscript{2}O to an artificial acceptor can be blocked more or less reversibly through different types of treatments (Cl\textsuperscript{-} depletion [11] (for review see [5], protein removal [1-10, 12-15], or bicarbonate depletion [23, 28]. In PS II reaction centers in which oxygen evolution was blocked by various treatments, the nanosecond reduction kinetics of Chl-a\textsuperscript{+} disappear and Chl-a\textsuperscript{+} is reduced slowly in the time range of microseconds [1,3,27].

Incubation with formate or acetate under a CO\textsubscript{2}-free atmosphere is a currently used tool for bicarbonate depletion, resulting in a reversible retardation of the electron transfer kinetics both from $Q_A$ to $Q_B$ and from $Q_B$ to the PQ-pool by a factor of about 10 (for recent reviews of bicarbonate depletion see [23, 28]). There is disagreement whether or not bicarbonate depletion also acts – to a minor extent – at the donor side of PS II [23, 28]. Acetate has also been used in experiments together with Cl\textsuperscript{-}-depletion [11, 22]. In our experiments the acetate concentration was 660 mM in the presence of 15 mM Cl\textsuperscript{-}. Under these conditions a complete inactivation of oxygen evolution and a retardation of the reduction kinetics of Chl-a\textsuperscript{+} to the microsecond time range have been observed. After the removal of acetate, O\textsubscript{2} evolution was totally restored together with the reappearance of the nanosecond reduction of Chl-a\textsuperscript{+}.

Materials and methods

PS II particles from Synechococcus sp. were prepared according to Schatz and Witt [19] and stored at 193 K in the dark. Under repetitive flash illumination oxygen evolution was measured with a Clark-type electrode. Single flash as well as repetitive flash-induced oxygen evolution was measured with a Zirconia oxygen sensor. Flash-induced absorption changes at 824 nm were measured using a spectrophotometer essentially described in [3] with variations described in [2]. The fluorescence artifact was negligible. At 820 nm we observed an additional light-induced absorption change in flash numbers higher than 2 (10–25% of the total signal) which was not related to Chl-a\textsuperscript{+}. Simultaneous measurements at 334 nm and 824 nm were performed with a spectrophotometer similar to the one described in [26].