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Mutations D1-E189K, R and Q of *Synechocystis* sp. PCC6803 are without influence on ns-to-ms electron transfer between OEC-Y_Z-P_{680} in photosystem II

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Introduction

The oxygen-evolving manganese cluster (OEC) of photosynthesis is oxidised by the photochemically generated primary oxidant ($P_{680}^{+\cdot}$) of photosystem II (PSII) via a tyrosine residue known as Y_Z (Tyr161 on the D1 subunit). The redox span between these components is rather small and probably tuned by protonic equilibria. The very efficient electron transfer from Y_Z to $P_{680}^{+\cdot}$ in nanoseconds requires the intactness of a hydrogen bonded network presumably involving the OEC, Y_Z, D1-His190, and neighbouring residues (Haumann et al. 1999). Recently it was argued that D1-Glu189 plays a critical role in maintaining this hydrogen bonded network and may help to position a group that accepts a proton from D1-His190 when Y_Z is oxidised by $P_{680}^{+\cdot}$(Debus et al. 2000). To examine the role of Glu189 further, we measured the rates of electron transfer from the OEC to Y_Z^{ox} and from Y_Z to $P_{680}^{+\cdot}$ in the mutants D1-E189Q, D1-E189R and D1-E189K of *Synechocystis* sp. PCC 6803, which were expected to differ electrostatically from D1-Glu189, the wild-type. The surprising result was that the electrostatic properties of these amino acids did not at all affect the electron transfer around Y_Z.

Materials and methods

Mutants were constructed as described previously (Chu et al. 1994). The modified wild-type-strain (WT*) lacks *apc*E and PSI function (Chu et al. 1995b). Oxygen evolving PSII core particles were prepared as described in Hays et al. (1999) with minor modifications: The membranes were applied to a 40 ml DEAE-Toyopearl 650s column and all buffers, except the equilibration buffer, contained 1 M glycine betaine. The equilibration buffer and the eluation buffer contained 5 and 50 mM $MgSO_4$, respectively. Electron transfer from OEC to Y_Z^{ox} was measured flash-photometrically at 360 nm with repetitive dark adapted PSII core particles (8 µM chl) in buffer DB1 (50 mM MES pH 6.5, 1 M sucrose, 25 mM $CaCl_2$, 10 mM $NaCl_2$, 1M glycine betaine, 0.06% β-DM [w/v]) and 200 µM DCBQ. The samples were excited by a Nd:YAG laser (532 nm, FWHM 6 ns, 100 ms between flashes). Reduction of $P680^{+\cdot}$ was measured under repetitive excitation (Nd:YAG laser, FWHM 3 ns, 532 nm, 100 ms between flashes) in DB1 buffer, 30 µM chl and 1 mM DCBQ. Flash induced release of O_2 was

measured polarographically with a centrifugable bare platinum electrode. Thylakoids were prepared according to Burnap et al. (1994) with minor modifications. Thylakoids were suspended at 25 µM in HMCS-HS buffer (HEPES 50 mM pH 7.2, 10 mM $MgCl_2$, 5 mM $CaCl_2$, 1 M sucrose 200 mM NaCl) and pelleted upon the platinum by centrifugation (1000g, 10 min, 20°C). The oxygen-evolution under continuous saturating illumination was measured as described in Hundelt et al. (1998).

Results

Highly active PSII core particles were isolated from cells of the wild-type* and of the mutants D1-E189K, R and Q. Oxygen evolution was almost unchanged in the mutants D189K and D1-E189R, but strongly diminished in D1-E189Q cells and core particles.

Table 1: Electron transfer rates (ETR) of wild-type* and mutant cells and core particles (in µmol O_2 / mg of chl / h)

Material	Wild-type*	D1-E189K	D1-E189R	D1-E189Q
ETR (Cells)	1031 (100%)	885 (86%)	828 (80%)	446 (43%)
ETR (Core particles)	2100 (100%)	2000 (95%)	1900 (90%)	1000 (48%)
Amplitude of $S_3 \Rightarrow S_0$ [a]	0.86 (100%)	0.81 (94%)	0.73 (85%)	0.37 (43%)

[a] determined from figure 2

We characterised the WT* by measuring the release of oxygen with thylakoids polarographically (Figure 1). The half rise time of the oxygen evolution was determined with 1.1 ms which was in good agreement with our spectroscopic measurements (Figure 2A) and with data from the literature that range from 1 to 1.5 ms [Dekker et al. (1984); Renger & Weiss (1986); Razeghifard & Pace (1997)].

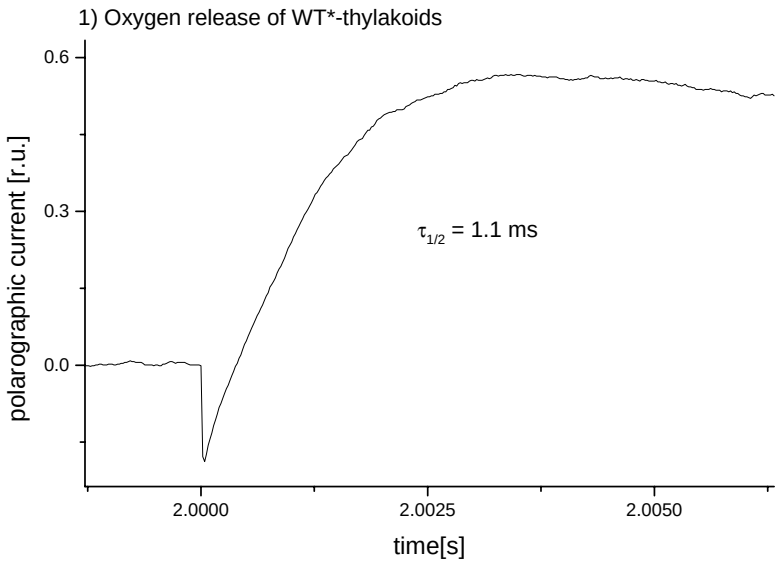

Figure 1: Polarographic current representing the oxygen producing electron transfer in the S-state transition $S_3 \Rightarrow S_0$. Flash induced oxygen release on the third flash was measured with dark adapted thylakoids. Time resolutions was 20 µs/point.

In the next step flash-spectrometric measurements of the S-state transition $S_3 \Rightarrow S_0$ were performed with WT* core particles and with core particles of the mutants D1-E189K, R and Q, which were expected to differ electrostatically from D1-E189, the wild-type* (Figure 2).

We analysed the difference between the absorption transients after the third flash ($S_3 \Rightarrow S_0$ plus $Q_A^{\cdot-}$ oxidation) and the fifth flash ($S_1 \Rightarrow S_2$ plus $Q_A^{\cdot-}$ oxidation), in order to delete the contribution of the $Q_A^{\cdot-}$ oxidation by DCBQ. The resulting curve was fitted (solid line) with one exponentially decaying phase ($S_3 \Rightarrow S_0$) and an unresolved negative jump ($S_1 \Rightarrow S_2$). The half rise time for WT* core particles was 1.1 ms (Figure 2A). The mutants showed similar half rise times with 1.2 ms for D1-E189Q (Figure 2B), 1.4 ms for D1-E189R (Figure 2C) and 1.2 ms for D1-E189K (Figure 2D). Therefore electron transfer from the Mn-cluster to Y_Z^{ox} was unaffected by these mutations at position 189 in the D1-subunit.

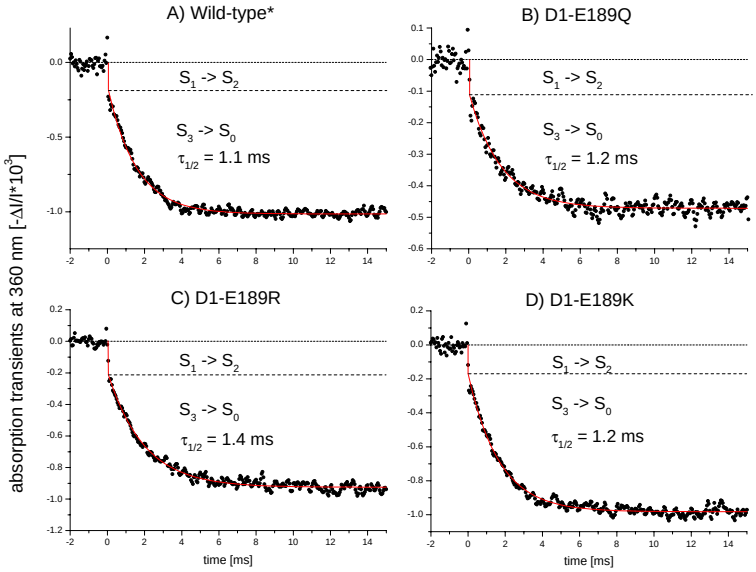

Figure 2: Kinetics of redox-transition $S_3 \Rightarrow S_0$. Difference between the third and fifth flash. Time resolution was 50 µs/point. Data were smoothed over every second point. PSII core particles of A) Wild type* B) D1-E189Q C) D1-E189R and D) D1-E189K. 50 transients were averaged in C – D and 55 in A.

The reduction of $P_{680}^{+\cdot}$ by Y_Z was measured at 827 nm with oxygen evolving core particles under repetitive conditions ($\Rightarrow$ equal distribution of the s-states, data from Clausen et al. [2001]).

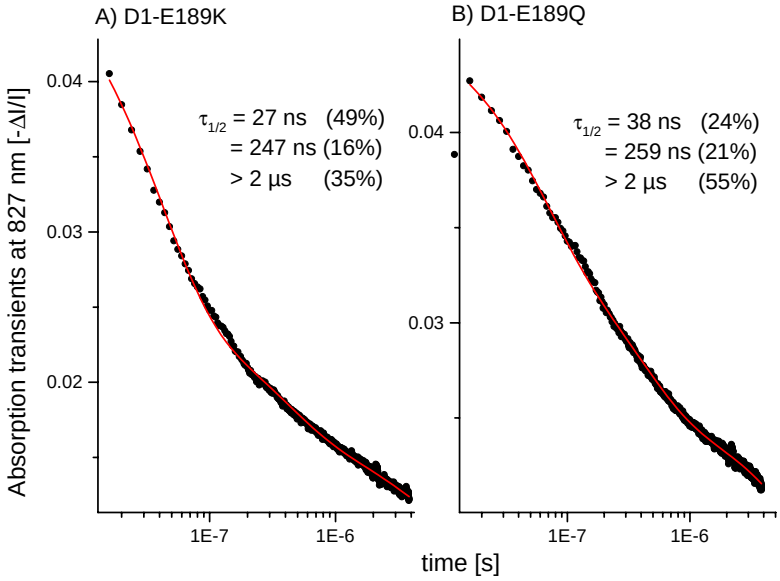

Figure 3: Reduction of $P_{680}^{+\cdot}$ by Y_Z. Time resolution = 4 ns/address. PSII core particles of A) D1-E189K and B) D1-E189Q. 1000 (A) and 800 (B) transients were averaged.

The absorption transients were fitted with two exponentially decaying phases and a stable offset of a µs-phase ($\tau_{1/2}$ > 2 µs) [Figure 3]. Core particles of D1-E189K showed two fast exponentially decaying phases with half times of 27 ± 1 ns and 247 ± 10 ns and a relative extent of the amplitude of 49% and 16%, respectively. The relative extent of the µs-phase was 35%. Absorption transients of D1-E189Q core particles were fitted with 38 ns ± 2 ns (24%), 259 ± 10 ns (21%) and 55% of the µs-phase. These half times were comparable to wild-type* core particles [34 ns (46%), 325 ns (18%) and > 2 µs (36%); Clausen et al. (2001)] and to those reported for other PSII preparations [Brettel & Witt (1983); Schlodder et al. (1984); Hundelt et al. (1998); Ahlbrink et al. (1998): 20 –40 ns (~50%), 100 – 320 ns (~20%) and > 2 µs (~30%)]. These results show that the half times of the ns-components of electron transfer between Y_Z and $P_{680}^{+\cdot}$ were almost unchanged between wild-type* and mutant PSII core particles. The extent of the µs-phase is a measure for the upper limit of the proportion of inactive centres. Its increase in the D1-E189Q mutant was in good agreement with the clearly decreased rate of oxygen evolution of D1-E189Q cells and core particles [Table 1 and Chu et al. (1995a)].

Discussion

Contrary to our expectations we did not see any electrostatic effect of the mutations D1-E189K, R and Q on the electron transfer reactions from the Mn-cluster to Y_Z and from Y_Z to P_{680}. The missing electrostatic effect of the mutations of D1-Glu189 may have at least two reasons. First, D1-Glu189 could be far away from the manganese cluster and Y_Z and/or second, D1-Glu189 could be located in a very hydrophobic environment so that all residues, namely E, K, R and Q, are uncharged. A discrimination between these possibilities has to wait for the assignment of amino acids to the crystal structure.

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