S13-024

Reaction coordinate of $P680^{+\bullet}$ reduction by Y_Z in PS II core complexes from spinach

P. Kühn[1], N. Iwanowski[2], R. Steffen[1], H.J. Eckert[1], K.-D. Irrgang[1], H.-J. Eichler[2], G. Renger[1]

[1]*Max-Volmer-Laboratorium für Biophysikalische Chemie der TU Berlin, PC 14, Strasse des 17. Juni 135, D-10623 Berlin, Germany. Fax +49 30 314 21122,* renger@pc-109ws.chem.tu-berlin.de

[2]*Optisches Institut der TU Berlin, P 1-1, Strasse des 17. Juni 135, D-10623 Berlin, Germany*

Keywords: Photosystem II, $P680^{+\cdot}$ reduction, activation energy, Ca^{2+} effect, relaxation processes

Introduction

Photosynthetic oxidation of two water molecules to molecular oxygen and four protons comprises three types of reactions (for a review, see Renger 1999): i) generation of a strongly oxidizing Chlorophyll-*a* cation radical ($P680^{+\bullet}$) by light induced charge separation, ii) transfer of this oxidizing redox equivalent to tyrosine residue Y_Z under formation of the neutral radical Y_Z^{ox} and iii) stepwise electron abstraction by Y_Z^{ox} from the water oxidizing complex (WOC). In addition to the redox active tetranuclear manganese cluster a single Ca^{2+}-ion is indispensable for the functional competence of the WOC (for a review, see Debus 1992). Different treatments were used to remove Ca^{2+} in order to study restoration of oxygen evolution capacity and rebinding of Ca^{2+} (Ghanotakis et al. 1984, Adelroth et al. 1995). A surprising heterogeneity of restoration and/or binding constants was found (Kalosaka et al. 1990, Adelroth et al. 1995) that rises questions on the origin of this phenomenon. It could reflect either a heterogeneity of WOCs or multiple effects of Ca^{2+} or a combination of both. In a recent report Ca^{2+} was inferred to be not only an essential constituent of the WOC but also to exert an important regulatory function in the mode of coupling between membrane energization and ATP-synthesis at limiting light intensities in relation to switching on nonphotochemical quenching under light stress. It was postulated that Ca^{2+} binding to the CF_0 part of ATP-ase exerts the regulatory control (Pan and Dilley 2000).
The present communication describes effects of low affinity Ca^{2+} binding and thermal activation on the multiphasic kinetics of $P680^{+\bullet}$ reduction by Y_Z in solubilized untreated spinach PS II core complexes with high oxygen evolving capacity.

Materials and Methods

PS II core complexes were isolated from market spinach by a procedure as described previously (Haag et al. 1990, Irrgang et al. 1998). Laser pulse (λ=532 nm, fwhm=3 ns) induced absorption changes at 820 nm were measured with a single beam flash photometer (Liu et al. 1993). The Ca^{2+} content was determined by atomic absorption spectroscopy (model A Analyst 800, Perkin Elmer). The assay medium contained: PS II core complexes (CC) with a chlorophyll concentration of 50 µg/ml and 100 µg/ml for measurements under repetitive and single flash excitation, respectively; 10 mM NaCl and 50 mM buffer (MES/NaOH at pH 5.0 and 6.5, HEPES/NaOH at pH 8.0 and 9.0). Artificial electron acceptors and either Ca^{2+} or Mg^{2+} were added as indicated in the figure captions.

Results and Discussion

Fig. 1 shows traces of flash induced absorption changes at 820 ns of spinach PS II core complexes measured in buffer solutions of four different pH-values and a Ca^{2+} background concentration of about 200 µM. The traces were monitored in two different time domains in order to illustrate the pH-dependence of the ns-kinetics (upper panel) and of the µs kinetics (lower panel).

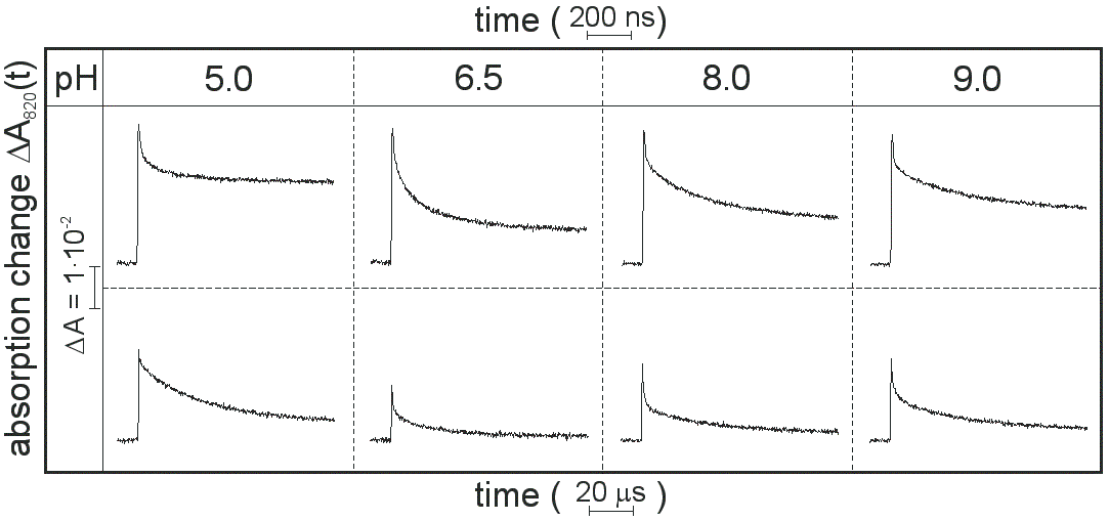

Fig. 1: Flash induced absorption changes at 820 nm monitored in two different time domains.

Two striking features emerge from this data: i) the normalized extent of ns kinetics, ΔA_{820}^{norm}(ns), is severely diminished at pH 5 concomitant with a complementary increase of µs kinetics and ii) in the alkaline region this effect on ΔA_{820}^{norm}(ns) is less pronounced. Surprisingly, even at pH 9 a significant fraction of the total relaxation occurs with kinetics slower than 1 µs. This interesting phenomenon, however, cannot be discussed in this short communication and will be analyzed in more detail in a forthcoming study.

The effect of Ca^{2+} addition on the $P680^{+\bullet}$ reduction in solubilized PS II core complexes was investigated by measuring absorption changes at 820 nm induced by repetitive laser flash excitation. The left panel of Fig. 2 below shows typical traces obtained at a background Ca^{2+} concentration of 200 µM at pH 5 (top) and 6.5 (bottom) and the effect of Ca^{2+} addition (40 mM).

A marked Ca^{2+} induced enhancement of ΔA_{820}^{norm}(ns) is observed at pH 5 whereas a less pronounced effect emerges at pH 6.5. In the right panel ΔA_{820}^{norm}(ns) is depicted as a function of the Ca^{2+} and Mg^{2+} concentrations. An inspection of this data reveals that: a) the stimulation is specific for Ca^{2+} and b) the effect saturates at about 10 mM in solutions of both pH 5 and 6.5. Based on these findings it is clear that solubilized PS II core complexes from spinach exhibit a Ca^{2+} demand that gives rise to an increased extent on ΔA_{820}^{norm}(ns) when the pH is lowered from 6.5 to 5.0. On the other hand, the affinity as reflected by the saturation curve is not or only marginally dependent on pH. The concentration dependence is reminiscent of the low affinity Ca^{2+} stimulation of oxygen evolution in samples where Ca^{2+} is depleted by a suitable treatment. In spinach PS II core complexes deprived of Ca^{2+} by a special treatment (2M NaCl-buffer 1mM EGTA 20 µM ionophore A 23187) three K_D values of 1-4 µM, 70-100 µM and 2.7-7 mM were gathered from a Scatchard plot of Ca^{2+} induced restoration of O_2-evolution rate (Kalosaka et al. 1990). The high affinity sites are expected to be occupied at a background Ca^{2+} content of 200 µM and therefore the observed effect on the $P680^{+\bullet}$ reduction

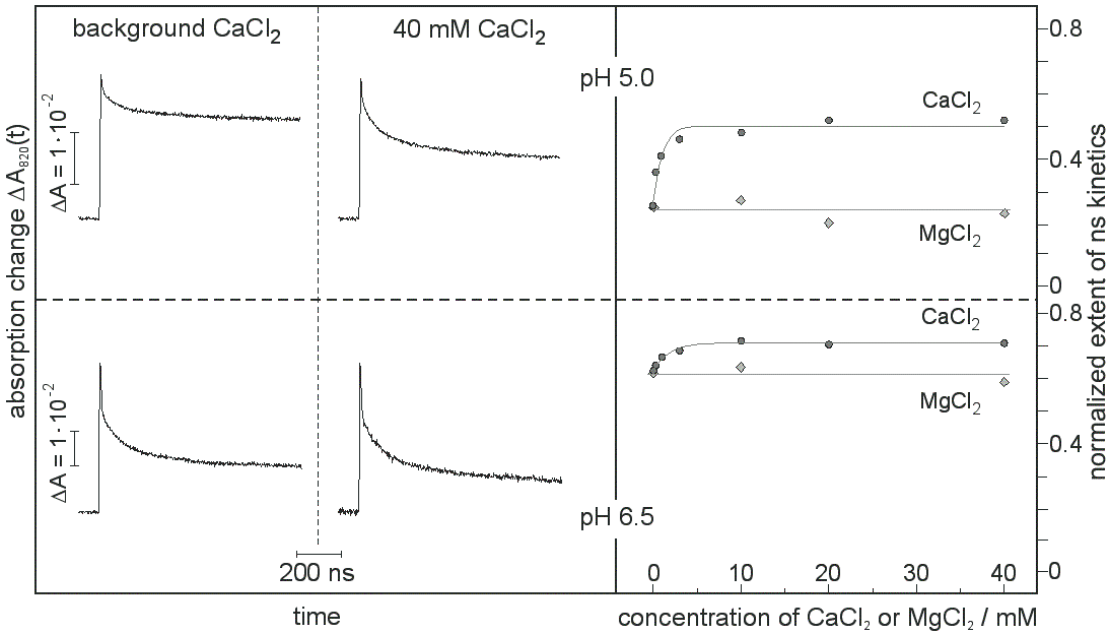

Fig. 2: Transient absorption changes $\Delta A_{820}(t)$ in solubilized PS II core complexes from spinach (left panel) and normalized extent of relaxation in the ns time domain as a function of $CaCl_2$ or $MgCl_2$ concentration (right panel)

reported in this study is assumed to be owing to saturation of low affinity site(s). This phenomenon raises questions on the origin of this effect. In a recent study the Ca^{2+} stimulation on the kinetics of $P680^{+\bullet}$ reduction has been assigned to the population of the single Ca^{2+} binding site of the WOC (Haumann and Junge, 1999). Alternatively a Ca^{2+} binding to other site(s) could also modulate the electron transfer rate from Y_Z to $P680^{+\bullet}$ by changing the relaxation reaction within the hydrogen bond network. An increased extent of µs-kinetics leads to enhanced probability of misses owing to more effective competition by the back reaction between $P680^{+\bullet}$ and $Q_A^{-\bullet}$ (for a discussion, see Christen et al. 1999).

Although a straightforward conclusion cannot be achieved at our current stage of knowledge, we favor the latter mechanism because it seems to be less probable that the WOC exhibits an intrinsic heterogeneity with respect to the Ca^{2+} binding affinity. This idea is also in line with our previous findings that in mildly trypsinized PS II membrane fragments Ca^{2+} binding specifically enhances the extent of ns kinetics (Renger et al. 1989) with affinity constants K_D of about 60 µM and 1.5 mM (Völker et al. 1987). It appears highly unlikely that quite different procedures give rise to a WOC heterogeneity in Ca^{2+} affinity with virtually the same binding constants. It is therefore assumed that Ca^{2+} modulates the hydrogen bond network which determines via relaxation processes the kinetics of $P680^{+\bullet}$ reduction.

Acknowledgement

The financial support by Deutsche Forschungsgemeinschaft (Sfb 498 TP C4) is gratefully acknowledged.

References

Adelroth P, Lindberg K and Andréasson L-E (1995) *Biochemistry* **34**, 9021-9027.
Christen G, Seeliger A. and Renger G (1999) *Biochemistry* **38**, 6082-6092.
Debus RJ (1992) *Biochim. Biophys. Acta* **1102**, 269-352.
Ghanotakis DF, Babcock GT and Yocum CF (1984) *FEBS Lett.* **167**, 127-130.
Haag E, Irrgang K-D, Boekema EF and Renger G (1990) *Eur. J. Biochem.* **189**, 47-53.
Haumann M and Junge W (1999) *Biochim. Biophys. Acta* **1411**, 121-133.
Irrgang K-D, Lekauskas A, Franke P, Reifarth F, Smolian H, Karge M and Renger G (1998) In: *Photosynthesis: Mechanisms and Effects* (Garab G, ed.), Vol. II, pp 977-980, Kluwer, Dordrecht.
Kalosaka K, Beck WE, Brudvig G and Cheniae G (1990) In: *Current Research in Photosynthesis* (Baltscheffsky M, ed) Vol. I, pp 721-724, Kluwer, Dordrecht.
Liu B, Napiwotzki A, Eckert HJ, Eichler HJ and Renger G (1993) *Biochim. Biophys. Acta* **1142**, 129-138.
Pan R-S and Dilley RA (2000) *Photosynth. Res.* **65**, 141-154.
Renger G, Eckert, H-J and Völker M (1989) *Photosynth. Res.* **22**, 247-256.
Renger G (1999) In: *Concepts in photobiology: Photosynthesis and Photomorphogenesis* (Singhal GS, Renger G, Govindjee, Irrgang K-D, Sopory SK, eds), pp. 292-329, Kluwer Academic Publishers, Dordrecht, Narosa Publishing Co., Delhi.
Renger G (2001) *These Proceedings.*
Völker M, Eckert, H-J and Renger G (1987) In: *Progress in Photosynthesis Research* (Biggins J, ed), Vol. I, pp 545-548, Martinus Nijhoff/Dr. W. Junk, Dordrecht.