

## 4. CONCLUSIONS

The disassembly of the Mn complex as induced by a temperature jump to 47 °C involves three distinct steps. During the first 5 min of heating, the quantitative release of the 18 kDa extrinsic protein from PSII occurs ((Pospisil et al., 2003) and this work). This process presumably is accompanied by a decrease in the affinity of the essential  $\text{Ca}^{2+}$  ion to its binding site and by the reduction of two  $\text{Mn}^{\text{IV}}$  ions to the  $\text{Mn}^{\text{III}}$  level resulting in a  $\text{Mn}^{\text{III}}_4(\text{di-}\mu\text{-O})_2$  complex (Barra et al., 2005).

This complex still evolves oxygen under continuous illumination in the presence of exogenous calcium, although only at about 30-40 % of the control activity. Apparently, the  $\text{Mn}^{\text{III}}_4$  complex can be rapidly oxidized in the light to reach the oxidation level of the  $S_1$  state ( $\text{Mn}^{\text{III}}_2\text{Mn}^{\text{IV}}_2$ ) and thereafter, the S-state cycle of water oxidation functions normally in those PSII centers that contain transiently bound  $\text{Ca}^{2+}$ .

The second step of the disassembly (Figure 4.1) causes the reduction of the manganese complex by two more electrons and the subsequent release of two  $\text{Mn}^{\text{II}}$  ions into the bulk (Pospisil et al., 2003; Barra et al., 2005) accompanied by the almost complete loss of  $\text{O}_2$  activity within about 15 min of heating. The remaining binuclear complex is in the  $\text{Mn}^{\text{III}}_2(\text{di-}\mu\text{-O}(\text{H}))$  configuration (Barra et al., 2005). This complex appears to be a comparably stable intermediate and is reduced only within hours of heating, leading to the release of the remaining two manganese ions as  $\text{Mn}^{\text{II}}$  into the bulk (Pospisil et al., 2003) and to the formation of apo-PSII with an unoccupied Mn/Ca binding site.

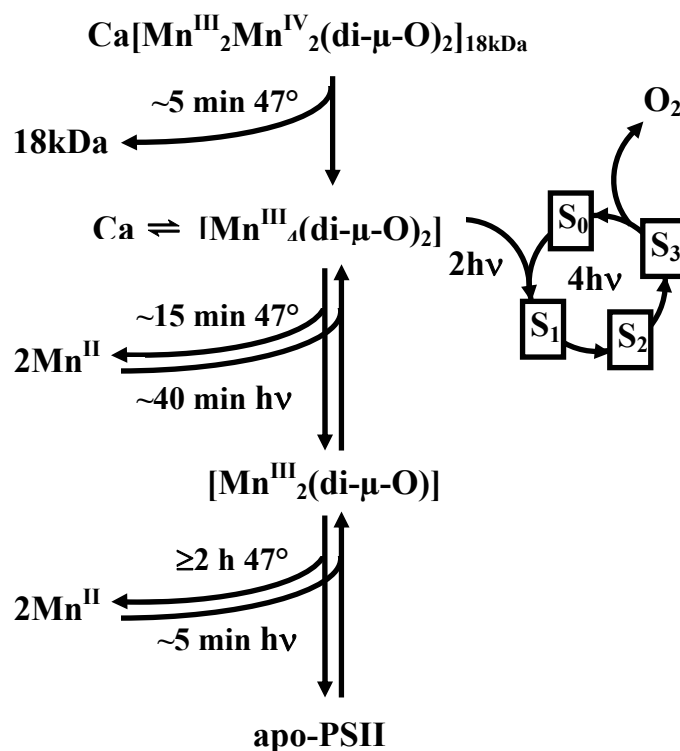


Figure 4.1 Kinetic-structural scheme for the stepwise disassembly of the Mn complex in response to a temperature jump.

In this study, I show that the binuclear Mn complex can be transformed by photoactivation to the functional  $\text{Mn}_4$  complex in the presence of two additional  $\text{Mn}^{\text{II}}$  ions per PSII (Figure 4.2). The findings in the present study and in our previous investigations (Pospisil et al., 2003; Barra et al., 2005) on the sequence of events during the heat-induced disassembly and on the subsequent reassembly of the Mn complex during photoactivation can be summarized as shown in Figure 4.2.

When apo-PSII created by the extraction of Mn either by hours of heating or by biochemical extraction is used as the starting material, a clear lag-phase of 5-10 min duration in the recovery of  $\text{O}_2$  activity during photoactivation suggests that also in this process a fairly stable intermediate is formed. According to the polarography and XAS results, this intermediate is likely to contain two  $\text{Mn}^{\text{III}}$  ions, which are connected, at least partly, by a di- $\mu$ -oxo bridge.

The binuclear center formed by photoactivation of apo-PSII is less stable (for about 15 min in the dark) than that formed after  $\sim 15$  min of heating (stable for hours), possibly due to the re-reduction of its bound  $\text{Mn}^{\text{III}}$  ions to  $\text{Mn}^{\text{II}}$  by reduced electron acceptor molecules.

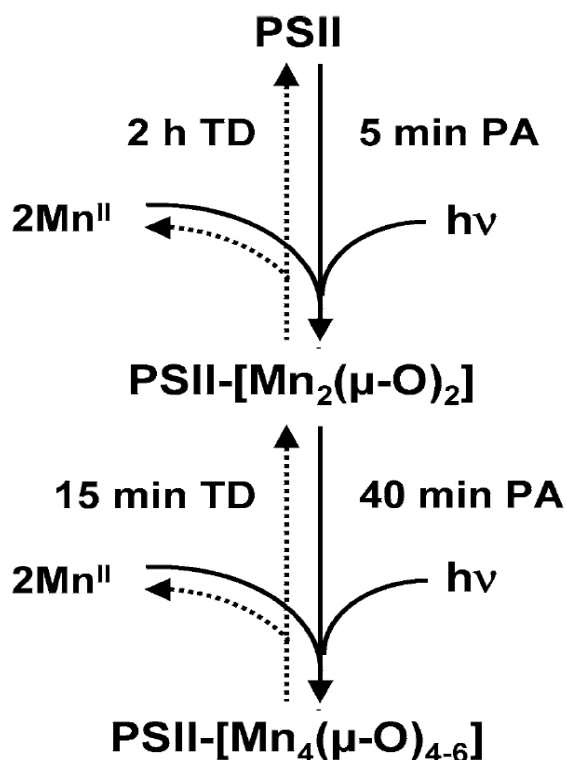


Figure 4.2 Kinetic scheme of the processes proposed to occur during the thermally induced disassembly of the Mn complex and its subsequent reassembly by photoactivation.

Previous investigations have provided evidence for a state where two Mn<sup>III</sup> ions are bound to PSII during photoactivation (Tamura and Cheniae, 1987; Zaltsman et al., 1997; Burnap, 2004). However, there was limited information on the structure of this state. We propose that the same binuclear Mn<sup>III</sup><sub>2</sub>(di-μ-oxo) complex is formed within the lag-phase of photoactivation observed in this work and during the first 15 min of the heat-induced disassembly (Barra et al., 2005). This intermediate may correspond to those postulated by other authors (states "IM<sub>2</sub>" in (Zaltsman et al., 1997) and "D" in (Tamura and Cheniae, 1987)). Accordingly, this binuclear complex may be considered as the key intermediate both in the disassembly and the assembly of the Mn complex.

The results in the present investigation suggest that *in vivo*, assembly of the Mn complex may not only occur in de-novo synthesized D1 protein incorporated into PSII. Reassembly of a functional Mn complex may also proceed after partial loss of Mn from PSII, thereby increasing its lifetime. It may be expected, that such a "self-maintenance" mechanism is a prerequisite also for stable and efficient (semi)artificial photosynthetic systems, along with further requirements, see (Dau and Haumann, 2005; Magnuson et al., 2006), to be employed in biomimetic and biotechnological applications.