Properties change of the Mn-cluster induced by replacement of Ca^{2+} with alkali metal cations

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Introduction

Photosynthetic oxygen evolution definitively requires Ca^{2+} for its activity. In the absence of Ca^{2+}, the oxidation process of the cluster beyond the S_2 is interrupted, concomitant with the oxidation of Y_Z and/or an auxiliary redox component(s) of PS II (Boussac et al. 1990, Ono and Inoue 1990, Hallahan et al. 1992, Mino et al. 1998). EXAFS (Cinco et al., 1998) and FTIR (Noguchi et al. 1995) studies indicate that Ca^{2+} is closely associated with the Mn cluster, presumably through a carboxylate bridge to form a multi-metal center. Mutagenesis studies suggest that Asp59 and 61 in the AB-loop of the D1 protein might bind Ca^{2+} (Chu et al. 1995). Recently, crystal structure of oxygen evolving PS II was reported, but no electron density could be assigned to the Ca^{2+} (Zouni et al. 2001). The identity of the ligand for Ca^{2+} binding and its function in water oxidation chemistry still remain unresolved. In general, the Ca^{2+} binding sites in enzymes can be occupied by other metal cations, and the function of Ca^{2+} can be evaluated by studying the influence of the cation substitution on the structure and function of the enzymes. Some of alkali metal cations have been suggested to associate with the Ca^{2+} site (Waggoner et al., 1989; Yocum, 1991) and may be useful tools for probing the function of Ca^{2+} in the water oxidation. However, no attempt has been made to characterize the effects of the binding of the monovalent cations on the structure and the function of the Mn cluster. The present study examines the effects of replacing Ca^{2+} with alkali metal cations on the properties of the Mn-cluster.

Materials and methods

O_2 evolving PSII membranes were prepared from spinach. To deplete Ca^{2+}, the membranes in a medium containing 2 M NaCl, 200 mM sucrose, 20 mM MES/NaOH (pH 6.5) at 0.5 mg of Chl/ml were incubated for 20-30 min at 0°C under fluorescent light, then Na-EDTA was added at 1 mM, and the mixture was further incubated for 5 min in the dark. The following manipulations proceeded in the dark or under dim green light unless otherwise noted. The Ca^{2+}-depleted membranes were washed three times with a large quantity of a medium containing 400 mM sucrose (Sigma Ultra), 20 mM Bis-tris/HCl (pH 6.5) (buffer A), and were incubated in the dark for more than 3 hr for relaxation to the S_1 state. To deplete the Mn-cluster, the 2 M NaCl washed membranes were treated with 1 mM NH_2OH. The resulted Mn-depleted membranes were washed and suspended in buffer A. Sample membranes were supplemented with cations (chloride salts),

phenyl-*p*-benzoquinone,NH_2OH and/or DCMU when indicated. Low-temperature X-band EPR spectra were measured with a Jeol JES-FE1XG EPR spectrometer or a Bruker E580 EPR spectrometer equipped with an Oxford-900 cryostat. FTIR spectra were measured with a Bruker IFS-66v/s spectrometer equipped with an MCT detector at 250K. Each single-beam spectrum was measured at 4 cm^{-1} resolution by averaging 300 scans (130s accumulation), before and after illumination ($\geq$ 620 nm). Thermoluminescence (TL) was measured using a home made apparatus. O_2 evolution was measured using a Clark-type oxygen electrode in buffer A.

Results

Fig. 1 shows the effects of alkali metal cations on the O_2 evolving activity in none-treated control (A) and in Ca^{2+}-depleted membranes restored by 1 mM Ca^{2+} (B). In the control membranes, O_2 evolution was preserved in the presence of cations although it was gradually suppressed at higher cation concentrations. The Ca^{2+}-dependent O_2 evolution in the Ca^{2+}-depleted membranes was reversibly inhibited by K^+, Rb^+ and Cs^+ in the following order: $K^+ = Rb^+ > Cs^+$. On the other hand, Li^+ and Na^+ suppressed the O_2 activity only by 10 % even at 100 mM. This is not due to the activity supported by these cations, since little activity was found if no Ca^{2+} was added. A double reciprocal plot of the Ca^{2+}-dependent O_2 evolution rate as a function of the Ca^{2+} concentration at varying cation concentrations (K^+, Rb^+ and Cs^+)
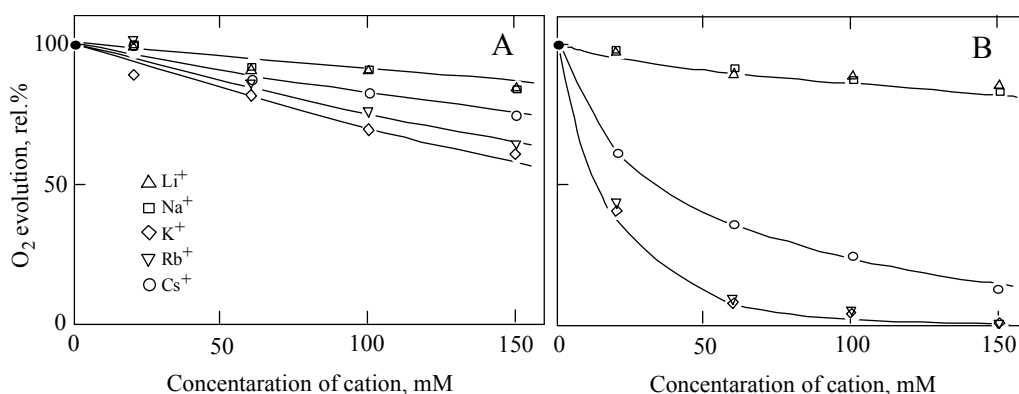

Fig.1. Effects of alkali metal cations on O_2 evolution in none-treated control (A) and Ca^{2+}-depleted PS II membranes (B).

yielded linear lines with a crossing point on the Y-axis (data not shown), indicating that these cations inhibits the O_2 evolution in competition with Ca^{2+}. Apparent Ki values were estimated to be 3 mM, 3 mM and 8 mM for K^+, Rb^+ and Cs^+, respectively. Data presented in Fig. 1B may indicate high Ki values (>> 100 mM) for Li^+ and Na^+. The affinities of the putative Ca^{2+} site for these ions must be very low. Notably, the effects of the cations were reversible since Ca^{2+}-dependent O_2 activity was restored after washing the excess cations.

Fig. 2 shows the TL glow curves of the Ca^{2+}-depleted membranes induced by illumination at 5°C for 5s in the presence of DCMU. Ca^{2+}-depleted membranes without metal cation supplementation generated the normal $S_2Q_A^-$ band at approximately 12°C. The TL peak temperature was upshifted to 38°C by supplementation with K^+, Rb^+ or Cs^+, although an appreciable shoulder remained in

Cs^+-supplemented membranes. Modifications of the TL band suggest that the binding of K^+, Rb^+ and Cs^+ to the Ca^{2+} site alters the redox properties of the OEC. Supplementation with Li^+ or Na^+ shifted the TL peak to 3 and 10°C, respectively. Since the affinity of these two cations for the Ca^{2+} site appears to be low, as shown in Fig.1, whether these changes are due to the binding of Li^+ or Na^+ to the Ca^{2+} site, or whether they are due to another effect of these cations remains unclear. Illuminating the Mn-depleted membranes at 77 K induced the $Q_A^-Y_D^+$ band at approximately 40°C in the presence of 300 mM K^+ (data not shown), suggesting the cation-induced upshifted TL band in K^+-, Rb^+- and Cs^+-substituted OECs may be ascribed to the charge recombination between Q_A^- and Y_D^+ pair; illumination induces the modified S_2 state whose oxidation potential becomes more negative than that of Y_D^+.
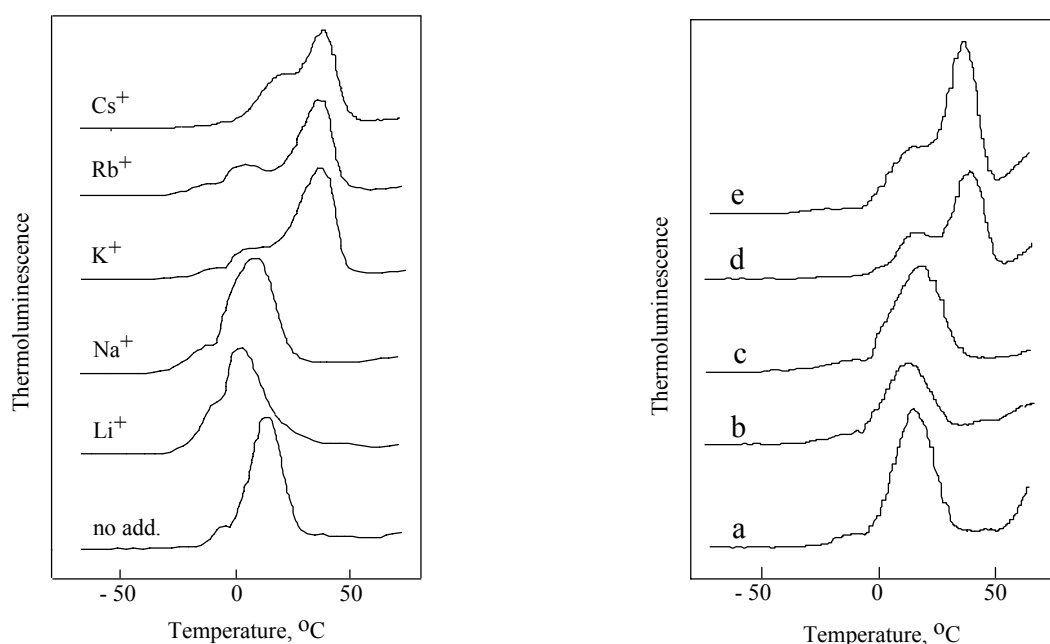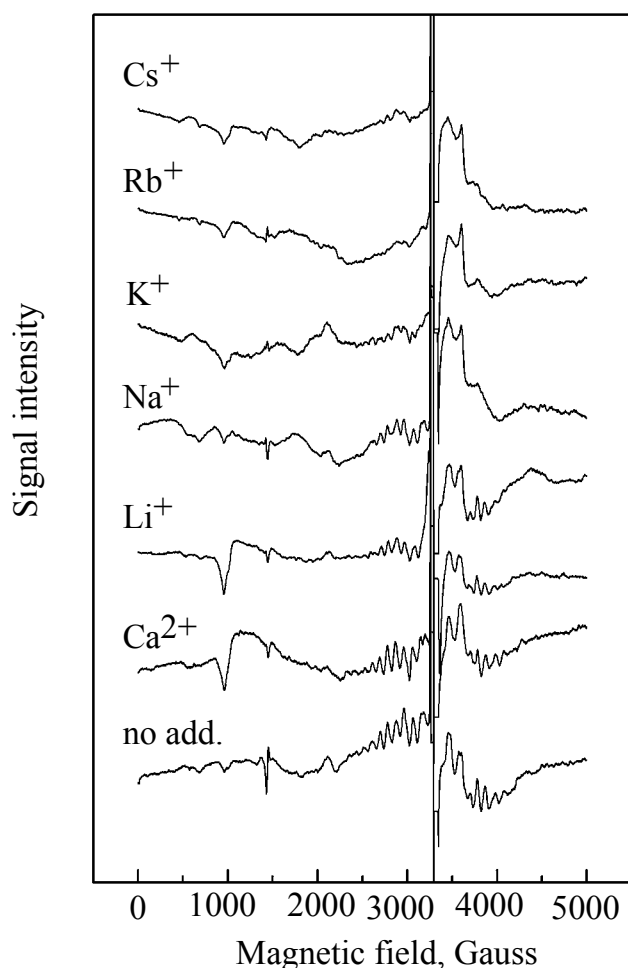

Fig. 2 (left) Effects of alkali metal cations on TL glow curves. Sample membranes were incubated with 300 mM respective cations at 0°C for 5min, then illuminated.
Fig. 3 (right) Effects of post K^+/Ca^{2+} addition on TL glow curves. Membranes were illuminated with no cation (a), 10 mM Ca^{2+}/10 mM K^+ (b), 300 mM K^+ followed by dark addition of 10 mM Ca^{2+} (final:10 mM K^+) (c), no cation followed by dark addition of 300 mM K^+ (d), 300 mM K^+ (e). Reaction mixture contained DCMU.

Fig. 3 shows the effects of dark/post-additions of K^+ and Ca^{2+} to the pre-illuminated membranes on TL glow curves, where K^+ was added after illuminating the Ca^{2+}-depleted membranes without cations supplementation, or Ca^{2+} was added after illuminating the depleted membranes supplemented with K^+. The post K^+-addition converted the $S_2Q_A^-$ band (curve a) to a high-temperature band (curve d), which was induced by the illumination in the presence of K^+ (curve e). Similarly, the post Ca^{2+}-addition converted the high-temperature band to the $S_2Q_A^-$ band (curve c). The results demonstrated that the high-temperature and $S_2Q_A^-$-bands are interconvertible in the dark by the addition of K^+ or Ca^{2+} ,due to the change in the oxidation potential of the S_2-sate Mn-cluster.

Fig. 4 shows the S_2 EPR spectra of the Ca^{2+}-depleted membranes (light minus dark) supplemented with 300 mM alkali metal cations. The membranes were illuminated at 0°C for 20s in the presence of DCMU. The Ca^{2+}-depleted membranes supplemented with no metal cation, Li^+ and Na^+ generated a multiline S_2 signal that are indistinguishable from that induced in the presence of 30 mM Ca^{2+}, although a g = 4 S_2 signal was not induced. In contrast, neither a multiline nor a g =4 S_2 signal was induced in the membranes supplemented with K^+, Rb^+ and Cs^+. $Fe^{2+}Q_A^-$ signals were similarly induced in all sample membranes, indicating that an electron was delivered from the oxidizing side of PS II to Q_A. Other spectral changes were not obtained reproducibly.

Fig. 4 Effects of 300 mM alkali metal cations on S_2 EPR spectra. Reaction mixture contained DCMU. Instrumental settings: temperature, 6K; microwave power, 2 mW: microwave frequency, 9.09 GHz; modulation frequency and amplitude, 100 kHz and 20 G, respectively.

Fig. 5 shows the g = 2 split-type EPR signals of the Ca^{2+}-depleted membranes. The sample membranes were illuminated at 0°C for 60s in the presence of phenyl-*p*-benzoquinone. Illumination induced a split-type signal at g = 2 with an approximate line width of 150 G in both the Ca^{2+}-depleted membranes supplemented with no cation and 300 mM K^+. It is of note that the g = 2 signal was not induced in the presence of DCMU. The split signal is originated from the interaction between Y_Z^+ and the Mn-cluster at the S_2 state. Therefore, the results indicate that the Mn-cluster s oxidized to the S_2 state in the presence of K^+ although the S_2 sate, thus formed, does not generate S_2 EPR signals, presumably due to faster relaxation of the S_2 signal, or spin state change of the Mn-cluster. This view is consistent with the dark conversion of the high-temperature TL band to the $S_2Q_A^-$ band by post-dark Ca^{2+} addition as shown in Fig. 3.
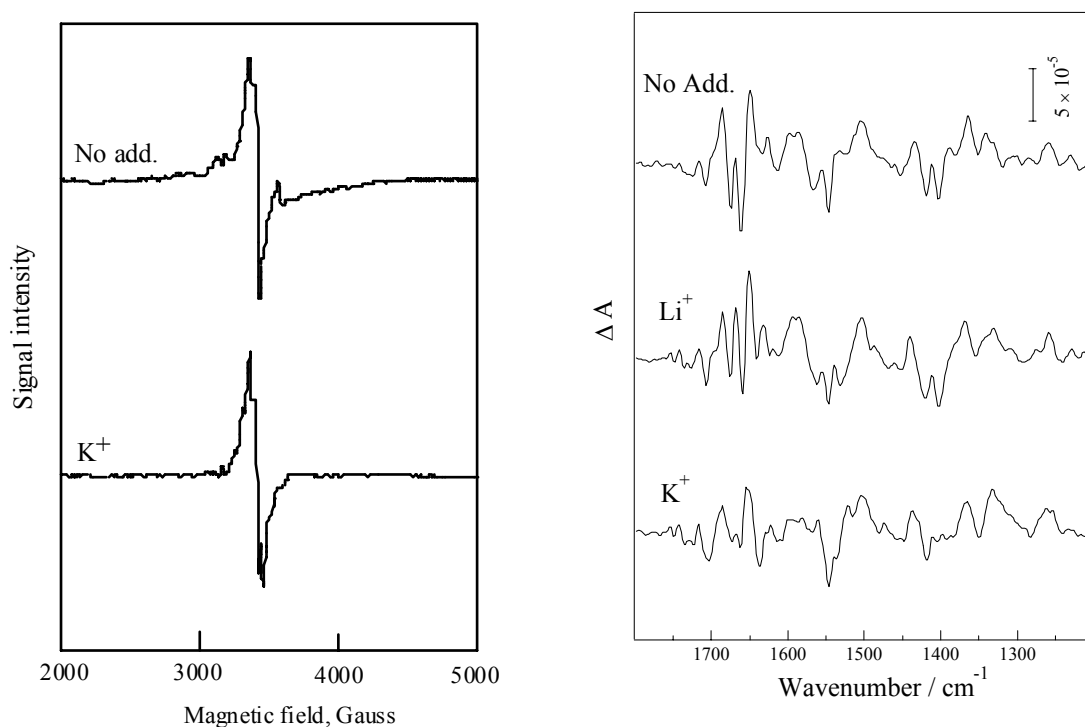
Fig. 5 (left) g = 2 split-type EPR signal. K^{+}-supplemented spectrum is presented after magnification by a factor of 1.5 in intensity. Instrumental settings: temperature, 8K; microwave power, 1 mW: microwave frequency, 9.50 GHz; modulation frequency and amplitude, 100 kHz and 16 G, respectively.

Fig. 6 (right) Light-induced S_2/S_1 difference FTIR spectra. Each double difference spectrum was obtained by subtracting Q_A^-/Q_A difference spectrum from the $S_2Q_A^-/S_1Q_A$ difference spectrum.

Fig.6 shows the FTIR spectra of the Ca^{2+}-depleted membranes induced by illumination at 250K for 5s. The double difference spectrun of the Ca^{2+}-depleted membranes showed the characteristic vibrational feature at 1365(+)/1404(-) cm^{-1} for the symmetric and at 1584(+)/1562(-) cm^{-1} for the asymmetric stretching vibrations which are attributable to the carboxylate ligand for the Mn-cluster. Additionally, the prominent bands for the conformational change of the protein backbone around 1690-1630 cm^{-1} (amide I) and 1590-1515 cm^{-1} (amide II) were seen. These features closely resemble those typical of S_2/S_1 difference spectrum in the none-treated control membranes. Supplementation of 300 mM Li^+ did not influence the spectrum, but 300 mM K^+ significantly affected the spectrum; the carboxylate bands disappeared almost completely and the amide I and II bands were largely suppressed and distorted.

Discussion

The present study demonstrated that alkali metal cations associate with the Ca^{2+}-site in their specific manner. Cations with an ionic radius larger than Ca^{2+}, such as K^+, Rb^+ and Cs^+, interact with the Ca^{2+} site, but Li^+ and Na^+, with an ionic radius smaller than Ca^{2+}, have much lower affinities for the Ca^{2+} site. In the K^+-, Rb^+- and Cs^+-substituted PSII, the Mn-cluster was oxidized to the S_2-state. However, thus formed S_2-state showed no multiline EPR signal, abnormally high oxidation potential and was not accompanied by the conformational change of the protein matrices.

Interestingly, Ca^{2+} depletion itself did not induce these abnormalities, indicating that Ca^{2+} participates in the water oxidation at higher process than the S_2 state. Therefore, it is likely that the anomalous S_2 properties found in K^+-, Rb^+- and Cs^+-substituted OEC do not ascribe to insufficiency of these cations as a functional replacement of Ca^{2+} but are caused by the secondary perturbation effects on the vicinity of the Mn-cluster and/or Mn-cluster itself. This must be induced by the binding of the cation, with larger ionic radius than Ca^{2+}, to the Ca^{2+}-site, which is so close to the Mn-cluster that the distortion of the Ca^{2+}-site influences the Mn-cluster and/or its ligands structurally .

Acknowledgments

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