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The S-state dependence of Cl^- binding to plant Photosystem II

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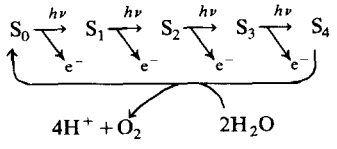
A combined single-turnover flash and ^{35}Cl NMR technique has been used to monitor S-state dependence of Cl^- binding to PS-II particles derived from mangrove (*Avicennia marina*). No detectable high-affinity binding was found to particles in the S_0 and S_1 states, but binding with an affinity comparable to that which activates O_2 evolution was found in the S_2 and S_3 states.

Millimolar levels of Cl^- stimulate oxygen-evolving activity in the plant chloroplast Photosystem II (PS-II) complex [1]. The enzyme responsible for this water-splitting reaction within the complex is probably a mangano protein, with approx. four Mn atoms per PS-II reaction centre [2]. The overall conversion $2H_2O \rightarrow O_2 + 4H^+ + 4e^-$ is now believed to occur in four one-electron steps [2,3], each driven by a single light quantum which energises primary-charge separation across the thylakoid membrane through the reaction centre (P-680) protein. With each electron withdrawal, the oxygen-evolving centre cycles through well-defined intermediate states (S states), as summarised below:



The S_4 state has a transient existence, but the other S states are long lived (more than 30 s at 20°C) and decay to S_1 in the dark. Extensive physical/chemical characterisation of these states has been carried out (see Ref. 2 for a review) and there is evidence for variations in the oxidation numbers of the Mn centres between S states [4], as well as in the EPR properties [5] of these states.

Recently, attention has focussed on the role of Cl^- in activating the water-oxidation reaction, and the mediation of this effect by certain membrane proteins associated with the PS-II complex (see Ref. 1 for a recent review). It appears that Cl^- always stimulates oxygen evolution activity in PS-II preparations and is absolutely required for activity at pH > 7. The Cl^- affinity of the activating site(s) decreases with increasing pH and drops by an order of magnitude on removal of the 16 and 23 kDa, NaCl-extractable proteins from the inner thylakoid membrane surface [6,7]. Such protein-depleted PS-II preparations can still achieve full oxygen evolution activity, compared with intact systems, but at higher Cl^- levels. However, despite extensive study, the detailed nature of Cl^- interaction with the water-splitting centre remains as yet obscure.

Since Cl^- is demonstrably labile in the PS-II system, it is possible that this property is vital for

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Abbreviations: Mes, 4-morpholineethanesulphonic acid; Chl, chlorophyll.

its catalytic role, i.e., that the ion binds functionally to only some states in the S cycle. In this study we use ^{35}Cl NMR to examine Cl^- interaction with each of the four long-lived ($S_0 - S_3$) states of the cycle. Typically, Cl^- binding to (cationic) protein sites reflects in a readily detectable relaxation enhancement of the total chloride population [8,9] even (as here) for free/bound ratios higher than 10^4, provided exchange rates are sufficiently high. Under such circumstances, the concentration of ions bound to a particular class (i) of sites is directly proportional to $\delta\nu_i$ [Cl], where [Cl] is the total Cl^- concentration and $\delta\nu_i$ that component of relaxation rate attributable to sites of class *i*.

PS-II particles were prepared from greenhouse grown mangrove (*Avicennia marina*) leaves as described by Preston and Critchley [10]. The particles were washed once in 50 mM Mes-KOH (pH 6.5)/5 mM EDTA, then resuspended to 200 μg/ml chlorophyll in the same buffer medium. This stock suspension was stored on ice in the dark. For NMR measurements, the particles were diluted to 30 μg/ml chlorophyll in the above buffer containing sodium chloride (20–80 mM) with 500 μM potassium ferricyanide and 130 μM phenyl-*p*-benzoquinone as electron acceptors. Unlike PS – II particles prepared from spinach, mangrove particles remained unaggregated and well suspended throughout the duration of the NMR run (2 h at 20°C). Typically, samples lost approx. 30% of initial oxygen-evolving activity over this period.

^{35}Cl NMR measurements were performed at 19.6 MHz on a Bruker CXP-200 wide bore instrument. To produce PS-II populations of defined S state, a 20 mm diameter, non-spinning NMR tube with a flat mirrored base was filled to a sample depth of 1 cm and illuminated from the top by an optical fibre bundle fed down the bore of the magnet. Single turnover flashes (approx. 15 μs duration) were generated with an EG & G FX-132 flash lamp and band-pass filter (600–650 nm).The chloride transverse magnetisation decay (T_2) was acquired using the standard Carr Purcell/Meiboom Gill sequence [11] with phase alternation between scans. At the low chlorophyll concentration required to allow uniform illumination throughout the sample (30 μg/ml) the maximum relaxation enhancement is only approx. 15% of the solution background at 30 mM Cl^-. To compensate for systematic variations (instrumental drift, dissolved O_2, etc.) the S_n state and S_1 state (dark state) spectra were obtained 'simultaneously' during a run. Scans from the S_1 state were accumulated for 10 s, the S_n state generated and scanned for the same period and the system allowed to relax in the dark to S_1 over 2.5 min (more than 5 half-lives in our system). This was repeated 40-times to give 1500 accumulations of each decay. All decays were monoexponential within experimental error. Binding to dark (S_1) state particles was determined on more concentrated (150 μg/ml chlorophyll) solutions, using 10 mm spinning tubes and the T_2 values determined directly from the line widths. All relaxation values are quoted as line-width equivalents (see fig. 2).

Fig. 1 shows Cl^- activation of O_2 evolution activity in the mangrove PS-II particle system at pH 6.5. At this pH, chosen for optimum long-term particle stability, there is residual O_2-evolving activity in the absence of exogenous Cl^-. Further, these particles are deficient in the 16 and 23 kDa proteins and the apparent $K_{1/2}$ of the Cl^- activating site(s) is approx. 5 mM. Fig. 2 shows Cl^-

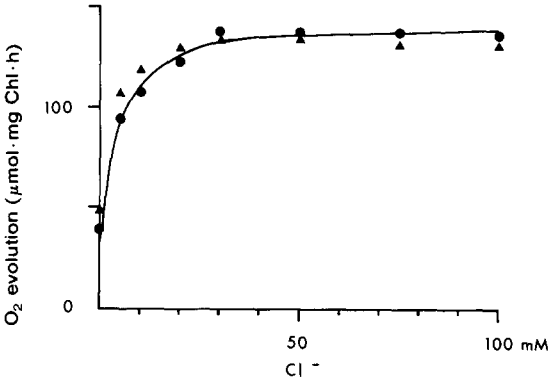

Fig. 1. Oxygen-evolution activity of mangrove PS-II particles at pH 6.5 as a function of chloride concentration [Cl^-] in the assay medium. Each point represents a single determination, different symbols correspond to different preparations. $K_{1/2}$ for chloride-stimulated activity is approx. 5 mM. Measurements performed at 25°C with a Clark type O_2 electrode (Rank Bros., Bottisham, U.K.) in red light (610 nm cut off) at a quantum flux density of 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$. Particle concentration, 10 μg/ml chlorophyll in assay medium containing NaCl (0–100 mM) /potassium ferricyanide (1.5 mM)/phenyl-*p*-benzoquinone (0.5 mM)/25 mM Mes-2-amino-2-methyl-1,3-propanediol. Chlorophyll was determined by the method of Arnon [17].

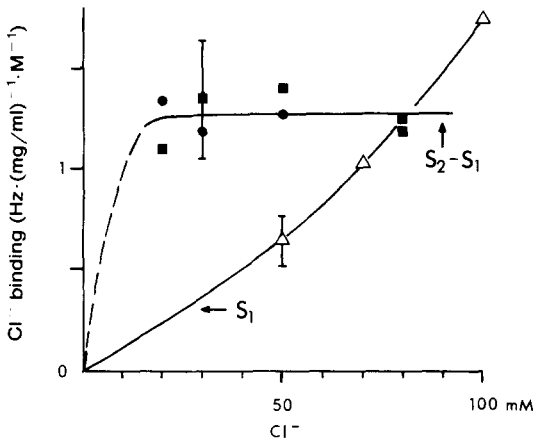

Fig. 2. Chloride binding to PS-II particles in suspension as a function of chloride concentration [Cl^-] in the medium (expressed as specific line width increment on chlorophyll basis, $\delta\nu$[Cl]/[Chl], where $\nu = \pi/T_2$, T_2 is the observed transverse-magnetisation decay time and [Chl] is the chlorophyll concentration). S_2-S_1 curve shows binding difference between dark states (S_1) and one-saturating-flash states (S_2). S_1 curve shows total binding to S_1-state particles. Symbols as in Fig. 1 $K_{1/2}$ for S_2-S_1 curve is less than 10 mM. Background ν value is approx. 10 Hz, and individual measurements are reproducible within ± 0.3 Hz at 30 mM Cl^-. Temperature, 20 ± 1°C for all data points.

titration of the specific particle-induced relaxation for both the S_1 state and the $S_2 - S_1$ states. This specific relaxation is directly proportional to anion binding (on a chlorophyll basis) for a given class

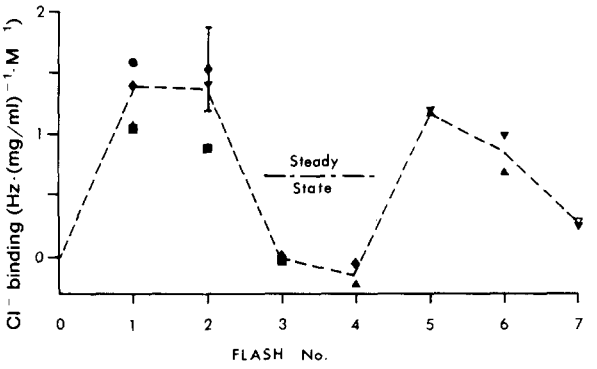

Fig. 3. Flash-number dependence of binding to S_n state minus binding to S_1 state (for same sample). Symbols as in Fig. 1. 'Steady-state' value obtained by scrambling states with one flash per 10 s during continuous (three scans/s) acquisition. Delay between flashes in a given train is 0.7 s and NMR accumulation commenced 1 s after final flash in train.

of sites under our conditions. The S_1 state shows only low affinity ($K_{1/2} \gg 0.1$ M) binding behaviour, but the S_2 state exhibits a class of high-affinity sites on top of the S_1 background. The $K_{1/2}$ value of this class is too low for precise determination in our system, but is less than 10 mM and so of a similar order to that of the O_2-evolution-activating sites(s). Fig. 3 shows a flash pattern for the $S_n - S_1$ binding at 30 mM Cl^-, extending out to seven flashes. The Cl^- binding shows a periodicity of 4, and is completely consistent with a Kok model involving functional Cl^- interaction with only the S_2 and S_3 states. The simplest interpretation suggests a near uniform (assumed S_1) population in the dark state, approximately equal high-affinity binding to the S_2 and S_3 states and more than 90% efficiency of turnover/flash.

While our data indicate that labile, high affinity Cl^- binding to PS II occurs only in the S_2 and S_3 states, we cannot exclude with NMR a slowly exchanging (very high affinity?) interaction with these or other S states. Although no direct evidence for such binding exists, and several studies [12–15] on whole chloroplasts suggest Cl^- depletion affects fluorescence and kinetic properties in only the S_2 and S_3 states, the full extent of 'Cl^- depletion' in such cases remains uncertain. However, it is a reasonable presumption that Cl^- functions by interacting closely with one or more of the Mn centres of the catalytic site. Very thorough extended X-ray absorption fine structure studies by Klein et al. [16] show that Cl is not a first shell ligand to the functional Mn of chloroplasts in the S_1 state, consistent with out failure to detect high-affinity Cl^- binding in this state.

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