Photosystem II: Thermodynamics and Kinetics of Electron Transport from $Q_{A}^-$ to $Q_{B}(Q_{B}^-)$ and Deleterious Effects of Copper(II)

G. Renger, H. M. Gleiter, E. Haag, and F. Reifarth
Max-Planck-Institut für biophysikalische Chemie, Technische Universität Berlin, Straße des 17. Juni 135, D-W-1000 Berlin 12, Bundesrepublik Deutschland
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Introduction

The key steps of photosynthetic water cleavage into dioxygen and metabolically bound hydrogen take place within photosystem II. The overall reaction sequence comprises (i) the light-induced generation of reducing and strongly oxidizing redox equivalents, (ii) the oxidation of water to dioxygen under proton release into the lumen, and (iii) the formation of bound hydrogen by reduction of plastoquinone to the quinol form coupled with proton uptake from the stroma (for reviews see [1, 2]). The latter process is a cooperative reaction of two electrons which occurs at a special plastoquinone binding niche (referred to as $Q_{B}$ site), located in polypeptide D1 of the PS II complex. Plastoquinone noncovalently binds to the $Q_{B}$ site and becomes reduced via a sequence of two univalent redox steps with $Q_{A}^-$ as electron donor. Only the semiquinone form $Q_{B}^-$ is comparatively strongly bound, while the association of fully reduced and protonated plastoquinone ($Q_{B}H_{2}$), with the $Q_{B}$ site is rather labile and $Q_{B}H_{2}$ readily exchanges with another PQ molecule of the pool thereby completing the reaction cycle (for a review see ref. [3]). The relationship between structure and binding properties of the $Q_{B}$ site is of special relevance for herbicide research because many potent herbicides used in agriculture bind to this unique PS II target, thereby blocking photosynthetic electron transport (for reviews see ref. [4–6]). Different procedures can be applied to modulate the properties of the $Q_{B}$ site in terms of its functional role and in herbicide binding. Marked changes can be obtained either by specific genetic manipulations [7, 8] or by less specific structural modifications like detergent treatment [9] or proteolytic degradation [10]. In the present communication two selected
topics will be addressed: (a) the thermodynamic and kinetic properties of the electron transfer from $Q_a^-$ to $Q_b^- (Q_b^-)$ and (b) the effect of bivalent metal ions on PS II.

Materials and Methods

Thylakoid and PS II membrane fragments were prepared from spinach as described in ref. [11–13]. Preparations of PS II core complexes were obtained by the procedure outlined in ref. [14]. For bivalent metal cation treatment, samples were incubated for 2 min in the dark prior to the measurements. The incubation medium contained PS II membrane fragments or PS II core complexes (20 μM Chl), 10 mM NaCl, 10 mM CaCl$_2$, and 20 mM MES/NaOH, pH 6.5. The rates and average yield per flash (ref. [15]) of oxygen evolution were measured with a Clark-type electrode with DCBQ (500 μM) as electron acceptor and at saturating continuous of flash light intensity. The manganese content of the sample was determined by atomic absorption (Perkin Elmer model AA 5 300).

Flash-induced changes of the fluorescence quantum yield were monitored with home-built equipment [16, 17]. Conventional fluorescence induction curves were measured as described in ref. [18].

Herbicide binding assays were performed with $[^{14}C]$atrazine as in ref. [19].

Results

Thermodynamics and kinetics of electron transport from $Q_a^-$ to $Q_b^- (Q_b^-)$

Fig. 1 shows typical changes in the relative fluorescence quantum yield induced by a saturating laser flash in dark adapted thylakoids at room temperature. The general feature of this signal is characterized by four parameters: (i) the relative fluorescence yield before the actinic flash, $F_o$; (ii) the maximum level of the relative fluorescence yield induced by the actinic flash, $F_m$; (iii) the terminal level 10 s after the actinic flash, $F_t$, and (iv) the decay kinetics of the flash-induced transient, $F(t)-F_t$ (the rise kinetics from $F_o$ to $F_m$ will not be discussed in this study).

Regardless of the detailed interpretation of the flash induced transient fluorescence emission, two

![Fig. 1. Laser flash induced change of the relative fluorescence quantum yield as a function of time in isolated thylakoids at 20 °C. Insert: logarithmic time scale. Experimental details as described in Materials and Methods.](image-url)
types of interesting information can be gathered from an analysis of this type of signal: (a) the relaxation $F(t) - F_e$ reflects the kinetics of $Q_A^-$ reoxidation by endogenous plastoquinone (PQ) or exogenous electron acceptors, and (b) the normalized level of the terminal relative fluorescence yield, $F_t/F_o$, provides a measure of the extent of $Q_A^-$ that remains reduced for 10 s after the flash.

As the normalized concentration $[Q_A^-](t)$ and $F(t)$ are nonlinearly related quantities [20], the value of $[Q_A^-](t)$ has to be calculated from $F(t)$ by a suitable procedure as outlined in ref. [9].

Two striking phenomena emerge, if the temperature of the sample decreases: the parameter $F_t/F_o$ increases, and the relaxation kinetics of $F(t) - F_e$ become markedly slower (data not shown). These effects can be used to analyze the thermodynamics and determine the activation energy of the $Q_A^-$ reoxidation. Fig. 2 depicts the normalized quantity $F_t/F_o$ as a function of sample temperature in isolated thylakoids (top) and PS II membrane fragments (bottom). The steep increase below $-10$ to $-20^\circ C$ reflects the blockage of $Q_A^-$ reoxidation in an increasing fraction of PS II [21, 22]. The simplest explanation of this phenomenon can be provided by the assumption of a temperature-dependent equilibrium between PS II centers that are active (A) or inactive (I) in respect to $Q_A^-$ reoxidation. If one ascribes the quantity $[Q_A^-](t = 10\, s)$ calculated from $F_t/F_o$ (see ref. [19]) to the fraction of inactive centers, the equilibrium constant, $K_{eq}$, is given by:

$$K_{eq} = \frac{[A]}{[I]} = \frac{1 - [Q_A^-](t = 10\, s)}{[Q_A^-](t = 10\, s)}$$  \hspace{1cm} (1)

The temperature dependence of an equilibrium constant is described by the Van't Hoff relation:

$$\frac{\delta \ln K_{eq}}{\delta T} = \frac{\Delta H^o}{RT^2}$$  \hspace{1cm} (2)

where $\Delta H^o$ is the standard enthalpy of the process and $R$ the gas constant.

If one assumes that $\Delta H^o$ can be considered nearly constant within the temperature range of $220\, K < T < 300\, K$, Eqn. (2) leads to:

$$\ln K_{eq}(T) = \text{const.} - \frac{\Delta H^o}{RT}$$  \hspace{1cm} (2a)

Fig. 3 presents a semilogarithmic plot of the $K_{eq}$ values calculated according to Eqn. (1) from the

Fig. 2. Normalized terminal fluorescence $F_t/F_o$ as a function of temperature in isolated thylakoids (top) and PS II membrane fragments (bottom). For further details see text.

Fig. 3. Semilogarithmic plot of calculated equilibrium constant $K_{eq}$ as a function of reciprocal temperature in isolated thylakoids (top) and PS II membrane fragments (bottom). For further details see text.
data of Fig. 2 as a function of the reciprocal temperature for thylakoids (top) and PS II membrane fragments (bottom). In both cases the data nicely fit a linear relation which gives rise to enthalpy values of

\[ \Delta H^\circ (\text{thylakoids}) = 95 \text{ kJ/mol}^{-1} \]
\[ \Delta H^\circ (\text{PS II membrane fragments}) = 60 \text{ kJ/mol}^{-1}. \]

Based on the relation

\[ \ln K_{\text{eq}} = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \]  

one can also calculate the entropy of the process leading to the transformation of inactive to active PS II centers (in terms of Q_A^- reoxidation). At \( K_{\text{eq}} = 1 \), the standard entropy of the transition is simply given by \( \Delta S^\circ = \Delta H^\circ / T_{K=1} \). Based on \( T_{K=1} \) values of 243 K (thylakoids) and 255 K (PS II membrane fragments) one obtains

\[ \Delta S^\circ (\text{thylakoids}) = 370 \text{ J K}^{-1} \text{ mol}^{-1} \]
\[ \Delta S^\circ (\text{PS II membrane fragments}) = 240 \text{ J K}^{-1} \text{ mol}^{-1}. \]

The calculations reveal that the electron transfer from Q_A^- to Q_B(Q_B^-) requires a significant degree of flexibility in the protein matrix (see Discussion).

In addition to this information on the thermodynamics of state transition I \( \rightleftharpoons \) A, measurements of the flash-induced changes of the relative fluorescence emission at different temperatures also permit an analysis of the reaction coordinate of Q_A^- reoxidation. The normalized parameter \( [F(t) - F_o] / F_o \) provides a measure of \([Q_A^- (t)]\) (see ref. [9]). In general, \([Q_A^- (t)]\) exhibits an at least bi-phasic decay of the form

\[ \frac{[Q_A^- (t)] - [Q_A^-]}{[Q_A^- (t = 0)] - [Q_A^-]} = a \exp(-k_1 t) + (1 - a) \exp(-k_2 t). \]

In thylakoids at room temperature \( a \) and \( k_1 \) were found to be 0.8 – 0.9 and 4000 s\(^{-1}\), respectively [9]. The rate constant \( k_1 \) describes the electron transfer form Q_A^- to a Q_B site with a bound Q_B or (Q_B^-), depending on the dark population before the first flash. Accordingly, an Arrhenius plot of the \( k_1 \) values provides direct information on the activation energy of this process. The temperature range is restricted to 265 K \( < T < 300 \) K, because at lower temperatures the uncertainties in \( k_1 \) determination become very large due to the drastic decrease of the total extent of Q_A^- reoxidation; while at temperatures above 300 K effects due to thermal deg-

radation have to be taken into account. The results depicted in Fig. 4 for thylakoids (top) and PS II membrane fragments (bottom) lead to activation energies of:

\[ E_A (\text{thylakoids}) = 30 \pm 5 \text{ kJ mol}^{-1} \]
\[ E_A (\text{PS II membrane fragments}) = 40 \pm 10 \text{ kJ mol}^{-1}. \]

**Effects of bivalent metal ions on PS II**

In the former part of this study the properties of the normal PS II acceptor side have been studied. Now, the effect of metal ions on PS II will be briefly analyzed. Conventional fluorescence induction curves were measured by illumination with continuous light (see Materials and Methods). Fig. 5 depicts the normalized variable fluorescence \( F_o / F_o \) as a function of metal ion concentration in the suspension of PS II membrane fragments (top) and PS II core complexes (bottom). The data show that marked effects in the \( \mu \) range arise only for Cu\(^{2+}\). The other cations are virtually without effect in this range in the case of PS II membrane frag-
ments while some inhibiting effects are observed for Zn\(^{2+}\) and Co\(^{2+}\) in PS II core complexes.

The effect of Cu\(^{2+}\) was analyzed in more detail by measuring the light-saturated rate of oxygen evolution, the binding properties of the PS II herbicide atrazine and the manganese content. Typical dependencies on Cu\(^{2+}\) concentration are summarized in Fig. 6 for the case of PS II membrane fragments. The data reveal that the manganese content of about 4 Mn per functionally competent oxygen-evolving complex remained virtually unaffected by the preincubation with 70 \(\mu\)M CuCl\(_2\) which causes a complete suppression of the activity. The Cu\(^{2+}\) concentration required for 50\% inhibition of oxygen evolution is of the order of 8–10 \(\mu\)M (in the case of PS II core complexes the \(I_{50}\) value is about 5 \(\mu\)M at the same total chlorophyll concentration of 20 \(\mu\)M). Measurements of [\(^{14}\)C]\(\)atrazine binding and data analysis via the conventional double reciprocal plot [19] lead to values of about 300 chlorophylls per atrazine-binding site and a \(K_D\) of about 60 \(nM\) in control PS II membrane fragments. Incubation with CuCl\(_2\) did not affect the number of binding sites (data not shown) but greatly reduced the binding affinity as shown by the sharp decrease of the reciprocal \(K_D\) value in Fig. 6. The effect on \(K_D\) is clearly more sensitive to CuCl\(_2\) than the rate of oxygen evolution. Analogous experiments could not be performed in PS II core complexes because already the control sample exhibited markedly reduced affinity and binding sites (data not shown) due to a severe modification of the Q\(_B\) site as reported recently [9].

The disparity between the dependencies on Cu\(^{2+}\) concentration of the inhibition of oxygen evolution and the effect on atrazine-binding affinity, respectively, shows that the former effect is probably not due to a blockage of the acceptor side. To clarify this point 830 nm absorption changes were measured which reflect the light-induced turnover of P680. The results obtained (data not shown) indicate that Cu\(^{2+}\) affects the function of the redox-active tyrosine Y\(_Z\) which mediates the electron transfer between the oxygen-evolving complex and P680\(^{\ast}\). A more detailed analysis will be presented in a forthcoming paper.

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**Fig. 5.** Normalized variable fluorescence as a function of bivalent metal cations in PS II membrane fragments (top) and PS II core complexes (bottom). The symbols represent: Cu\(^{2+}\) (●), Zn\(^{2+}\) (X), Cd\(^{2+}\) (○) and Co\(^{2+}\) (□). Experimental details as described in Materials and Methods.

**Fig. 6.** Oxygen evolution rate (○) and calculated equilibrium constant (●) normalized to the control values at [Cu\(^{2+}\)\(]=0\) and manganese content as a function of [Cu\(^{2+}\)] concentration. Experimental details as described in Materials and Methods.
Discussion

The present study shows that the $Q_A^-$ reoxidation by $Q_B(Q_B^-)$ is frozen due to the transition from an "active" (A) into an "inactive" (I) state of the PS II acceptor side. The formation of the "active" state is an enthalpy-driven process which enhances the flexibility as reflected by the significant entropy increase. This transition either strongly affects the redox equilibrium $Q_A^-Q_B\rightleftharpoons Q_A^+Q_B^-$ or gives rise to structural changes in the protein matrix of functional relevance as discussed previously [23]. Further experiments are required to clarify this point. It is interesting to note that $\Delta H^0$ and $\Delta S^0$ of the state I $\rightarrow$ state A transition are significantly higher in thylakoids than in PS II membrane fragments. This finding strongly supports the idea that treatment with Triton X-100 modifies the protein structure near the $Q_B$ site. Indirect lines of evidence for this conclusion provide the differences in atrazine binding reported previously [24] and the retardation of $Q_A^-$ reoxidation [9]. Based on the assumption of nearly the same normalized $Q_B$ population in dark adapted thylakoids and PS II membrane fragments, the present data reveal that the activation energy of the electron transfer from $Q_A^-$ to $Q_B$ in PS II membrane fragments exceeds the corresponding value in thylakoids by about 30% (see Fig. 3). Within the framework of the classical Marcus theory [25, 26] of thermally activated nonadiabatic electron transfer, this difference can be explained by a higher reorganization energy in the Triton X-100-modified PS II acceptor side. Accordingly, different lines of evidence support the idea that Triton X-100 affects the protein matrix of the D1/D2 heterodimer at the acceptor side.

The second part of this communication presents data on the interaction of Cu$^{2+}$ with PS II. Among different bivalent metal ions (Zn$^{2+}$, Co$^{2+}$, Cd$^{2+}$, Cu$^{2+}$) only Cu$^{2+}$ exerts marked effects in the $\mu$m concentration range. Recently, also Hg$^{2+}$ was shown to exert marked effects at comparable concentration as will be presented elsewhere (U. Wacker and G. Renger, in preparation).

In latest reports Cu$^{2+}$ was inferred to interact with the PS II acceptor side probably near the Fe$^{2+}$ $Q_A$ Pheo region [27, 28]. Other studies came to the conclusion that Cu$^{2+}$ modifies the $Q_B$ site [29].

Our results indicate that Cu$^{2+}$ interferes in PS II with both, the acceptor and the donor side. The finding that Cu$^{2+}$ affects the affinity of atrazine without diminishing the number of binding sites is in line with the idea of a $Q_B$ site modification. A different pattern was recently observed for the effect of Cu$^{2+}$ on DCMU binding. It was shown that Cu$^{2+}$ does not affect the affinity constant but leads to a decreased number of binding sites [28]. This differences between DCMU and atrazine binding in response to Cu$^{2+}$ might be due to different binding determinants as is also reflected by the effects of an exchange of Ser 264 and Phe 255 by site directed mutagenesis on the resistance to both inhibitor types [8]. At present, the molecular mechanism of Cu$^{2+}$ modifications near the $Q_B$ site is not resolved. Further experiments are required to clarify this effect. In addition to the modulation of herbicide binding Cu$^{2+}$ also affects the donor site probably at the level of the redox active tyrosine residue $Y_7$ which functionally connects the water oxidizing complex with P680*. In comparison to atrazine binding higher Cu$^{2+}$ concentrations are required to elicit the latter effect. In summary the results of the present study reveal that Cu$^{2+}$ interacts with at least two different targets in PS II. In this way Cu$^{2+}$ provides an interesting tool to modulate the PS II reaction pattern.

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