Photosystem II: Thermodynamics and Kinetics of Electron Transport from Q_A^- to $Q_B(Q_B^-)$ and Deleterious Effects of Copper(II)

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Studies on thermodynamics and kinetics of electron transfer from Q_A^- to $Q_B(Q_B^-)$ were performed by monitoring laser flash induced changes of the relative fluorescence emission as a function of temperature ($220\ K < T < 310\ K$) in isolated thylakoids and PS II membrane fragments.

In addition, effects of bivalent metal ions on PS II were investigated by measuring conventional fluorescence induction curves, oxygen evolution, manganese content and atrazine binding mostly in PS II membrane fragments. It was found: a) the normalized level of the fluorescence remaining 10 s after the actinic flash (F_t/F_0) steeply increases at temperatures below -10 to -20 °C, b) the fast phase of the transient fluorescence change becomes markedly retarded with decreasing temperatures, c) among different cations (Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}) only Cu^{2+} exhibits marked effects in the concentration range below 100 μM and d) Cu^{2+} decreases the normalized variable fluorescence, inhibits oxygen evolution and diminishes the affinity to atrazine binding without affecting the number of binding sites. The content of about four manganeses per functionally competent oxygen evolving complex is not changed by $[Cu^{2+}] < 70$ μM.

Based on these findings it is concluded: i) a temperature dependent equilibrium between an inactive (I) and active (A) state of Q_A^- reoxidation by $Q_B(Q_B^-)$ is characterized by standard enthalpies ΔH° of 95 kJ mol^{-1} and 60 kJ mol^{-1} and standard entropies ΔS° of 370 kJ K^{-1} mol^{-1} and 240 kJ K^{-1} mol^{-1} in isolated thylakoids and PS II membrane fragments, respectively, ii) the activation energies of Q_A^- reoxidation by plastoquinone bound to the Q_B site are about 30 kJ mol^{-1} (thylakoids) and 40 kJ mol^{-1} (PS II membrane fragments) in $220\ K < T < 300\ K$, and iii) Cu^{2+} causes at least a two-fold effect on PS II by modifying the atrazine binding affinity at lower concentrations (~ 5 μM) and interference with the redox active tyrosine Y_Z at slightly higher concentration (~ 10 μM) leading to blockage of oxygen evolution.

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 D 1 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_BH_2), with the Q_B site is rather labile and Q_BH_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

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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

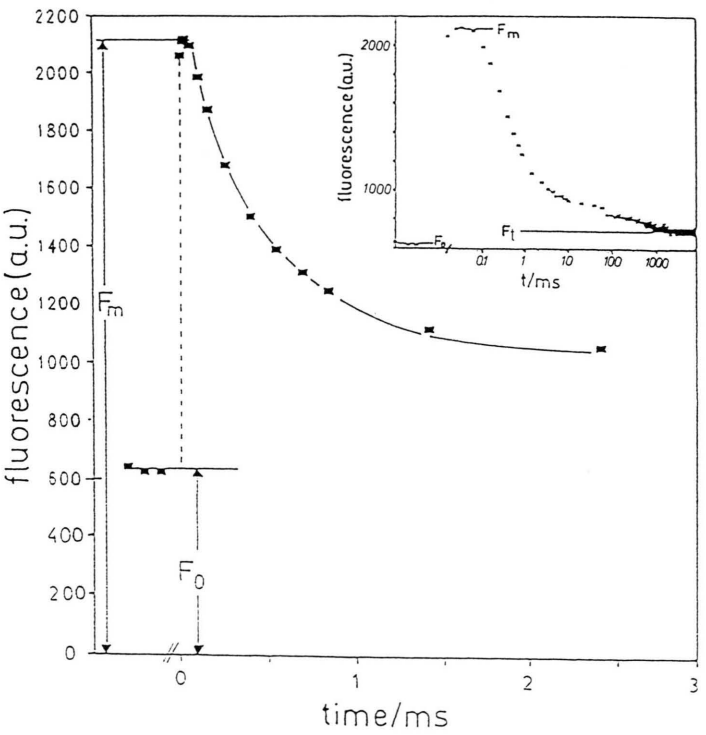

Fig. 1. Laser flash induced change of the relative fluorescence quantum yield as a function of time in isolated thylakoids at 20 °C. Insert: logarithmic time scale. Experimental details as described in Materials and Methods.

types of interesting information can be gathered from an analysis of this type of signal: (a) the relaxation $F(t)$-F_t 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_t 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 °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:

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

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

$$\frac{\delta \ln K_{eq}}{\delta T} = \frac{\Delta H^\circ}{RT^2} \qquad (2)$$

where ΔH° is the standard enthalpy of the process and R the gas constant.

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

$$\ln K_{eq}\,(T) = \text{const.} - \frac{\Delta H^\circ}{RT}. \qquad (2a)$$

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

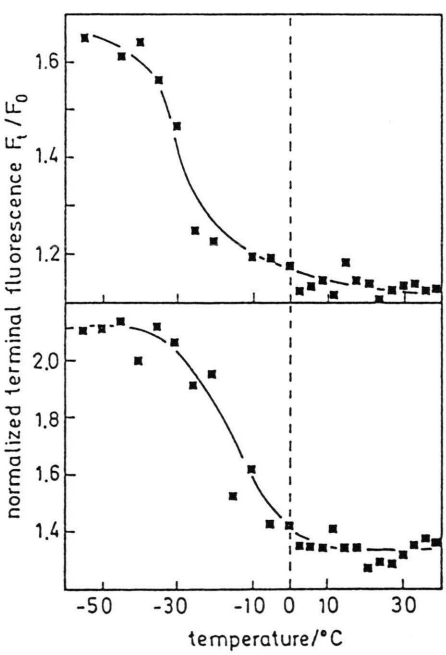

Fig. 2. Normalized terminal fluorescence F_t/F_o as a function of temperature in isolated thylakoids (top) and PS II membrane fragments (bottom). For further details see text.

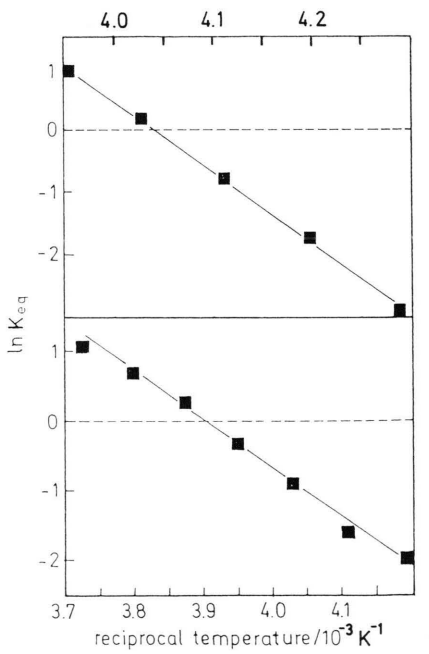

Fig. 3. Semilogarithmic plot of calculated equilibrium constant K_{eq} as a function of reciprocal temperature in isolated thylakoids (top) and PS II membrane fragments (bottom). For further details see text.

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

ΔH° (thylakoids) = 95 kJ/mol^{-1}
ΔH° (PS II membrane fragments) = 60 kJ/mol^{-1}.

Based on the relation

$$\ln K_{eq} = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \qquad (3)$$

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 $K_{eq} = 1$, 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

ΔS° (thylakoids) = 370 J K^{-1} mol^{-1}
ΔS° (PS II membrane fragments) = 240 J K^{-1} 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 $[F(t)-F_t/F_o$ provides a measure of $[Q_A^-(t)]$ (see ref. [9]). In general, $[Q_A^-(t)]$ exhibits an at least biphasic decay of the form

$$\frac{[Q_A^-(t)]-[Q_A^-]_t}{[Q_A^-(t=0)]-[Q_A^-]_t} = a\exp(-k_1 t) + (1-a)\exp(-k_2 t). \qquad (4)$$

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 degradation 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:

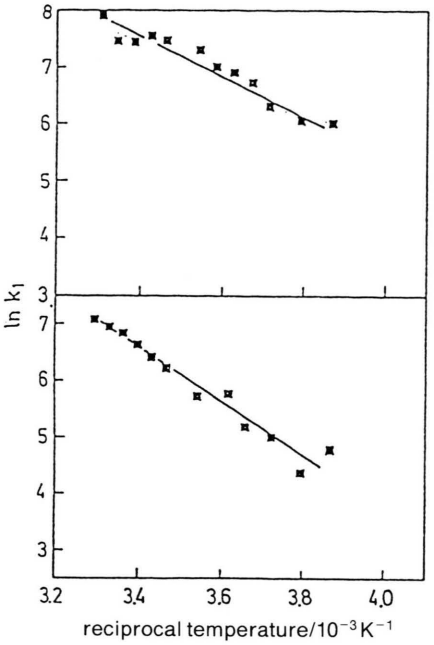

Fig. 4. Semilogarithmic plot of the rate constant k_1 of the fast relaxation kinetics of the transient fluorescence yield as a function of reciprocal temperature in isolated thylakoids (top) and PS II membrane fragments (bottom). For further details see text.

E_A (thylakoids) = 30 ± 5 kJ mol^{-1}
E_A (PS II membrane fragments) = 40 ± 10 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_v/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 μM range arise only for Cu^{2+}. The other cations are virtually without effect in this range in the case of PS II membrane frag-

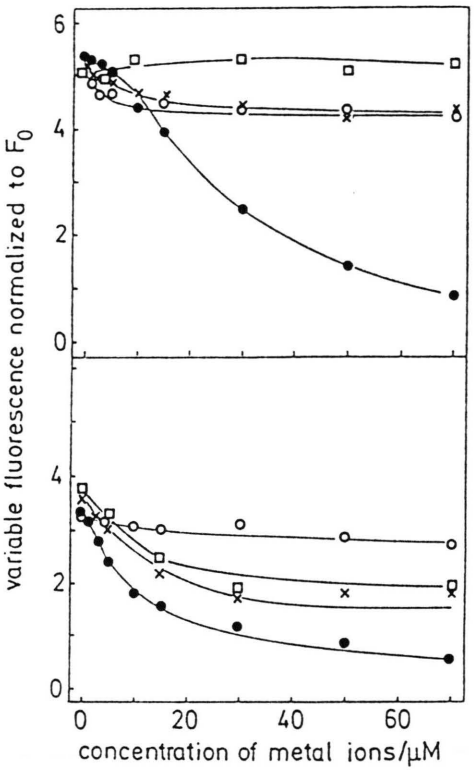

Fig. 5. Normalized variable fluorescence as a function of bivalent metal cations in PS II membrane fragments (top) and PS II core complexes (bottom). The symbols represent: Cu^{2+} (●), Zn^{2+} (X), Cd^{2+} (○) and Co^{2+} (□). Experimental details as described in Materials and Methods.

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 μM $CuCl_2$ 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 μM (in the case of PS II core complexes the I_{50} value is about 5 μM at the same total chlorophyll concentration of 20 μM). 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 $P680^+$. A more detailed analysis will be presented in a forthcoming paper.

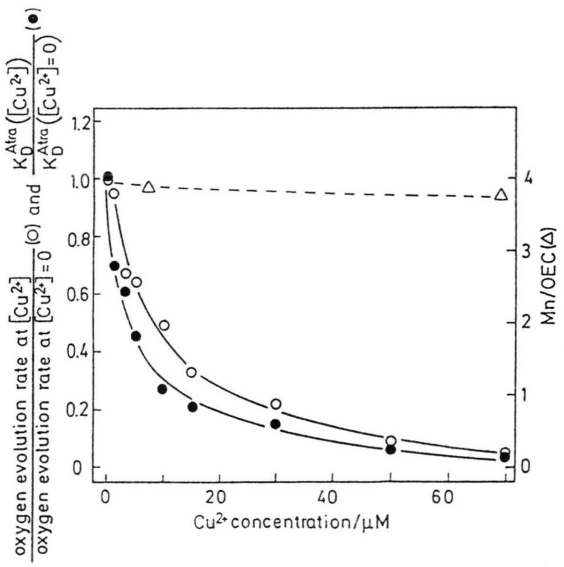

Fig. 6. Oxygen evolution rate (○) and calculated equilibrium constant (●) normalized to the control values at $[Cu^{2+}] = 0$ and manganese content as a function of $[Cu^{2+}]$ concentration. Experimental details as described in Materials and Methods.

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 ΔH° and ΔS° 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 μM 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_Z 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.

Acknowledgements

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