Effects of Potassium-(picrate)-(18-crown-6) on the Photosynthetic Electron Transport

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The effects of potassium-(picrate)-(18-crown-6) on the electron transport of photosystem II was investigated in isolated pea thylakoids. Low concentrations of the compound inhibited the fast decay of fluorescence yield associated with electron transfer between the primary (Q_A) and secondary (Q_B) quinone electron acceptor and increased the intermediary level of fluorescence to the F_{max} level. The decay half-time of fluorescence yield measured in the presence of DCMU ($S_2Q_A^-$ charge recombination) decreased from about 1.8 s to about 0.3 s in thylakoids treated with potassium-(picrate)-(18-crown-6). While the inhibition of electron transport by DCMU gave rise to the appearance of a thermoluminescence band at about +10°C ($S_2Q_A^-$charge recombination) addition of potassium-(picrate)-(18-crown-6) resulted in a thermoluminescence band at about -10°C. Increasing concentrations of potassium-(picrate)-(18-crown-6) diminished the fluorescence yield and the -10°C TL band and abolished the Signal II_s and Signal II_f EPR signals of the tyrosine-D and tyrosine-Z electron donors, respectively. The phenolic-type inhibitor, potassium picrate had the same effect on thermoluminescence and on the tyrosine EPR signals. It is concluded that potassium-(picrate)-(18-crown-6) is a phenolic type inhibitor owing to its picrate constituent. At low concentrations picrate and potassium-(picrate)-18-crown) not only block the electron transport between Q_A and Q_B but they probably decrease the midpoint redox potential of Q_A, as well. At high concentrations they also inhibit the light-induced oxidation of the tyrosine-D and tyrosine-Z donors.

Introduction

It has recently been shown that the potassium picrate complex of crown (K-pic-18-crown-6) is an inhibitor of the electron transport chain of PS II (Sabat *et al.*, 1991; Mohanty *et al.*, 1991). Electron transport rate and fluorescence experiments revealed that this complex reversibly affects oxygen evolution. It has been concluded that the inhibitory site of K-pic-18-crown-6 is probably located between the redox active tyrosine-Z donor and the reaction center chlorophyll, P680 (Sabat *et al.*, 1991). Since addition of chloride ion releaved the effect of K-pic-18-crown-6 it was suggested that the site of action of this complex is on the Cl^- pool in the thylakoid membranes. Potassium picrate, which is used for the synthesis of K-pic-18-crown-6, is a trinitrophenol compound and belongs to the family of phenolic-type inhibitors (Oettmeier, 1992; Bowyer *et al.*, 1991). Partial electron transport rate measurements and displacement experiments carried out with ^{14}C-labelled picric acid indicated an inhibition site for this compound at the reducing side of PS II similar or identical to that of diuron and phenolic inhibitors (Oettmeier and Masson, 1982). This suggests that the inhibitory effects of K-pic-18-crown-6 can be attributed to the picrate constituent. In the present work we reinvestigated the effects of K-pic-18-crown-6 on PS II. Thermoluminescence and EPR experiments re-

Abbreviations: ADRY, reagents which accelerate the deactivation reactions of the water-splitting system; D1 and D2, reaction center proteins of PS II; DCPIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea; DPC, 1,5-diphenylcarbazide; F_o, initial fluorescence; F_{max}, maximal fluorescence; MV, methyl viologen; P 680, reaction center chlorophyll of PS II; Q_A, primary quinone acceptor of PS II; Q_B, secondary quinone acceptor of PS II; Q band, TL band associated with $S_2Q_A^-$ charge recombination; B band, TL band associated with $S_2Q_B^-$ charge recombination; PS II, photosystem II; S_2 and S_3, oxidation states of the water-splitting system; TL, thermoluminescence.

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vealed that K-pic-18-crown-6 behaves like a phenolic inhibitor owing to its picrate constituent. It was found that at low concentrations K-pic-18-crown-6 and potassium picrate inhibited the electron transport between Q_A and Q_B and probably shifted the redox potential of Q_A to negative direction. At high concentrations they influenced the functions of the tyrosine-D and tyrosine-Z donors, as well.

Materials and Methods

Thylakoid membranes were isolated as in (Sabat *et al.*, 1991) from 1–2 month old spinach or 2–3 week old green peas grown in a green house. The membranes were suspended in a medium containing 0.4 M Sucrose/ 15 mM NaCl/ 5 mM $MgCl_2$ and 50 mM HEPES buffer of pH 7.5. Chemical treatments of thylakoid membranes were carried out by incubating the membranes for 3 min in the dark in the presence of various concentrations of chemicals. Oxygen-evolving PS II particles were isolated from pea thylakoids (Völker *et al.*, 1985). For manganese depletion the PS II particles were incubated at 0°C in 0.8 M Tris buffer at pH 8.0 for 30 min at a chlorophyll concentration of 50 μg/ml. The Tris-treated particles were washed two times in the suspension buffer and centrifuged for 45 min at 45 000×*g*.

The rate of photosynthetic oxygen evolution was measured at saturating light intensity by using a Hansatech oxygen measuring system. The assay medium contained 0.1 M D-sorbitol, 10 mM K_2HPO_4, 20 mM NaCl, 4 mM $MgCl_2$, 2 mM EDTA, 50 mM HEPES at pH 7.5 and thylakoids carrying 50 μg chlorophyll in a final volume of 1 ml. Different parts of the electron transport chain were studied by addition of electron acceptors and donors: 100 μM MV (PS I + PS II), 2 mM ascorbate/ 50 μM DCPIP/100 μM MV in the presence of 5 μM DCMU (PS I). The DCPIP-Hill activity was measured with the help of an Aminco spectrophotometer used in the split-beam mode. The DCPIP photoreduction was assayed by recording absorbance changes at 590 nm. In the assay, 50 μM DCPIP (from water to DCPIP) or 500 μM DPC and 50 μM DCPIP (from DPC to DCPIP) were added. The intensity of the red actinic light was 300 W m^{-2}.

Fluorescence induction and relaxation measurements were carried out in a PAM-101 spectrofluorometer.

TL glow curves were measured in a home-built apparatus similar to that described by Tatake *et al.* (1971) at a light intensity of 10 W m^{-2}. Following illumination the samples were heated in the dark at a rate of 20°C/min and the emitted TL light intensity was measured with an EMI 9558B photomultiplier. The signals were amplified by a differential amplifier and fed to an X-Y recorder. Electron paramagnetic resonance spectra were measured with a Brucker ECS-106 spectrometer. X-band spectra were recorded at room temperature with 9.45 GHz microwave frequency and 100 kHz modulation frequency. Data collection and processing were performed by using the EPR spectrometer data acquisition software.

Results

In order to find the action sites of K-pic-18-crown-6 partial electron transport rate measurements were carried out. Incubation of spinach thylakoid membranes with increasing concentrations of K-pic-18-crown-6 severely inhibited the whole-chain electron transport from water to the PS I acceptor, methylviologen (Fig. 1). The electron transport rate measured from reduced 2,6-dichlorophenolindophenol (DCPIP) to methyl viologen involving plastoquinone, the cytochrome *b/f* complex and PS I was insensitive to micromolar concentrations of K-pic-18-crown-6. However, the PS II electron transport reactions measured either from water to DCPIP or from diphenylcarbazide (DPC) to DCPIP were almost completely inhibited by 10 μM K-pic-18-crown-6. Since DPC donates electrons directly to the tyrosine-Z donor and DCPIP accepts electrons at Q_B we can conclude, in agreement with Sabat *et al.* (1991), that K-pic-18-crown-6 inhibits PS II electron transport in this section of the electron transport chain. Although Sabat *et al.* (1991) could not find any inhibitory effect of potassium picrate (used for synthesis of K-pic-18-crown-6) we observed almost complete inhibition of PS II electron transport (from water to phenyl-*p*-benzoquinone) in the presence of 10 μM potassium picrate (not shown). On the other hand 10 μM 18-crown-6 did not affect the electron transport. Consequently, we suggest that the picrate constituent is responsible for the inhibitory effect of K-pic-18-crown-6.

The inhibitory site of K-pic-18-crown-6 was more precisely determined by fluorescence relax-

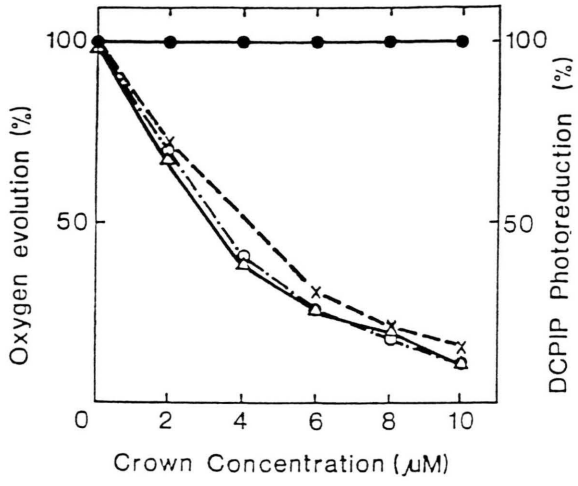

Fig. 1. Effect of K-picrate-18-crown-6 on various partial electron transport reactions. x--x, water to MV; Δ--Δ, water to DCPIP; o--o, DPC to DCPIP; ●--●, $DCPIPH_2$ to MV. The assay medium contained 0.1 M D-sorbitol, 10 mM K_2HPO_4, 20 mM NaCl, 4 m $MgCl_2$, 2 mM EDTA, 50 mM HEPES pH 7.5 and thylakoids carrying 50 μM chlorophyll in 1 ml. Concentrations of the acceptors and donors were: 100 μM MV, 50 μM DCPIP/2 mM ascorbate, 500 μM DPC. 100% rates in the $H_2O \rightarrow MV$ and $DCPIPH_2 \rightarrow MV$ reactions were 220 and 960 μmol O_2 consumed per mg chlorophyll per h, respectively. Control rates in the $H_2O \rightarrow$ DCPIP and DPC → DCPIP photoreductions were 170 and 190 μmol of DCPIP (mg of Chl)$^{-1}$ h^{-1}, respectively.

ation measurements. In uninhibited thylakoids the decay of fluorescence yield apparently consisted of two components (Fig 2, curve A). The fast one is characteristic of the Q_A to Q_B electron transfer and decays in some hundred microseconds. The slow one can be attributed to backreaction of Q_A^- with the positively charged donor side of PS II. When the electron transport was inhibited between Q_A and Q_B by DCMU the reduced Q_A molecules underwent charge recombination with the S_2 state of the water-splitting system. Consistently, in the presence of DCMU the fast relaxing component completely disappeared and only the slow component was observed in the decay curve (Fig. 2, curve D). The effect of K-pic-18-crown-6 on the decay curve was similar to that of DCMU. After addition of 5 μM K-pic-18-crown-6 the amplitude of the fast component was greatly reduced and the contribution of the slow component increased (Fig. 2, curve B). 10 μM K-pic-18-crown-6 almost completely eliminated the fast component with a concomitant enhancement of the amplitude of the slow component (Fig. 2, curve C). Potassium picrate had the same effect on fluorescence relaxation as K-pic-18-crown-6 (not shown). Thus, at low concentrations both K-pic-18-crown-6 and potassium picrate inhibit the electron transport at the acceptor side of PS II between Q_A and Q_B.

The results of fluorescence relaxation measurements were confirmed by fluorescence induction measurements. Upon DCMU addition the intermediary level of fluorescence, F_i was lifted to the F_{max} level converting the slow rise of fluorescence, characteristic of uninhibited thylakoids (Fig. 3, curve E, solid line), into an accelerated rise (Fig. 3, curve E, dashed line). Similarly to DCMU, 5 μM K-pic-18-crown-6 also enhanced the F_i level of fluorescence (Fig. 3, curve F) indicating an inhibition of electron transport between Q_A and Q_B. However, 10 μM K-pic-18-crown-6 (Fig. 3, curve G) not only lifted the F_i level almost to the F_{max} level but also decreased the F_{max} level. This phenomenon suggests an inhibition of electron trans-

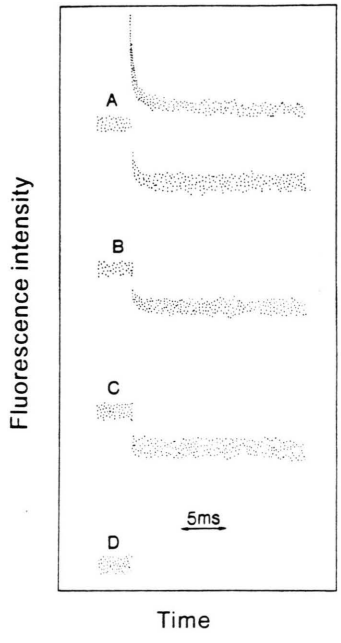

Fig. 2. Fluorescence relaxation kinetics of green pea thylakoids following a single turnover xenon flash. A: control thylakoids; B: 5 μM K-picrate-18-crown-6; C: 10 μM K-picrate-18-crown-6; D: 10 μM DCMU. The thylakoids were suspended in the suspension buffer at a chlorophyll concentration of 10 μg/ml.

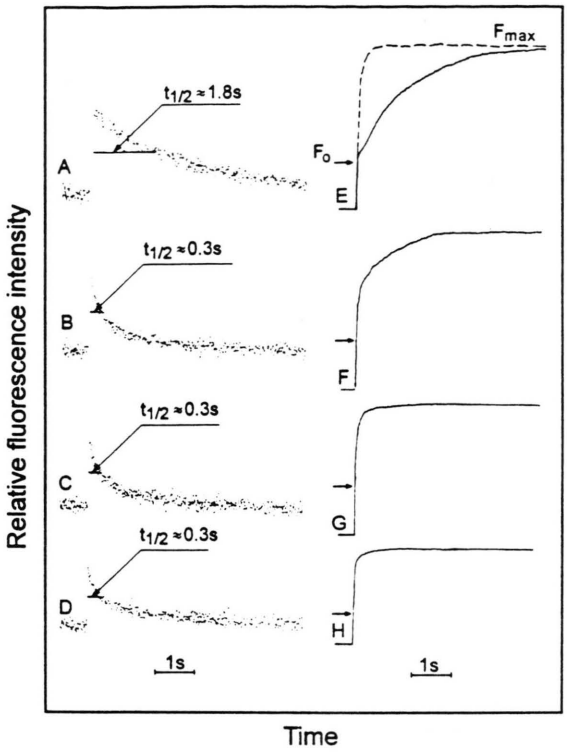

Fig. 3. Fluorescence relaxation kinetics (left hand side) and fluorescence induction transients (right hand side) of pea thylakoids as a function of K-pic-18-crown-6 concentration. A and E: treated with 10 μM DCMU (solid line represents control thylakoids); B and F: 5 μM K-pic-18-crown-6; C and G: 10 μM K pic-18-crown-6; D and H: 200 μM K-pic-18-crown-6. Chlorophyll concentration of the samples is 10 μg/ml.

port not only at the acceptor but at the donor side of PS II, as well. At a K-pic-18-crown-6 concentration of 200 μM the F_o level also began to decrease (Fig. 3, curve H). Potassium picrate had the same effects on the fluorescence induction transients as potassium-(picrate)-(18-crown-6) (not shown).

Although both 10 μM DCMU and 5 μM K-pic-8-crown-6 lifted the F_i level of fluorescence almost to the same F_{max} level the corresponding decay half-times in the second time region were considerably different. The decay half-time of fluorescence yield attributed to the interaction of Q_A^- and the S_2 state of water-splitting system was about 1.8 s in the DCMU-treated thylakoids (Fig. 3, curve A). In the presence of 5 μM K-pic-18-crown-6 the decay half-time decreased to about 0.3 s (Fig. 3, curves B, C and D) indicating a decrease in the redox distance between the interacting negatively charged acceptor and positively charged donor molecule. Similarly as in fluorescence induction (Fig. 3, curve H), 200 μM K-pic-18-crown-6 considerably quenched the fluorescence decreasing the overall fluorescence yield (Fig. 3, curve D).

The thermoluminescence method proved to be a very sensitive technique for investigation of the effects of inhibitors on electron transport (Sane and Rutherford, 1986; Demeter and Govindjee, 1989; Vass and Inoue, 1992). Therefore, we investigated the effects of K-pic-18-crown-6 and potassium picrate on the thermoluminescence of spinach thylakoids. In the thermoluminescence glow curve of uninhibited thylakoids the main TL band (designated as B band) appeared at about + 30°C (Fig. 4, curve A, solid line). The B band originates from charge recombination occurring between the positively charged S_2/S_3 states of the water-splitting system and the negatively charged secondary quinone acceptor, Q_B^- (Rutherford *et al.*, 1982;

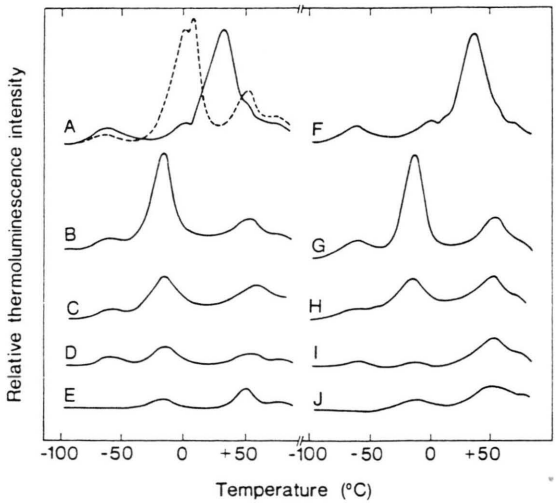

Fig. 4. Effect of K-pic-18-crown-6 on the thermoluminescence of pea thylakoids. A: control (solid line); treated with 10 μM DCMU (dashed line); B: treated with 10 μM K-pic-18-crown-6; C: treated with 10 μM K-pic-18-crown-6 and 10 μM DCMU; D: treated with 500 μM K-pic-18-crown-6; E: treated with 10 μM K-pic-18 crown-6 and excited at 77 K for 30 s; F: treated with 10 μM 18 crown-6; G: treated with 10 μM K-picrate; H: treated with 10 μM K-picrate and 10 μM DCMU; I: treated with 500 μM K-picrate; J: treated with 10 μM K-picrate and excited at 77 K for 30 s. Thermoluminescence was excited (except curves E and J) by illuminating the samples (50 μg Chl/0.4 ml) with continuous white light for 30 s at -80°C. Following illumination samples were heated in the dark with a heating rate of 20°C/min.

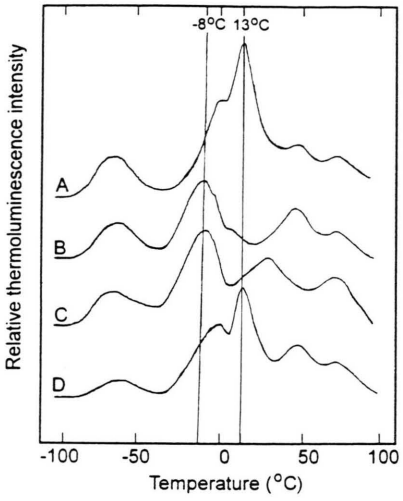

Fig. 5. Displacement of picrate by DCMU at the binding site. A: treated with 10 μM DCMU; B: treated with 10 μM picrate; C: treated with 1 μM picrate; D: treated with 1 μM picrate and 20 μM DCMU. Samples contained 50 μg Chl/0.4 ml and were excited at -80°C with continuous white light for 30 s. Thermoluminescence was measured at a heating rate of 20 °C/min.

Demeter and Vass, 1984). After DCMU addition the B band was replaced by an other band appearing at about 10°C (Fig. 4, curve A, dashed line). This band is designated as Q band and originates from $S_2Q_A^-$ charge recombination (Rutherford *et al.*, 1982; Demeter and Vass, 1984). We note, that the trough at the top of the band at about 0°C is a distortion caused by the solid-liquid phase transition of water (Vass *et al.*, 1981). The appearance of the Q band in the glow curve at about +10°C is characteristic of the effect of urea/triazine type inhibitors on the thermoluminescence of thylakoids (Droppa *et al.*, 1981; Vass and Demeter, 1982). 10 μM K-pic-18-crown-6 also abolished the B band with a simultaneous appearance of a band at about – 10°C (Fig. 4, curve B). The effect of 10 μM potassium picrate was the same as that of 10 μM K-pic-18-crown-6 (Fig. 4, curve G). In contrast to this, 18-crown-6 (used for the synthesis of K-pic-18-crown-6) did not influence the appearance of the B band (Fig. 4, curve F). These observations substantiate that the picric constituent can be accounted for the effect of K-pic-18-crown-6 on thermoluminescence.

If the excitation of TL occurred at 77 K, that is below the treshold temperature of the S_2 state formation, the -10°C band could not be charged (Fig. 4, curves E and J) suggesting that the water-splitting system may participate in the generation of the -10°C band.

Similarly as it was observed in fluorescence (Fig. 3, curves D and H), increasing concentrations of K-pic-18-crown-6 or potassium picrate quenched the thermoluminescence resulting in a decrease in the amplitude of the TL band at -10°C. 500 μM K-pic-18-crown-6 or 500 μM potassium picrate almost completely eliminated the – 10°C band (Fig. 4, curves D and I, respectively).

Addition of DCMU to the potassium picrate-treated sample (1 μM picrate plus 20 uM DCMU; Fig. 5, curve D) replaced the -10°C band (appear-

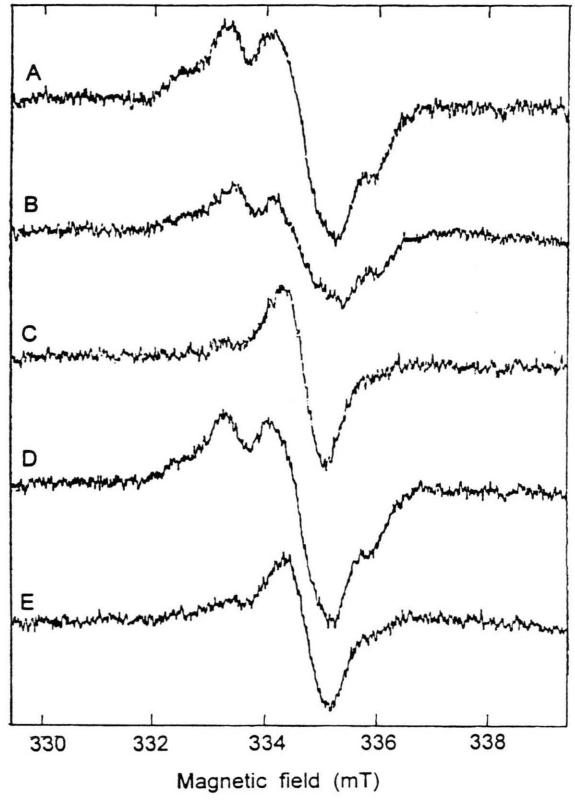

Fig. 6. Effect of K-pic-18-crown-6 and its constituents on the EPR signals of tyrosine-Z and tyrosine-D donors in manganese depleted PS II particles. A: control in the light (sum of the tyrosine-Z and tyrosine-D signals); B: control after 2 min dark incubation (tyrosine-D signal); C: treated with 1 mM K-pic-18 crown-6; D: treated with 1 mM 18-crown-6; E: treated with 1 mM K-picrate. Samples contained 3.5 mg Chl/ml. Conditions for EPR measurements: Room temperature, microwave power 10 mW, modulation amplitude 2 G, modulation frequency 100 kHz.

ing at -8°C in the presence of 1 μM picrate; Fig. 5, curve C) with a band appearing at about +13°C band characteristic of DCMU treatment (Fig. 5, curve A). Similarly, the +13°C band induced by 1 μM DCMU was exchanged to the -8°C band after addition of 20 μM K-pic-18-crown-6 (not shown). These observations indicate that picrate can displace DCMU from its binding site and vice versa. It is of note, that at high concentrations of picrate (more than 10 μM) addition of DCMU did not result in the replacement of the -8°C band with the +13°C band but decreased the amplitude of the -8°C band (not shown).

The effects of K-pic-18-crown-6 and potassium picrate on fluorescence and thermoluminescence suggest that at high concentrations they also have an inhibitory site at the donor side of PS II. Fig. 6, curve A shows the sum of the EPR signals of the oxidized tyrosine-D and tyrosine-Z donors in Tris-treated PS II particles during illumination of the sample at room temperature. In the dark the EPR signal of tyrosine-Z^+ relaxed very quickly and only the EPR signal of the oxidized tyrosine-D donor could be observed (Fig. 6, curve B). Both 500 μM K-pic-18-crown-6 or potassium picrate abolished the tyrosine-D and tyrosine-Z signals and only an unidentified radical signal remained in the EPR spectrum (Fig. 6, curves C and E, respectively). A similar radical was observed earlier in the presence of the phenolic inhibitor, dinoseb (Rutherford *et al.*, 1984). 18-crown-6 had not any effect on the tyrosine signals (Fig. 6, curve D).

Discussion

In the present study we found that K-pic-18-crown-6 inhibits the photosynthetic electron transport between the donation site of DPC and the acceptory site of 2,6-dichlorophenol indophenol, that is between tyrosine-Z and Q_B. A more precise localization of the inhibitory sites was achieved by fluorescence relaxation measurements. Low concentrations of K-pic-18-crown-6 abolished the fast decaying component of fluorescence yield characteristic of the $Q_A \rightarrow Q_B$ electron transfer suggesting an inhibition of electron transport at the acceptor side of PS II between Q_A and Q_B. Fluorescence induction measurements confirmed this conclusion. Similarly to the effect of DCMU, the rise of fluorescence yield was accelerated by the addition of K-pic-18-crown-6 indicating a fast accumulation of electrons in the Q_A pool. Thus, at low concentrations (5–10 μM), K-pic-18-crown-6 (or potassium picrate) inhibits the electron transport of PS II between Q_A and Q_B. At the same concentrations the F_i level of fluorescence was lifted to the F_{max} level indicating an active water-splitting system at the donor side of PS II.

Due to the inhibition of the $Q_A \rightarrow Q_B$ electron transfer in the presence of K-pic-18-crown-6 light excitation can induce only one transition of PS II during which the water-splitting system is converted from the S_1 to the S_2 state and one electron is transferred to Q_A. Hence, it follows that the -10°C TL band appearing after the addition of K-pic-18-crown-6 can be assigned to $S_2Q_A^-$ charge recombination. In the presence of DCMU the $S_2Q_A^-$ charge recombination (designated as Q band) resulted in a TL band at about +10°C (Fig. 4A, dashed line). This means, that the redox distance between the interacting negatively charged acceptor (Q_A^-) and the positively charged donor (S_2) undergoing charge recombination is smaller in the presence of K-pic-18-crown-6 than in the DCMU-treated membrane. The discrepancy can be explained by assuming that either the redox potential of Q_A became more negative or the redox potential of the S_2 state increased after K-pic-18-crown-6 addition. Another possibility that either the donor or acceptor component of the $S_2Q_A^-$ redox couple was replaced by another electron transport component decreasing the redox span between the interacting components.

Addition of 20 μM DCMU to the 1 μM picrate-treated thylakoids resulted in the replacement of the -10°C band with the +10°C band and vice versa. The displacement experiments suggest overlapping binding sites of the two compounds in the Q_B binding niche at the acceptor side of PS II. The same conclusion was also reached by Oettmeier and Mason (1982) who observed that the ^{14}C-labelled picric acid is competitively displaced from the thylakoid membrane by DCMU and the phenolic inhibitor, ioxynil. It is unlikely that displacement of a compound by an other in the Q_B binding niche at the acceptor side of PS II would result in a modification not only at the acceptor but at the donor side of PS II, as well. Therefore, we suggest, that the appearance of the Q band at about +10°C and -10°C in the presence of DCMU and K-pic-18-

crown, respectively, can be attributed to different modifications in the redox state of Q_A caused by inhibitor binding.

It has been reported that the DCMU/triazine type inhibitors bind to the D1 protein, but the binding domain of the phenolic-type inhibitors involves the CP 47 chlorophyll-protein complex and an unidentified 41 kDa protein, as well (Oettmeier, 1992; Oettmeier *et al.*, 1982). The phenolic inhibitors displace the bicarbonate anion at the non-heme iron and convert the $g = 1.9$ EPR form of the quinone-iron complex to the $g = 1.82$ form (Bowyer *et al.*, 1991; Rutherford *et al.*, 1984). It can be assumed that binding of phenolic inhibitors can cause a decrease in the midpoint oxidation-reduction potential of Q_A, which is reflected in a lower peak temperature of the Q TL band than that observed in the presence of DCMU-type inhibitors. A shift in the midpoint redox potential of Q_A to negative direction can also explain the shortened decay half-time of fluorescence relaxation observed in the presence of K-pic-18-crown-6 (0.3 s) as compared to that of DCMU-treated membranes (1.8 s).

The above explanation concerning the variability in the peak position of the Q TL band have already been reported (Droppa *et al.* 1981; Vass and Demeter, 1982). Taking into account that the phenolic inhibitors can also influence the donor side of PS II (Bowyer *et al.*, 1991; van Assche, 1984) an alternative explanation can be provided for the origin of the -10°C thermoluminescence band and for the shortened decay half-time of fluorescence yield observed in picrate- and K-pic-18-crown-6-treated thylakoids. Assuming that at low concentrations picrate inhibits the formation of the S_2-state but does not inhibit the light-induced oxidation of the tyrosine-Z donor we can assign the -10°C TL band to charge recombination of Q_A^- with the oxidized tyrosine-Z donor. The midpoint redox potential of tyrosine-Z is more positive than that of the S_2 state thus the redox span is smaller for the tyrosine-$Z^+Q_A^-$ redox couple (associated with the -10°C TL band) than that of the $S_2Q_A^-$ redox pair (responsible for the Q band at +10°C). Our second explanation concerning the origin of the -10°C TL band is in correlation with EPR observations showing that in Tris-washed chloroplasts where the water-splitting sytem is impaired the charge recombination half-time of Q_A^- with the oxidized tyrosine-Z is 0.3 s (Yerkes *et al.*, 1983). This value agrees well with the decay half-time of the fluorescence yield (charge recombination of Q_A^- with the positively charged donor side of PS II) observed in the presence of K-pic-18-crown-6 or potassium picrate (Fig. 3). However, we note, that an unambiguous determination of the origin of the -10°C TL band would require parallel EPR and TL measurements of the S_2 state multiline signal and the -10°C TL band, respectively, as a function of picrate concentration. If the -10°C band is indeed associated with $S_2Q_A^-$ charge recombination then the same picrate concentration which is favourable for the induction of the -10°C band should not diminish or abolish the S_2 multiline signal.

At higher concentrations of K-pic-18-crown-6 or potassium picrate the decrease of the F_{max} level of fluorescence and the diminished TL band at -10°C indicate an effect of these inhibitors on the donor side of PS II, as well. However, the inhibition of electron transport between Q_A and Q_B, the acceleration of fluorescence rise and decceleration of fluorescence decay preceeded the decrease of the F_{max} and F_o levels of fluorescence. The transformation of the B band into the -10°C band was also completed at much lower K-pic-18-crown-6 concentration than that required to decrease the F_{max} level. Thus the acceptor side of PS II is inhibited at much lower concentration of K-pic-18-crown-6 or picrate than the donor side.

The decrease of F_{max} and F_o fluorescence levels at high concentrations of K-pic-18-crown-6 resembles the effect of ADRY reagents on fluorescence induction (McCauley, 1987). Chemicals which accelerate the deactivation of the charge storage S states of the water-splitting complex, Y (Renger and Inoue, 1983; Renger *et al.*, 1973; Schenck *et al.*, 1982; Babcock and Sauer, 1973) are referred to as ADRY reagents. It has been suggested that ADRY reagents and protonophores might induce a cyclic electron transport around PS II via cytochrome b-559 by oxidizing plastoquinone and other acceptor components of PS II and reducing the donor side (Arnon and Tang, 1988). A cyclic electron transport can drain electrons away from Q_A resulting in a quenching of the F_{max} level of fluorescence (Fig. 3). At low concentrations the ADRY reagents are electron donors to the S_2 and S_3 states of the water-splitting system causing their

fast relaxation to the S_0 and S_1 states. Reduction of the S_2 and S_3 states to the S_0 and S_1 states can be reflected in the disappearance of the B, Q and -10°C TL bands, as it was observed in the present work and by Renger and Inoue (1983) as well. At high concentrations the ADRY reagents can probably reduce not only the positively charged S_2 and S_3 states but the oxidized tyrosine-D and tyrosine-Z donors, as well. It has been reported that the phenolic-type dinoseb abolished Signal II_s associated with the oxidized radical of tyrosine-D on the D2 protein of PS II (Rutherford *et al.*, 1984). CCCP also accelerated the decay of Signal II_f, the EPR signal of tyrosine-Z (Yerkes *et al.*, 1983). The abolishment of the tyrosine EPR signals at higher potassium picrate or K-pic-18-crown-6 concentrations can be explained by the ADRY character of these compounds. The failure of DPC to restore electron transport and fluorescence yield can also be attributed to the inhibition of the action of tyrosine-Z in thylakoids treated with K-pic-18-crown-6.

The present comparative electron transport rate, fluorescence, TL and EPR experiments demonstrate that K-pic-18-crown-6 behaves like a phenolic-type inhibitor and its effects on photosynthetic electron transport can be attributed to its phenolic-type, trinitrophenol constituent. On the other hand 18-crown-6 has not any effect on the electron transport.

On the basis of the observations we can conclude that at low concentrations K-pic-18-crown-6 and potassium picrate reduce the redox potential of Q_A and interrupt the electron transport at the acceptor side of PS II between Q_A and Q_B. At high concentrations these chemicals inhibit the light-induced oxidation of the tyrosine-D and tyrosine-Z donors at the donor side of PS II.

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Arnon D. I. and Tang G. M.-S. (1988), Cytochrome *b*-559 and proton conductance in oxygenic photosynthesis. Proc. Natl. Acad. Sci. USA **85,** 9524–9528.

van Assche C. J. (1984), Photosynthetic electron transport inhibitors: some problems related to an accurate determination of the molecular site of action. Z. Naturforsch. **39c,** 338–341.

Babcock G. T. and Sauer K. (1973), Electron paramagnetic resonance signal II in spinach chloroplasts II. Alternative spectral forms and inhibitor effects on kinetics of Signal II in flashing light. Biochim. Biophys. Acta **325,** 504–519.

Bowyer J. R., Camilleri P. and Vermaas W. F. J. (1991), Photosystem II and its interaction with herbicides. In: Herbicides (N. R. Baker and M. P. Percival, eds.). Elsevier, Amsterdam, pp. 27–85.

Demeter S. and Govindjee (1989), Thermoluminescence from plants. Physiol. Plantarum **75,** 121–130.

Demeter S. and Vass I. (1984), Charge accumulation and recombination in photosystem II studied by thermoluminescence. I. Participation of the primary acceptor Q and secondary acceptor B in the generation of thermoluminescence of chloroplasts. Biochim. Biophys Acta **764,** 24–32.

Droppa M., Horváth G., Vass I. and Demeter S. (1981), Mode of action of photosystem II herbicides studied by thermoluminescence. Biochim. Biophys. Acta **638,** 210–216.

McCauley S. W., Melis A., Tang G. M. S. and Arnon D. I. (1987), Protonophores induce plastoquinol oxidation and quench chloroplast fluorescence: evidence for a cyclic, proton conducting pathway in oxygenic photosynthesis. Proc. Natl. Acad. Sci. USA **84,** 8424–8428.

Mohanty P., Sabat S. C. and Padhye S. (1991), Chemical probes for water oxidation complex in photosystem II. Reversible chloride anion pairing by K (picrate) (18-crown-6). In: Proc. Indo-US Workshop, New Delhi, January 8–12. Oxford & IBH Publishing Co., New-Delhi, Bombay, Calcutta, pp. 335–348.

Oettmeier W. (1992), Herbicides of photosystem II. In: The Photosystems: Structure, Function and Molecular Biology (J. Barber, ed.). Elsevier, Amsterdam, pp. 349–408.

Oettmeier W. and Masson K. (1982), Picrate as an inhibitor of photosystem II in photosynthetic electron transport. Eur. J. Biochem. **122,** 163–167.

Oettmeier W., Masson K. and Johanningsmeier U. (1982), Evidence for two different herbicide-binding proteins at the reducing side of photosystem II. Biochim. Biophys. Acta **679,** 376–383.

Renger G., Bouges-Bocquet B. and Delosme R. (1973), Studies on the ADRY agent-induced mechanism of the discharge of the holes trapped in the photosynthetic watersplitting enzyme system Y. Biochim. Biophys Acta **292,** 796–807.

Renger G. and Inoue Y. (1983), Studies on the mechanism of ADRY agents (agents accelerating the deactivation reactions of water-splitting enzyme system Y) on thermoluminescence emission. Biochim. Biophys Acta **725,** 146–154.

Rutherford A. W., Crofts A. R. and Inoue Y. (1982), Thermoluminescence as a probe of photosystem II photochemistry. The origin of the flash-induced glow peaks. Biochim. Biophys Acta **682,** 457–465.

Rutherford A. W., Zimmermann J. L. and Mathis P. (1984), The effect of herbicides on components of the PS II reaction centre measured by EPR. FEBS Lett. **165,** 156–162.

Sabat S. C., Vijayavergiya V., Tripathy B. C. and Mohanty P. (1991), Inhibitory effect of crown compound on photoelectron transport activity of beet spinach thylakoid membranes. Z. Naturforsch. **46c,** 87–92.

Sane P. V. and Rutherford A. W. (1986), Thermoluminescence from photosynthetic membranes. In: Light Emission of Plants and Bacteria (Govindjee, J. Amesz and D. C. Fork eds.). Elsevier, Amsterdam, pp. 259–294.

Schenck C. C., Diner B. A., Mathis P. and Satoh K. (1982), Flash induced carotenoid radical cation formation in photosystem II. Biochim. Biophys. Acta **680,** 216–227.

Tatake V. G., Desai T. S. and Battacharjee S. K. (1971), A variable temperature cryostate for thermoluminescence studies. J. Phys. E. Sci. Instr. **4,** 755–757.

Vass I. and Demeter S. (1982), Classification of photosystem II inhibitors by thermodynamic characterization of the thermoluminescence of inhibitor-treated chloroplasts. Biochim. Biophys. Acta **682,** 496–499.

Vass I., Horváth G., Herczeg T. and Demeter S. (1981), Photosynthetic energy conservation investigated by thermoluminescence. Activation energies and half-lives of thermoluminescence bands of chloroplasts determined by mathematical resolution of glow curves. Biochim. Biophys. Acta **634,** 140–152.

Vass I. and Inoue Y. (1992), Thermoluminescence in the study of photosystem II. In: The Photosystems: Structure, Function and Molecular Biology (J. Barber, ed.). Elsevier, Amsterdam, pp. 259–294.

Völker M., Ono T., Inoue Y. and Renger G. (1985), Effect of trypsin on PS II particles, correlation between Hill-activity, Mn-absorbance and peptide pattern. Biochim. Biophys. Acta **806,** 25–34.

Yerkes C. T., Babcock G. T. and Crofts A. R. (1983), A Tris induced change in the midpoint potential of Z, the donor to photosystem II, as determined by the kinetics of the back reaction. FEBS Lett. **158,** 359–363.