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Studies on the reaction mechanism of tetraphenylboron at the Photosystem II donor side in isolated spinach chloroplasts

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In the present study the effect of low tetraphenylboron (TPB^-) concentrations on the flash-induced oxygen yield pattern in dark-adapted spinach chloroplasts has been analysed. The following was found. (1) TPB^- at a concentration of less than ten molecules per Photosystem II strongly damps the characteristic oscillation pattern. (2) TPB^- induces an accelerated decay of S_2 and S_3 that is concentration dependent. In contrast to the acceleration of the deactivation reactions of the water-splitting enzyme system Y agent (ADRY agent) 2-(3-chloro-4-trifluoromethyl)-anilino-3,5-dinitrothiophene (ANT 2p) the acceleration by TPB^- disappears after preflashing. (3) TPB^- does not induce a bivalent reduction of S_2 and S_3 of the type $S_{i+2} + TPB^- \rightarrow S_1$ + oxidation products. (4) The effect of TPB^- on the decay kinetics of S_2 and S_3 can be quantitatively described by a mobile-type mechanism as discussed recently for ANT 2p (Hanssum B., Dohnt G. and Renger, G. (1985) Biochim. Biophys. Acta 806, 210–220) if one additionally accounts for the irreversible oxidative consumption of TPB^-. (5) Cyclic voltammetry in buffer solution reveals that TPB^- becomes irreversibly oxidized with redox potential of the order of +0.7 and +1.0 V, respectively. Similar measurements with different 2-anilino-3,5-dinitrothiophene derivatives support the idea that also ANT 2p becomes irreversibly oxidized. Based on these data the mechanism of TPB^- and ADRY agents on the donor side of system II is discussed.

Introduction

Photosynthetic water oxidation to molecular oxygen occurs via a four-step univalent reaction sequence that is catalyzed by a manganoprotein refered to as water-oxidizing enzyme system (for a recent review, see Ref. 1). The kinetics of these reactions are known, but the mechanistic details, especially the chemical nature of the intermediate redox states as well as the structure of the water-oxidizing enzyme system are highly unresolved questions. One way to attack the problem is a well-defined modification of the reaction pattern by selectively acting agents. This permits a kind of 'specific labeling' of characteristic properties of the different redox states. Among these types of substances ADRY agents [2] are of special interest, because they were found to affect very specifi-

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Abbreviations: ANT 2a, 2-(4-chloro)anilino-3,5-dinitrothiophene; ANT 2f, 2-(4-dimethylamino)anilino-3,5-dinitrothiophene; ANT 2p, 2-(3-chloro-4-trifluoromethyl)anilino-3,5-dinitrothiophene; NH_2OH, hydroxylamine; PS II, Photosystem II; P-680, photoactive chlorophyll *a* complex of Photosystem II; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride; TPB^-, tetraphenylboron; ADRY, acceleration of the deactivation reactions of the water-splitting enzyme system Y; Cyt, cytochrome.

cally the lifetimes of S_2 and S_3 [3], which represent those redox states of the water-oxidizing enzyme system that are caused by the accumulation of two and three oxidzing redox equivalents, respectively. Previous studies led to the conclusion that ADRY agents do no simply lower the overall activation-energy barrier of the reaction coordinate of the endogenous S_2 and S_3 decay, but, moreover, open a pathway for funneling electrons from a not yet identified internal donor to S_2 and S_3 [3]. Additionally, ADRY agents were inferred to act as mobile catalysts [4]. This mode of action is highly supported by a recent kinetic analysis [5]. However, the mechanistic details of the ADRY agent-induced destabilization of S_2 and S_3 are unknown. Two experimental findings appear to be relevant for further considerations about the mode of action of ADRY agents: (a) ADRY agents enhance the reduction rate of the oxidized donor component D_1^{ox} (or Z^{ox}) in chloroplasts that are completely deprived of their oxygen-evolving capacity [6,7], and (b) the formation of lipophilic anions seems to be essential for the ADRY effect [8]. Based upon the latter property the ADRY effect was inferred to be essentially electrostatic in its origin [9]. According to this idea, the anion form affects the redox potential of an intermediary donor component, which is involved in the ADRY-agent-catalyzed reaction pathway of accelerated S_2 and S_3 decay. As a consequence any lipid soluble anion was predicted to function as ADRY agent, and especially tetraphenylboron (TPB^-) was referred to as a 'super-ADRY compound' [9]. On the other hand, TPB^- is known to act simultaneously as a powerful PS II electron donor [10,11], which in contrast to many other compounds (diphenylcarbazide, hydroquinone/ascorbate, etc.) also competes in normal chloroplasts very efficiently with the water-oxidizing enzyme system for redox equivalents produced photochemically by P-680 [11]. Therefore one might conclude that the ADRY agents undergo a transient oxidation-reduction cycle, so that the ADRY effect would simply be caused by a redox-type catalytic effect. In this case the ADRY agents would be oxidized by S_2 and S_3 directly or via $D_1^{ox}(Z^{ox})$ and rapidly rereduced by an endogenous donor component, because the most powerful ADRY substances catalyze several hundreds of turnovers per ADRY molecule [6,12]. In contrast to that, TPB^- becomes irreversibly oxidized. Based on the redox properties of TPB^-, two interesting mechanistic aspects have to be considered for its reaction with the PS II donor side.

(a) The oscillation pattern of the O_2-evolution could be shifted in a very definite and well-controlled manner, analogously to the well-known effect of low hydroxylamine concentrations [13]. This could occur if TPB^- binds with sufficiently high affinity to the PS II donor side and in addition, reacts rapidly enough with oxidizing redox equivalents produced by P-680 photooxidation, so that an advancement of the S_i clock is prevented stoichiometrically.

(b) As TPB^- was found to undergo electrochemically an irreversible two-electron oxidation [14], one could ask whether TPB^- provides a tool for bivalent decay processes $S_3 \rightarrow S_1$ and $S_2 \rightarrow S_0$, similar to corresponding reactions in algae that are inferred to occur via an internal component C [15].

Accordingly, the present study was performed in order to analyze the effect of TPB^- on the reaction pattern of system II in normal chloroplasts with intact water-oxidizing enzyme system. Oxygen yield measurements were carried out to show whether TPB^- at a concentration in the range of 1–5 TPB^- molecules per system II effectively competes with the S_i-state advancement or whether it modifies the decay kinetics of S_2 and S_3 in a similar way as real ADRY agents. Additionally, cyclic voltammetry with TPB and ANT 2p was performed as an attempt to clarify whether ADRY agents really act as redox catalysts in accelerating the S_2 and S_3 decay.

Materials and Methods

Isolation of thylakoids from spinach (*Spinacea oleracea*) was performed as in Ref. 11 except for the centrifugation procedure. Thylakoids were centrifuged at $8000 \times g$ for 30 s in both steps. The number of intact water-oxidizing enzyme systems was found to be 1 per approx. 500 chlorophylls, as determined by conventional flash-induced yield measurements [2].

Oxygen was detected with an unmodulated Joliot-type oxygen electrode [17]. The thylakoid suspension (chlorophyll concentration, approx. 1

mM) on the Pt-electrode was separated by a single dialysis membrane from the upper buffer compartment containing the Ag|AgCl reference electrode. Thylakoids were isolated, stored on ice and transferred to the electrode in darkness. After 5 min for equilibration on the electrode, the thylakoids were illuminated with a series of saturating flashes (10 μs, half-width) using a Stroboslave 1539-A (General Radio). The signals were digitally recorded with a Nicolet Explorer III storage oscilloscope. The amplitudes were used to indicate the amount of oxygen produced. For calculations on the relative S_i state concentrations according to the Kok model [18], the procedure described extensively in Ref. 5 was used. The thylakoid suspension was directly mixed with TPB^- before being used in the experiment. The buffer solution contained 10 mM NaCl/5 mM $MgCl_2$/0.3 M sorbitol/50 mM Tricine-NaOH (pH = 7.5). The cyclic voltammetry was performed with a polarographic analyzer No. 174A (Princeton Applied Research, PAR) in combination with an universal programmer No. 175 (PAR). The scan rates varied from 200 to 400 mV/s. For measuring the oxidation potentials a glassy carbon electrode (Tecator) was used with an Ag|AgCl reference electrode. To the aqueous buffer solution (the same as for the other experiments except that sorbitol was omitted), 100 μM anilinothiophenes or TPB^- were added.

Results

As tetraphenylboron was found to compete efficiently with the water-oxidizing enzyme system for redox equivalents produced via P-680 photooxidation [11], one might expect that TPB^- exerts a similar effect as hydroxylamine [13] on the characteristic oscillation pattern of flash-induced oxygen evolution provided that TPB^- is also bound close to PS II and reacts sufficiently fast, even at rather low concentrations. To test this idea, experiments were performed at a ratio of 5 TPB^- molecules per PS II. The data presented in Fig. 1 clearly show that TPB does not function like hydroxylamine. TPB^- remarkably damps out the characteristic oscillation pattern rather than shifting it towards higher flash numbers. After TPB^- consumption during the flash train and subsequent dark adaptation a second illumination leads to almost the same oscillation of the oxygen yield as in the control chloroplasts. These findings suggest that (a) the affinity of TPB^- to the donor side of PS II seems to be comparatively low, and (b) TPB^- accelerates the decay of S_2 and S_3. However, according to previous data [10,11] the latter effect is expected to be accompanied by an irreversible TPB^- consumption in contrast to real ADRY agents. If this is the case, the decay kinetics of S_2 and S_3 induced by TPB^- should be strongly dependent on the number of preillumination flashes leading to irreversible TPB^--oxidation, whereas for real ADRY agents the S_2 and S_3 decay kinetics should be invariant to preflashing. Experiments were performed in chloroplasts preincubated with 1 TPB^- molecule per PS II. Under these conditions one preflash should destroy a large amount of the total TPB^--amount, provided that this compound efficiently competes with the internal deactivation. The data depicted in Fig. 2 show the expected pattern. They reveal that the S_2 decay becomes remarkably increased by TPB^-, but the acceleration almost completely disappears after one preflash, following by 3 min dark adaption. In contrast to that, the S_2 decay kinetics, in the presence of the most powerful ADRY agent, 2-(3-chloro-4-trifluoromethyl)-anilino-3,5-dinitrothiophene (ANT 2p) was found to remain unaf-

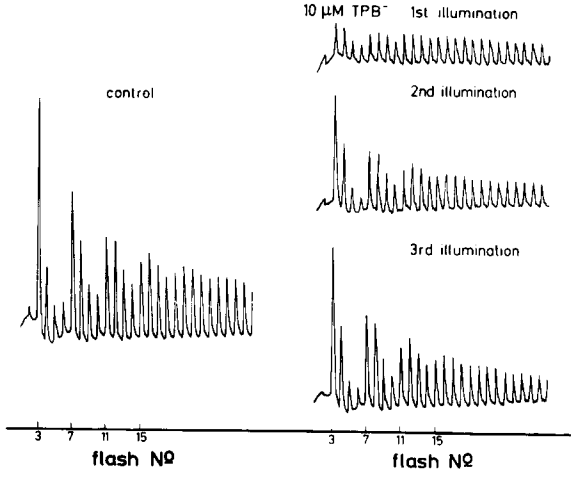


Fig. 1. Traces of the polarographic signals obtained by illumination of dark-adapted spinach chloroplasts with a flash train of 30 flashes.

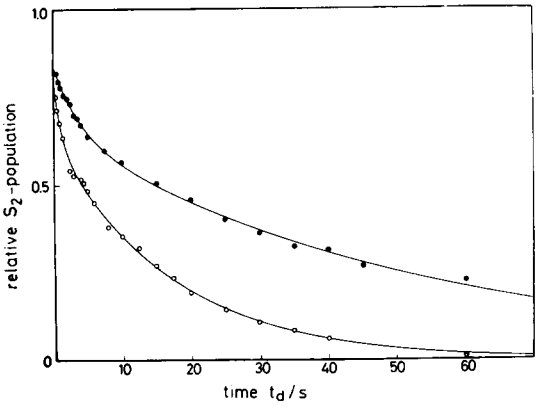


Fig. 2. Normalized S_2-population after one flash as a function of time t_d in isolated spinach chloroplasts in the presence of 2 μM TPB^- (○). In the case of preflashing (●) one flash was given 3 min before the onset of the experiment.

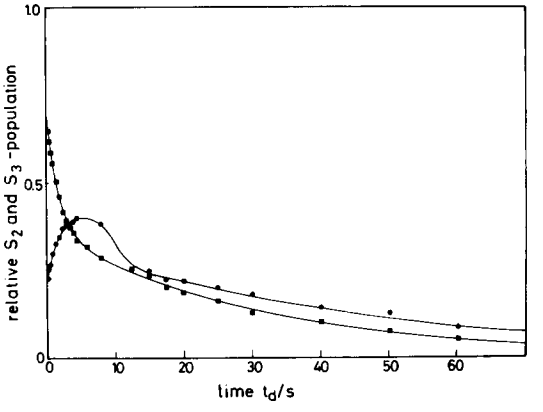


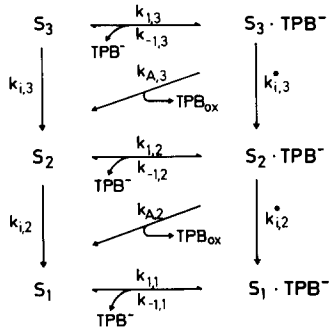
Fig. 3. Normalized S_2 and S_3 population after two flashes as a function of time t_d in isolated spinach chloroplasts in the presence of 2 μM TPB^-. Dots represent $S_2(t_d)$, full squares describe $S_3(t_d)$.

fected by the same preflashing (data not shown). It should be mentioned that, in contrast to ANT 2p, the more water-soluble TPB^- escapes from the chloroplasts through the dialysis membrane of the Joliot-type electrode. The half-time of this process was estimated to be of the order of 10–20 min. Accordingly, the experiments in the presence of TPB^- were performed under well-controlled experimental conditions in order to account for this effect.

Based on the redox properties of TPB^- an interesting mechanistic aspect arises for its effect on intermediary redox states of the water-oxidizing enzyme system. Titration experiments of oxygen evolution under repetitive flash excitation led to the conclusion that TPB^- acts as univalent reductant for the PS II donor side [11]. On the other hand, electrochemical studies unambiguously revealed that TPB^- becomes oxidized via a bivalent reaction giving rise to intramolecular biphenyl formation [14]. The latter process is especially interesting as it could open the possibility of TPB^--induced two-electron decay reactions of the type $S_3 \rightarrow S_1$ and $S_2 \rightarrow S_0$. Recently, evidence was presented for the occurrence of this type of reactions in algae, where an unknown redox couple C/C^{2+} was postulated to be responsible for the above-mentioned reactions [15]. In order to test the possibility of the induction of bivalent S_2 and S_3 decay reactions by TPB^-, experiments were performed to detect the time-course of $S_3(t)$ during the decay process. The experimental protocol was the same as that previously described for ANT 2p [5]. The numerical evaluation of the experimental data according to the Kok scheme (as outlined in Ref. 5) which are presented in Fig. 3, reveals a remarkable transient increase of $S_2(t)$ that can be explained only if the decay of S_3 occurs via a univalent reaction sequence $S_3 \rightarrow S_2 \rightarrow S_1$ also in the presence of TPB^-. This result favors the idea that TPB^- does not support a bivalent decay mechanism of S_2 and S_3, but functions as a univalent electron donor. This conclusion is corroborated by the fact that, after one preillumination flash and sufficient dark relaxation, the water-oxidizing enzyme system still attains the state S_1 (see Fig. 2) rather than state S_0 as would be expected from a bivalent $S_2 \rightarrow S_0$ transition (data not shown).

The flash-induced irreversible TPB^- consumption by the donor side has another consequence for the details of the decay kinetics at low concentrations: during the TPB^--induced S_2 and S_3 decay the TPB^- concentration declines continuously, in contrast to the situation for the ANT 2p catalyzed reactions. The data reported so far suggest that phenomenologically the effect of TPB^- on the decay kinetics of S_2 and S_3 could be described by a mechanism where TPB^- acts as irreversible and mobile one-electron donor. Accord-

ingly, the following reaction scheme is derived:



Scheme I

In order to test whether this scheme is pertinent to describe quantitatively the TPB^--induced acceleration of the S_2 and S_3 decay, experiments were performed at different TPB^- concentrations. For the numerical fitting of the data a Runge-Kutta procedure was used that is analogous to that described in Ref. 5 (assuming the same simplification: $k_{1,3} = k_{1,2} = k_{1,1} = k_1$; $k_{-1,3} = k_{-1,2} = k_{-1,1} = k_{-1}$; $k_{A,3} = k_{A,2} = k_A$; $k_{i,2} = k^*_{i,2}$ and $k_{i,3} = k^*_{i,3}$), except that the irreversible decrease of TPB^- concentration during the reaction course was taken into account. TPB^- was assumed to disappear via a univalent redox reaction. Figs. 4 and 5 show that a sufficient quantitative description of the S_2 and S_3 decay can be achieved at different TPB^- concentrations with the following set of kinetic parameters

$3.5 \cdot 10^5\ M^{-1} \cdot s^{-1} < k_1 < 4.5 \cdot 10^5\ M^{-1} \cdot s^{-1}$

$5\ s^{-1} < k_{-1} < 11\ s^{-1}$

$1.5\ s^{-1} < k_A < 2\ s^{-1}$

The parameter variation is small enough in order to be consistent with the model, especially if one considers that the set of differential equations was solved numerically and that small deviations of the actual TPB^- concentration could arise due to the unavoidable dialysis effect. However, it should be mentioned that, due to the problems arising by the dialysis effect, the parameter fit is not as precisely as in the case of ANT 2p (see Ref. 5). Accordingly, we can only say that the scheme provides a sufficient quantitative description of the data. Further conclusions (e.g., a determination of the TPB^- affinity to its target site) cannot be drawn from the present numerical analysis.

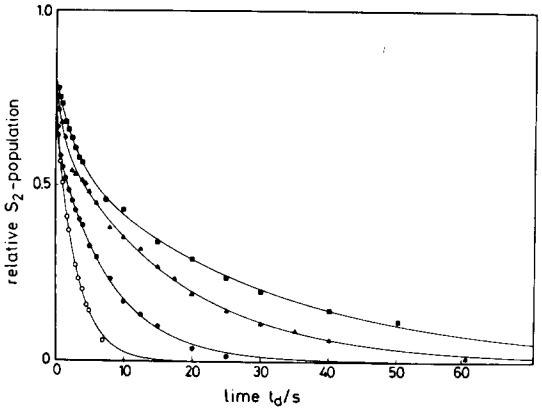


Fig. 4. Normalized S_2 population after one flash as a function of time t_d in isolated spinach chloroplasts in the presence of 1–4 μM TPB^-. ■, 1 μM; ▲, 2μM; ●, 3 μM, ○, 4 μM TPB^-, respectively. The curves were obtained by numerical evaluation of the reaction scheme as described in the text.

The reaction behaviour of TPB^- acting as irreversible PS II electron donor vs. the reversible catalytic effect of real ADRY agents raises the question whether or not ADRY agents really function as redox catalysts. As an attempt to clarify this point experiments were performed in order to determine the redox properties of ANT 2p and TPB^- by means of cyclic voltammetry. The data obtained are depicted in Fig. 6. The lower trace

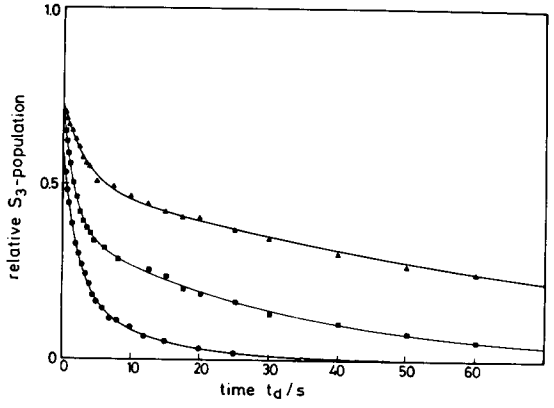


Fig. 5. Normalized S_3 population after two flashes as a function of time t_d in isolated spinach chloroplasts in the presence of 1–4 μM TPB^-. ▲, 1 μM; ■, 2 μM; ●, 4 μM TPB^-, respectively. The curves were obtained by numerical evaluation of the reaction scheme as described in the text.

shows that in the scanned voltage range, the aqueous tricine buffer system does not undergo an electrochemical reaction which could interfere with the response of the substances to be analyzed in this study. In the case of TPB^- a well-resolved oxidation peak followed by a smaller hump is observed. The corresponding redox potentials are of the order of +0.7 and +1.0 V, respectively. For the moment we cannot explain mechanistically the details of the curve shape in the oxidative pathway, but this point is not relevant for the problem of the TPB^- mechanism in thylakoids. The more interesting point is the absence of a redox peak in the reductive sweep. This result is in agreement with previous conclusions [14] and highly supports the idea that TPB^- becomes irreversibly oxidized also by Photosystem II in the thylakoid membrane as discussed previously [11].

In a similar way we tried to answer the question, whether or not ANT 2p is able to act as a redox type catalyst for the S_2 and S_3 decay (see Ref. 5). The middle trace of Fig. 6 reveals a pronounced oxidation peak of the order of 1.0 V, accompanied by a very small hump at significant lower voltages. The present data do not permit to draw conclusions about the significance of this hump. A further problem is the fact that in the case of ANT 2p the electrode surface becomes modified, so that after a new sweep the position of the oxidation peak is slightly shifted. Therefore, we cannot analyze the mechanism of electrochemical ANT 2p oxidation. The data only show that ANT 2p is an oxidizable substance with a redox step ranging from +0.9 to +1.0 V. A much more interesting problem for the ADRY mechanism, however, is the question whether ANT 2p becomes reversibly oxidized. The reductive sweep reveals a very small hump which could not be resolved as a reduction step. In some cases this hump was totally absent. Very similar results were obtained for ANT 2a, whereas ANT 2f shows pronounced peaks in the reductive pathway (data not shown). In this respect it is important to note that the highly redox-active species ANT 2f does not reveal ADRY-type activity [2], even at a ratio of 50 molecules per Photosystem II (Weiss, W. and Renger, G., unpublished results). Accordingly, on the basis of our measurements is appear unlikely that the ADRY effect of ANT 2p is due to a reversible oxidative-reductive redox reaction. Therefore, these data do not support a redox catalyst mechanism for ANT 2p and related ADRY agents.

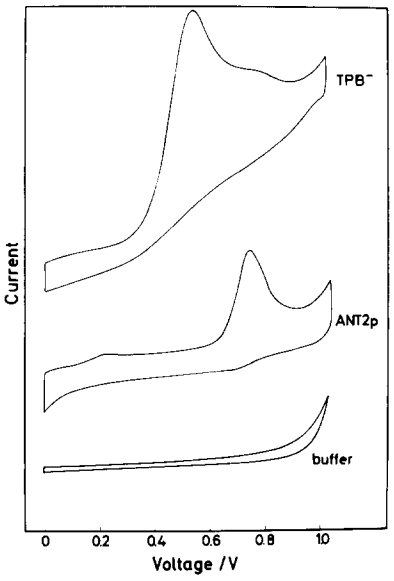


Fig. 6. Cyclic voltammogram of TPB^-, ANT 2p and control buffer at pH 7.5, the voltage scale is based on the Ag|AgCl reference electrode.

Discussion

The present study analyzes the mode of kinetic modification of the PS II donor side by tetraphenylboron in normal chloroplasts with intact water oxidizing enzyme system. The functional organization of photosynthetic water oxidation as a four-step univalent reaction sequence (see Ref. 18) implies that oxygen evolution can be reversibly affected in two ways by exogenous components without destroying the water-oxidizing enzyme system or blocking its redox turnover: (a) Modification of the life-times of the trapped intermediary redox states (S_2, S_3) and (b) Efficient competition with water by substrate analogues at the catalytic site.

The experimental data described in this paper show, that TPB^- affects the life-time of the redox states of S_2 and S_3 in a similar manner as real ADRY agents (see Ref. 5) rather than to react as a substrate (water) analogue that becomes competitively bound and subsequently oxidized at the

catalytic manganese center of the water-oxidizing enzyme system. In marked contrast to real ADRY agents, however, TPB^- can support only a single redox turnover, because it becomes irreversibly oxidized. This raises questions about the nature of the endogenous redox component that directly interacts with TPB^-.

Taking into account redox equilibria at the PS II donor side, generally at least three oxidized endogeneous components of the normal electron pathway have to be considered as reactive species: $P\text{-}680^+$, $D_1^{ox}(Z^{ox})$ or the higher redox states S_2 and S_3 of the water-oxidizing enzyme system. A direct interaction of TPB^- and S_2, S_3 appears unlikely for the three following reasons. (a) Electrochemical studies revealed a bivalent TPB^--oxidation in the form $2 \oplus + TPB^- \rightarrow DPB^+ +$ biphenyl [14]. Therefore two-electron type decay reactions of S_2 and S_3 according to the equation $S_{i+2} + C \rightarrow S_i + C_{ox}$ ($i = 0$; 1) could occur, because, for energetical reasons, at least S_3 should be able to perform this process. The present data, however, unambiguously show that this is not the case. In agreement with previous findings [11,19], TPB^- was confirmed to function as 1 electron donor in PS II. (b) TPB^- at low concentrations exerts an effect on the oscillation pattern of flash induced oxygen evolution (see Fig. 1), that is markedly different from that of low hydroxylamine concentrations [13]. Accordingly, both components seem to react via different mechanisms. Hydroxylamine at low concentrations probably functions as a substrate type analog [20], which becomes directly oxidized at the catalytic manganese center. In contrast to that, TPB^- acts as a mobile reductant of the PS II donor side thereby inducing statistical univalent S_2 and S_3 decay. The binding affinity as well as the exchange rate of TPB^- appear to be rather low according to our numerical analysis. (c) TPB^- was shown to be an efficient electron donor in Tris-washed chloroplasts [11], i.e., the functional integrity of the water-oxidizing enzyme system is not required for a TPB^- reaction with the PS II donor side.

Based on these arguments, $D_1^{ox}(Z^{ox})$ or $P\text{-}680^+$ might be a more likely candidate for a direct interaction with TPB^-. At a first glance the direct redox reaction with $D_1^{ox}(Z^{ox})$ seems to offer the most simple explanation for the TPB^--effect on the PS II donor side in normal as well as in Tris-washed chloroplasts. However, the reaction pattern appears to become more complex if one takes into account the recent discovery of flash-induced carotenoid oxidation induced by TPB^- and ADRY agents like ANT 2p or FCCP [21,22]. Latest data show that also phenolic-type herbicides induce carotenoid oxidation [23]. Interestingly enough, phenolic compounds, e.g., 2,4-dinitropheol, were found to affect simultaneously oxygen evolution in a similar way as ADRY agents [8,24]. Therefore, carotenoid oxidation seems to be related to the ADRY activity and some other PS II effects (disappearance of EPR-signal II_f [25] and cytochrome-*b*-559 transformation from high- to low-potential form [26,27], as pointed out by Mathis and Rutherford [23]). These authors proposed that all these changes of the PS II reaction pattern could originate in a lowered redox potential of a carotenoid, due to inhibitor binding to reaction center polypeptide(s), as suggested in a previous report [22].

Based upon the above-mentioned consideration it seems to be attractive to speculate that the PS II donor side effects induced by phenolic herbicides, TPB^- and real ADRY agents are caused by basically the same underlying mechanism: the opening of a bypass electron-transport pathway that funnels oxidizing redox equivalents from PS II via a carotenoid as transient redox component ('redox valve') to internal (cytochrome *b*-559?) or external e.g., benzidine [22] or TPB^-) donors. This generalized scheme, however, bears several kinetical problems. Carotenoid oxidation was found to occur without any lag phase with rise times of 10–35 μs in normal as well as in Tris-washed chloroplasts. This implies that at least in Tris-washed chloroplasts $D_1^{ox}(Z^{ox})$ cannot be involved as intermediate for Car^+ formation, because the electron transfer from $D_1(Z)$ to $P\text{-}680^+$ requires at least a few microseconds [28]. On the other hand, however, the proposed carotenoid 'valve' for oxidizing redox equivalents at PS II cannot efficiently compete with $P\text{-}680^+$-reduction by $D_1(Z)$ *, unless ad-

* If $D_1(Z)$ symbolizes the functional group together with its apoprotein matrix and $D_1^{ox}(Z^{ox})$ its oxidized form, the proton release coupled with $D_1(Z)$-oxidation [29,30] has to be taken into account.

ditional kinetic effects arise for the reaction P-680$^+$ $D_1(Z) \rightarrow$ P-680 $D_1^{ox}(Z^{ox}) + H^+$, e.g., either retardation of the forward reaction or acceleration of the backward reaction. This could explain the fact that at ANT 2p concentrations, where a significant ADRY effect is observed [5,6], the extent of Car^+ formation is relatively small (Weiss, W. and Renger, G., unpublished data). It was found that Car^+ formation is reached only at ANT 2p concentrations that markedly affect the PS II electron transport, whereas the ADRY effect of ANT 2p is already observed at 10–100-fold lower concentrations. In order to clarify the kinetic problems and the mechanistic details, more extended measurements of different activities (ADRY effect; Car^+ formation; Cyt *b*-559 oxidation; etc.) as a function of concentration of the modifying species are required under strictly comparable conditions. This, however, is out of the scope of the present study and will not be outlined here.

Regardless of the kinetical and mechanistic details the central point of the discussion is the assumption that allosteric effects induced by transient binding of specific compounds to PS II reaction center (or closely related) polypeptides are able to open a side pathway for oxidizing redox equivalents. This could occur by changing the redox potential of carotenoids (or another intrinsic component) via electrostatic effects (see Ref. 9) or by any other mechanism that modifies reaction coordinates. Certainly, an extraction of oxidizing redox equivalents in PS II can also occur via a direct redox reaction with the interacting exogenous species, but it has to be emphasized that this is only one among several possibilities. Therefore, different classes of agents, giving the same net result of dissipating oxidizing redox equivalents in PS II, might function via different mechanisms. If, for example, the formation of lipophilic anions is an essential property to induce a specific mechanism (see Ref. 9), then it is meaningless to compare mechanistically TPB^- and real ADRY agents with TMPD, because – despite of giving rise to the same effect (for details of the TMPD-effect, see Refs. 31 and 32) – TMPD does not form lipophilic anions.

Based on these considerations and on the experimental data presented in this study as well as in previous reports [4–6] TPB^- and ADRY agents like ANT 2p are assumed to act in similar way. These agents probably induce side pathing of oxidizing redox equivalents at PS II that could include a carotenoid as redox active species [22,23]. However, in contrast to real ADRY agents TPB^- becomes in addition irreversibly oxidized by a univalent redox reaction. The oxidant, that directly interacts with TPB^- is still not yet unambiguously clarified. Finally, it should be mentioned that according to our kinetic analysis TPB^- as well as ANT 2p [5] act as very mobile species that become only transiently bound. This is comparable to several rapidly exchanging PS II electron-transport inhibitors [33].

Acknowledgements

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