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EFFECT OF SOME ARTIFICIAL ELECTRON DONORS AND ACCEPTORS ON THE FUNCTIONING OF THE PHOTOSYNTHETIC OXYGEN EVOLVING SYSTEM

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Summary. Use of isolated pea chloroplasts and excitation with short saturating flashes showed that after addition of the reducing (ascorbate+DCPIP) couple the deactivation rate of S_2- and S_3 oxidised states decreased while in the presence of PBQ a significant increase was observed. The effect of these two reagent types on S_i states distribution and on the values of "misses" and "double hits" parameters are also presented. The observed increase of "misses" in the presence of (ascorbate + DCPIP) and a decrease in the presence of PBQ are discussed in relation with changes in the deactivation rate constants of the different S_i states.

***Key words**:* photosynthetic oxygen evolution, electron transport donors and acceptors, S_i state deactivations

***Abbreviations**:* DCPIP – 2,6-dichlorophenol indophenol; OES – oxygen evolving system; PBQ – p-benzoquinone; PSII – photosystem II; Q – primary acceptors of PSII

Introduction

Different chemical agents – electron acceptors, donors, inhibitors and "uncouplers" (Izawa, 1980; Trebst, 1980) have been applied during the investigation of photosynthetic electron transport. The couple (ascorbate + DCPIP) is usually applied as elec-

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tron donor for photosystem I (PSI) while the *p*-benzoquinone (PBQ) is an excellent electron acceptor for photosystem II (PSII). On the other hand these substances lead to some essential changes in the redox state of the investigated chloroplast suspensions and consequently act non-specifically on the investigated photochemical reactions.

In the present paper the action of the electron acceptor *p*-benzoquinone and of the electron donor – the couple (ascorbate+DCPIP) on the kinetics of photosynthetic oxygen evolving reactions was investigated using excitation with short, saturating flashes.

Materials and Methods

Fresh chloroplasts (thylakoids) from pea (*Pisum sativum*) were isolated according to Camm and Green (1980) and were suspended in a medium containing 0.4 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$ and 40 mM Hepes-NaCl, pH 7.5.

Oxygen flash yields were recorded at room temperature with a rate oxygen electrode of Joliot type (Joliot P. and Joliot A., 1968) and an universal polarograph OH-105 (Radelkis, Hungary). The samples were approximately 0.08 ml in volume and the chlorophyll concentration was about 0.3 mg/ml. The concentrations of the donor couple were ascorbate – 1.0 mM + 0.2 mM DCPIP and the concentration of the electron acceptor – *p*-benzoquinone was 10^{-4} M.

Oxygen flash yields were examined with a train of short saturating flashes (4J, $t_{1/2}$= 8 ms) at 0.5 s spacing and different (10 min – 5 s) dark inervals between flash trains.

The initial dark distribution of the S_i states as well as the misses and double hits parameters according to the model of Kok et al. (1970) were estimated using a minimisation procedure of the least-square deviation by personal computer.

Results and Discussion

Experimentally obtained oxygen flash yields in suspensions of isolated pea chloroplast after 5 min dark incubation in the control (triangles), and after PBQ (asterisks) or (ascorbat+DCPIP) (squares) addition are presented on Fig. 1.

The results show that the couple (ascorbate+ DCPIP) leads to a greater increase in Y_4 (oxygen yield after the fourth flash) amplitude than in Y_3 (oxygen yield at the third flash). It is also observed that the yields at the first flash are higher than those after the second flash, which could be explained by higher concentrations of the centres in the S_3 state in comparison to the S_2 state. Analogous abnormal yield at the first flash is reported by Lavorel and Seibert (1982) in OES II particles. No significant changes are observed after addition of PBQ except an increase of yield at the 3rd flash and a decrease at the 5th and 6th flashes.

Fig. 1. Oxygen flash yields after 10 min dark incubation and 500 ms spacing between flashes in pea chloroplasts: Control – triangles; in the presence of 10^{-4} M *p*-benzoquinone – asterisks, and in the presence of 1.0 mM ascorbate + 0.2 mM DCPIP – squares

Table 1 presents the initial dark distribution after 10 min dark incubation of the S_i states and the values of "misses" (α) and "double hits" (β). Data show that the couple (ascorbate+DCPIP) increases the number of the centres in S_0 and decreases those in S_1 states in comparison to the control. The action of PBQ is the opposite. At the same time data show that even after 10 minutes dark incubation of the chloroplast suspensions a significant number of the centres after (ascorbate+DCPIP)-treatment remain in higher oxidised S_i states (about 10% in S_2 and 13.5% in S_3). As far as the value of misses is concerned the two substances have also an opposite effect. The donor couple increases while the acceptor reduces the percent of misses. Both substances have little effect on the double hits parameter. Apparently the higher value of the misses as well as the higher percent of the centres in S_0, S_2 and S_3 states in case of (ascorbate + DCPIP) leads to smaller value of the amplitude and to a significant dampening of the oscillations (Fig. 1).

Table 1. Effect of *p*-benzoquinone and (ascorbate + DCPIP) on the values of Kok's model parameters*

Sample	S_0(%)	S_1(%)	S_2(%)	S_3(%)	Misses	Double hits
Control	39.8±1.31	58.2±2.41	0.0±0.1	2.0±0.0	0.135±0.02	0.034±0.00
Asc.+DCPIP**	35.6±4.57	41.0±2.31	9.9±1.2	13.5±2.0	0.177±0.02	0.033±0.01
PBQ***	34.6±0.90	63.7±1.21	0.0±0.5	1.7±0.0	0.115±0.00	0.028±0.03

*Average data from four independent experiments
**1.0 mM ascorbate + 0.2 mM DCPIP
***10^{-4}M *p*-benzoquinone

Fig. 2. Oxygen flash yields patterns in control pea chloroplast depending on the dark intervals (see legend) between flash trains

On Fig. 2, 3 and 4 are presented oxygen flash yield patterns obtained after different spacing between flash sequences in the control, in (ascorbate+DCPIP) and PBQ,

Fig. 3. Oxygen flash yields patterns in PBQ treated pea chloroplast depending on the dark intervals (see legend) between flash trains

respectively. The are no significant differences between flash yield oscillations in control (Fig. 2) and PBQ (Fig. 3) treated suspensions except the higher value of the yield at the 3rd and lower value at the 6th flash in case of PBQ. In both suspensions the yield after 5 minutes darkness between flash trains at the first flash is negligible and consequently this time is sufficient for deactivation of S_3 state. In the presence of the reducing couple (ascorbate+DCPIP) the behaviour of the oxygen evolving system is drastically changed. First, even after 10 minutes darkness oxygen yields following the first flashes are significant, i.e. the S_3 state is not fully reduced. Second, as pointed out the oxygen yields at the second flashes are lower than those at the first flashes and the yields at the 4th flashes are higher than those at the 3rd flashes. With decreasing the dark interval between flash trains the situation is changed and after 100 s darkness the yield at the 2nd flash is higher in comparison to the yield at the first flash and simultaneously the maximum oxygen flash yield is observed at the 3rd flash.

Fig. 4. Oxygen flash yields patterns in (ascorbate+DCPIP) treated pea chloroplasts depending on the dark intervals (see legend) between flash trains

Figs. 5 and 6 show the deactivation kinetics of the higher oxidised S_2 and S_3 states, respectively. These results show also that the deactivation of both states is reduced in case of (ascorbate+DCPIP) while in the presence of PBQ the process is enhanced.

Table 2. Effect of PBQ and (ascorbate+DCPIP) on the deactivation rate constants of S_2 and S_3 states*

Sample	$k_2(s^{-1})$	$k_3(s^{-1})$
Control	$7.7x10^{-3}$	$3.3x10^{-2}$
Asc.+DCPIP**	$6.5x10^{-3}$	$2.1x10^{-2}$
PBQ***	$2.8x10^{-2}$	$6.5x10^{-2}$

*Average data from four independent experiments
**1.0 mM ascorbate + 0.2 mM DCPIP
***10 mM *p*-benzoquinone

Data on Table 2 show that the higher oxidised state (S_3) is deactivated with a higher rate constant (k_3) than this of the S_2 state (k_2). Both rate constants decrease by the action of the (ascorbate + DCPIP) while the effect of PBQ is in opposite direction.

The results are in agreement with the data of Bouges-Bocquet (1973) and

of Delrieu and Rosengard (1988) and in the same time contradict the statement of Velthuys and Visser (1975) regarding the initial dark distribution of centres. In contrast to the data of Bouges-Bocquet our results show significant changes in the values of the misses in the presence of both substances ((ascorbate + DCPIP) and PBQ) while she has found a change only in the presence of the reducing couple.

Fig. 5. Kinetic of S_2 state deactivation in control chloroplasts (squares) and after treatment with (ascorbate + DCPIP) – triangles and PBQ – rhomboids.

Obviously, in the presence of PBQ, which is an effective electron acceptor from the reducing side of PSII, the rate of reoxidation of the primary electron acceptor (Q) is accelerated and thus the electron flow from the reaction centres of this system is enhanced leading to higher rate of oxygen evolution and to a decrease in photochemical misses (Table 1). On the other hand in the presence of the reducing couple (ascorbate + DCPIP), which is an effective electron donor to PSI, a reduced level in the

Fig. 6. Kinetic of S_3 state deactivation in control chloroplasts (squares) and after treatment with (ascorbate + DCPIP) – triangles and PBQ – rhomboids

rate of Q reoxidation, an inhibition to the functioning of PSII reaction centres, as well as an increase in the level of misses (Table 1) should be expected. Simultaneously, in the presence of the two kind of chemical agents some changes in the redox state of the system should arise which would probably lead to changes in the deactivation rate constants of the oxygen evolving centres (Table 2).

In conclusion it should be pointed out that the increase in the value of deactivation rate constants of the two states (S_2 and S_1) in the presence of PBQ and its decrease in case of (ascorbate + DCPIP) on one hand, and the changes in the value of the misses on the other hand cannot be explained satisfactorily. It is logical to assume that when the rate of deactivation reactions increases the value of the misses should be also increased and vice versa, which is not the case. This contradiction is analogous to that pointed out by Lavorel (1978), that contrary to the expectation the value of

the misses in suspension of intact unicellular algae (*Chlorella* and *Scenedesmus*) are higher than these in isolated chloroplast suspension. These contradictions as well as the absolute value of the misses reaching 15–30%, having in view almost 100% quantum efficiency of photosynthetic machinery, present some difficulties and insufficient extent of experimental verification of the generally accepted Kok's model.

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