S 8-012

Influence of herbicide binding on the yield of singlet oxygen production in photosystem II and on charge recombination kinetics in the T4 mutant of *Rhodopseudomonas viridis*

A. Krieger-Liszkay[1], C. Fufezan[1] and A. W. Rutherford[2]

[1]*Institut für Biologie II, Biochemie der Pflanzen, Universität Freiburg, Schänzlestr. 1, 79104 Freiburg, Germany*
[2]*Section de Bioénergétique, DBCM, CNRS URA 2096, CEA Saclay, 91191 Gif-sur-Yvette, France*

Keywords: oxidative stress, charge recombination, chlorophyll triplet, singlet oxygen, photosystem II

Introduction

Herbicide binding influences the redox potential (E_m) of the Q_A/Q_A^- redox couple in photosystem II. Phenolic herbicides lower the E_m by 45 mV, while DCMU raises it by 50 mV (Krieger-Liszkay and Rutherford, 1998). Earlier work shows that phenolics increase photodamage, while DCMU protects against it (Pallet & Dodge, 1980; Nakajima et al., 1996). We suggested previously (Krieger-Liszkay & Rutherford, 1998) that the recombination pathway in PSII is modulated by the binding of herbicides to the Q_B binding site. Lowering of the redox potentials by binding of phenolics should increase a true back reaction between $P680^+Q_A^-$ involving the repopulation of the primary charge pair. The $P680^+Ph^-$ radical pair can decay via the formation of the triplet state of P680. This triplet is relatively long-lived and has the potential to react with O_2, forming toxic singlet oxygen, $^1O_2$. An increase of the redox potential by DCMU should favour a recombination via a direct safe route, thereby lowering the yield of ^{3}Chl and subsequent $^1O_2$ formation.

We measured the yield of $^1O_2$ formation in the presence of herbicides by EPR spectroscopy, using 2,2,6,6-Tetramethyl-piperidine as a spin trap. Furthermore we measured $P^+Q_A^-$ recombination kinetics by flash-induced absorption spectroscopy using the PSII herbicide-sensitive T4 mutant of *Rhodopseudomonas viridis* (Sinning et al., 1989).

Materials and methods

PSII enriched membrane fragments from spinach were prepared as described by Berthold et al. (1981). $^1O_2$ was measured by EPR using as trap 2,2,6,6-tetramethyl-piperidine (TEMP) which forms the stable nitroxide radical TEMPO (Hideg et al., 1994). 100 µgChl/ml were incubated in the presence of 10 mM TEMP for 15 min in red light ($\lambda > 610$ nm) at 20°C in a buffer containing 0.3 M sucrose, 15 mM NaCl, 25 mM MES, pH 6.5. Flash-induced absorption changes at 1250 nm were measured using the T4 mutant of *Rps viridis* at room temperature and at 80 K essentially as described by Drepper et al. (1996) using appropriate filters and photodiode. Chromatophores of the T4 mutant were suspended in a buffer containing 10 mM NaCl, 50 mM MOPS, pH 7.0 and 0.8 mM $K_3[Fe(CN)_6]$ and 0.2 mM $K_4[Fe(CN)_6]$ to keep the cytochromes oxidised.

Results and discussion

Figure 1 shows that illumination of PSII enriched membrane fragments in the presence of TEMP and herbicides results in an EPR signal which is characteristic for the nitroxide radical

TEMPO which is formed by the reaction between $^1O_2$ and TEMP. When bromoxynil was added prior to the illumination, the signal was approximately two times larger than in the presence of DCMU at the given light intensity. These data show that addition of bromoxynil stimulates the production of $^1O_2$.

To further investigate the pathway of the $P^+Q_A^-$ recombination reaction in the presence of the different herbicides, reduction kinetics of P^+ were studied in the T4 mutant of *Rps viridis* by flash-induced absorption spectroscopy at 1250 nm at room temperature and 80 K.

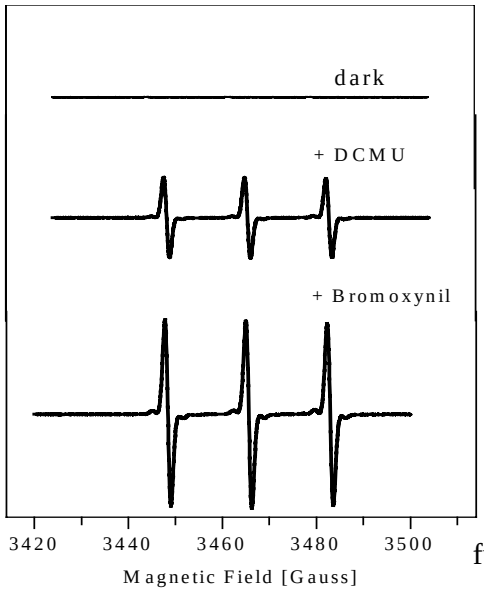

Figure 1 Spin trapping of singlet oxygen by TEMP. PSII enriched membrane fragments were illuminated for 15 min with red light (1800 µmolE m^{-2} s^{-1})

It has been demonstrated for the bacterial reaction centre that the free-energy gap between the $P^+Q_A^-$ radical pair and the P^+Ph^- radical pair has a major influence on the back reaction pathway. When the gap between $P^+Q_A^-$ and P* is smaller than 0.8 eV, the back reaction via P^+Ph^- dominates while under conditions where the gap is larger than 0.8 eV, a direct recombination pathway dominates (Woodbury et al., 1986; Gunner et al.1986).

As shown in Figure 2 (left side), the P^+ reduction kinetics are multiphasic at 22°C. The best fit was obtained with a sum of three exponential functions with half-times of 0.28 ms, 1.17 ms and 47 ms in the presence of bromoxynil. The slowest phase reflects the $P^+Q_B^-$ recombination (Gao et al., 1991) in those centres in which Q_B was not replaced by a herbicide. The two fast phases reflect the $P^+Q_A^-$ recombination. Similar half-times were observed for the wildtype of *Rps viridis* in the presence of o-phenantroline (Shopes and Wraight, 1989). The two phases of $P^+Q_A^-$ recombination were attributed by Gao et al. (1991) to a heterogeneity of PSII centres induced by protonation and thereby a stabilisation at the level of the primary charge pair.

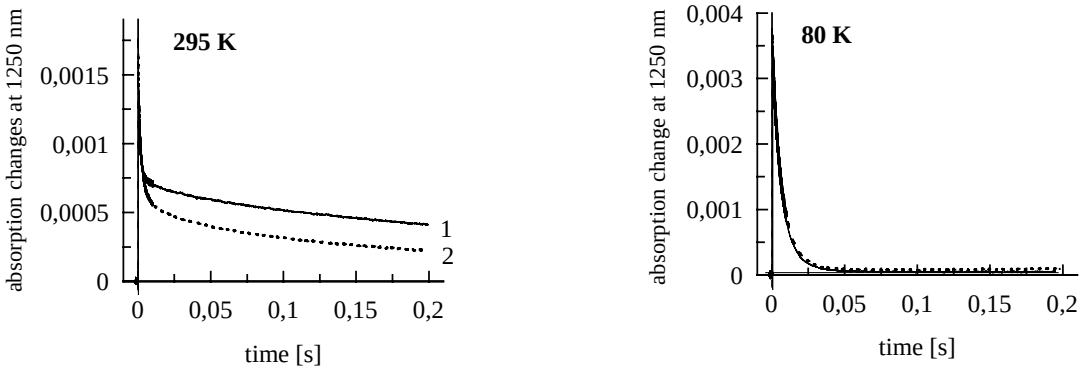

Figure 2 Flash-induced absorption changes measured at 1250 nm with chromatophores of the T4 mutant. Solid line (1): presence of 20 µM DCMU; Dashed line (2) presence of 100 µM bromoxynil. For the measurements at 80 K a 2.5 fold higher concentration of chromatophors was used.

According to the model shown in Fig. 3, two states of P^+Ph^- exist which differ in their free energy level. Three pathways of $P^+Q_A^-$ recombination are possible: pathway I via the higher free energy state of P^+Ph^-, pathway II via the lower free energy state of P^+Ph^-, pathway III

direct recombination via electron tunnelling. The half times measured at room temperature consist of the sum of the reactions I and III or II and III, respectively. At room temperature, the contribution of reaction III to the half times is so small that it can be neglected so that they reflect in first assumption reaction I or II.

Table 1 Half times and amplitudes of the three compounds (c_i) of the P^+ reduction in the T4 mutant of *Rps viridis* measured at 295 K and 80 K

	T [K]	*c1 $t_{1/2}$ [ms]*	*c2 $t_{1/2}$ [ms]*	*c3 $t_{1/2}$ [ms]*	*A1 (%)*	*A2 (%)*	*A3 (%)*
Bromoxynil	295	0.28	1.17	47	54	37	9
	80	3.1	6.2		45.9	54.1	
DCMU	295	0.56	1.63	20	35	59	6
	80	3.6	7.6		21.9	78.1	

When the temperature was lowered to 80 K (Figure 2, right side), the reduction kinetics could be described by two exponential function, which reflect the different pathways of $P^+Q_A^-$ charge recombination (see Fig. 3, indirect recombination via P^+Ph^- versus direct recombination). At low temperature the situation is different. Reaction I is strongly temperature dependent (data not shown), while reaction II shows a weaker temperature dependence and still takes place at T < 150 K. The half time of 3 ms reflects the sum of reaction II and III at 80 K. The half time of 6 ms at 80 K reflects the sum of reaction I and III, where III is dominating.

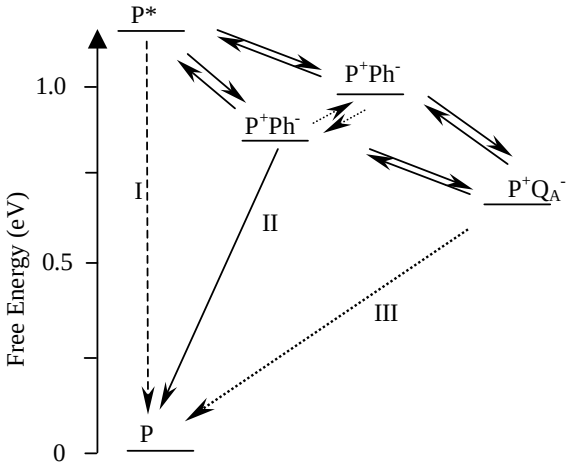

Figure 3 Possible pathways of $P^+Q_A^-$ recombination in *Rps viridis*; Pathway I: recombination via a high energy level of P^+Ph^-; pathway II, recombination via a lower energy level of P^+Ph^-; pathway III, direct recombination by electron tunneling. The conversion between the P^+Ph^- states is assumed to be slow.

At 22 °C the reduction of P^+ is slower in the presence of DCMU than in the presence of bromoxynil (see Figure 2, Table 1). The slower overall P^+ reduction is mainly caused by a different distribution of the two phases of the kinetics of $P^+Q_A^-$ recombination, but only a small increase of half times is observed. However, the amplitude of the fast phase (c1) is almost half the one obtained in the presence of bromoxynil, i.e. c2 is dominating the overall P^+ reduction kinetics in the presence of DCMU. At 80 K a smaller difference in the P^+ reduction kinetics is observed in the presence of the two different herbicides. This indicates that the faster phase (c1) of the $P^+Q_A^-$ recombination seen at room temperature disappears at

cryogenic temperatures. In the presence of DCMU, the slower phase (c2) which reflects the direct recombination occurs with a higher amplitude than in the presence of bromoxynil.

It has been described earlier that charge recombination of $P^+Q_A^-$ in *Rps viridis* occurs by two competing pathways: a direct electron-tunnelling pathway dominating at temperatures below 250 K with $t_{1/2}$ of 9 ms and a thermally activated process which proceeds via the formation of the P^+Ph^- radical pair (Shopes and Wraight, 1987). The half times of the electron transfer reactions involved in this direct pathway seem to be only slightly modified by the herbicides (see Table 1). At room temperature, the kinetics of the $P^+Q_A^-$ recombination are slowed down by a factor of three in the T4 mutant in the presence of DCMU compared to bromoxynil indicating a shift of the redoxpotential of Q_A in the same direction as measured in PSII. In the presence of DCMU a higher activation energy is needed and, therefore, the faster phase of the $P^+Q_A^-$ via P^+Ph^- recombination is most affected. However, the data obtained with the T4 mutant show that the direct pathway of $P^+Q_A^-$ recombination plays only a minor role at room temperature even in the presence of DCMU. We assume that the shift in the redox potential of Q_A in the T4 mutant of *Rps viridis* is not large enough to favour the direct recombination pathway. In PSII, however, the yield of $^1O_2$ formation is drastically lowered in the presence of DCMU, indicating that in PSII the competition is much more finely balanced between back reaction pathways and the direct recombination between P^+ and Q_A^-.

Acknowledgements

We would like to thank Roy Lancaster (MPI Frankfurt) for providing us with chromatophores of the T4 mutant and Friedel Drepper (Universität Freiburg) for his help with the optical measurements.

References

Bertold, D.A., Babcock, G.T., Yocum, C.F. (1981) *FEBS Lett.* **160**, 159-164.
Drepper, F., Hippler, M., Nitschke, W., Haehnel, W. (1996) *Biochemistry* **35,** 1282-1295.
Gao, J.L., Shopes, R.J., Wraight, C.A. (1991) *Biochim. Biophys. Acta* **1056,** 259-272.
Gunner, M.R., Robertson, D.E., Dutton, P.L. (1986) *J. Phys. Chem.* **90**, 3783-3795.
Hideg, E., Spetea, C., Vass, I. (1994) *Photosynth. Res.* **39**, 191-199.
Krieger-Liszkay, A., Rutherford, A.W. (1998) *Biochemistry* **37**, 17339-17344.
Nakajima, Y., Yoshida, S., Ono, T. (1996) *Plant Cell Physiol.* **37**, 673-680.
Pallet, K.E., Dodge, A.D. (1980) *J. Exp. Bot.* **31**, 1051-1066.
Shopes, R.J., Wraight, C.A. (1987) *Biochim. Biophys. Acta* **893**, 409-425.
Sinning, I., Michel, H., Mathis, P., Rutherford, A.W. (1989) *FEBS Letts.* **256**, 192-194.
Woodbury, N.W., Parson, W.W., Gunner, M.R., Prince, R.C., Dutton, P.L. (1986) *Biochim. Biophys. Acta* **851**, 6-22.