Oxidation of Q_A^- and of Q_B^- of photosynthetic reaction centers by an artificial acceptor

Ileana Agalidis and Bruno R. Velthuys

Laboratoire de Photosynthèse, CNRS, 91190 Gif-sur-Yvette, France

Reveived 6 January 1986

Bacterial reaction centers and photosystem II particles reduce *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine with a rate constant that depends on the state of the acceptor complex. We assume interference by detergent to explain the dissimilar behavior of the two types of material. Using this assumption, we summarize: Q_A (with an empty Q_B site) is more reactive than $Q_A^- \cdot X$ (with an occupied Q_B site; X = quinone, herbicide, or, we assume, detergent). $Q_A^- \cdot X$ is more reactive than $Q_A \cdot {}_B^-$.

Reaction center (Rps. sphaeroides) *Photosystem II* *Quinone reduction*
N,N,N′,N′-*Tetramethyl*-p-*phenylenediamine*

1. INTRODUCTION

PS II reaction centers and photosynthetic reaction centers of purple bacteria such as *Rhodopseudomonas sphaeroides* bind quinone at two sites. The quinone bound at the 'Q_A' site serves as an electron acceptor for the photoactive porphyrin part of the reaction center. It is bound tightly [1]. The quinone bound at the 'Q_B' site serves as an electron acceptor of Q_A^-. It leaves the reaction center frequently, but only as a quinone or dihydroquinone, not as a semiquinone [2]. In PS II, the quinones are plastoquinone (PQ) and in *Rps. sphaeroides*, ubiquinone (Q_{10}). The main characteristic of both molecules is that they are strongly hydrophobic due to a long hydrocarbon side chain [3].

Herbicides such as DCMU (effective only for PS II), ametryn and terbutryn compete with quinone for binding at the Q_B site and in this way prevent oxidation of Q_A^- [4,5]. It is unclear, however, whether they interfere with the head group of the quinone or its bulky side chain. The latter assumption was favored by some authors [6,7]. To clarify this point we have examined the reduction of the small artificial acceptor $TMPD^+$ in the absence and presence of herbicide. Our observations suggest that herbicide does compete with this acceptor and prevents it from oxidizing Q_A^- via the Q_B site. However, unlike PQ and Q_{10}, $TMPD^+$ can also oxidize Q_A^- via another route, bypassing the Q_B site. Surprisingly, a third route of reduction of artificial acceptors, reduction by Q_B^-, is much slower than direct reduction by Q_A^-.

2. MATERIALS AND METHODS

Bacterial reaction centers were extracted from wild-type *Rps. sphaeroides* strain Y as described [8] but with an extra DEAE-cellulose chromatography step. Q_B-less reaction centers were prepared according to Okamura et al. [9]. The reaction center concentration was determined using $\epsilon_{800nm} = 288\ mM^{-1} \cdot cm^{-1}$ [10].

PS II particles were prepared from spinach with

Abbreviations: TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Caps, 3-(cyclohexylamino)propanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; LDAO, lauryldimethylamine *N*-oxide; PS, photosystem

Published by Elsevier Science Publishers B.V. (Biomedical Division)

Triton X-100 and treated sequentially with NaCl and urea [11] and NH_2OH. They were a gift from Dr N. Murata. The particles lack donor-side components beyond the secondary donor Z. We noticed that they also lack Q_B. In the absence of donors and acceptors, Z^+ and Q_A^- back-react with a characteristic rate, producing luminescence. By comparing the flash-induced luminescence in the absence and presence of DCMU (fig.1), we estimate that ~90% of the centers (assuming 100% in the presence of DCMU) go no further than $Z^+ \cdot Q_A^-$, i.e. they do not form $Z^+ \cdot Q_B^-$.

Flash-induced absorbance changes were measured with a single-beam spectrophotometer of local design. The actinic flashes were produced by a xenon flashlamp and were of ~10 µs half-time.

3. RESULTS AND DISCUSSION

3.1. *Reduction of TMPD$^+$ by Q_B-less PS II particles*

We used NH_2OH/urea-washed particles that appear to lack PQ at the Q_B site (see section 2). Flash illumination of the particles oxidizes the secondary donor Z and reduces Q_A. In the presence of a mixture of TMPD and TMPD$^+$, Z^+ oxidizes TMPD and Q_A^- reduces TMPD$^+$. We measured these reactions by monitoring dye absorbance at 570 nm.

The oxidation of TMPD is more rapid than the reduction and is seen first. The rate constant for the reduction is ~40 $mM^{-1} \cdot s^{-1}$ (fig.2A). In the presence of DCMU, the rate drops to ~1.3 $mM^{-1} \cdot s^{-1}$. Exactly the same degree of inhibition is obtained with other inhibitors, e.g. the phenolic inhibitor ioxynil (not shown).

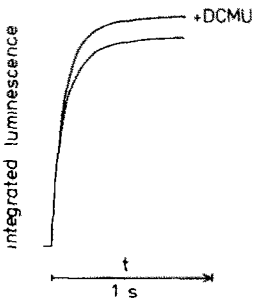

Fig.1. Integrated luminescence of NH_2OH/urea-washed PS II particles. Integration was started 10 ms after illumination by a single flash. Without or with DCMU (1 µM), as indicated. Hepes-NaOH, 2.5 mM (pH 7.5); NaCl, 10 mM; versene, 10 µM; chlorophyll concentration, 2 µg/ml.

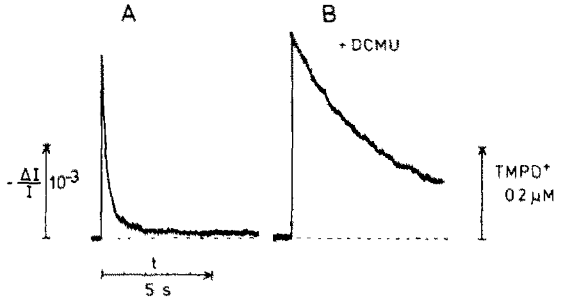

Fig.2. Oxidation and reduction of TMPD by NH_2OH/urea-treated PS II particles. Illumination by a single flash. Mes/NaOH, 25 mM (pH 6.1); TMPD, 500 µM; $K_3Fe(CN)_6$, 150 µM; DCMU, 0 µM (A) or 2.5 µM (B); chlorophyll, 60 µg/ml. The recordings were corrected for baseline slant due to slow oxidation of TMPD by oxygen. Measuring wavelength, 570 nm; optical path length, 2 mm. We estimate ϵ(TMPD$^+$ minus TMPD) = 11 $mM^{-1} \cdot cm^{-1}$. We obtained this value by oxidizing TMPD with known amounts of ferricyanide.

The above results suggest that in the absence of herbicides TMPD$^+$ reacts with Q_A^- predominantly via the Q_B cavity. The remaining reaction of lower rate in the presence of herbicide may bypass the Q_B cavity.

3.2. *Reduction of TMPD$^+$ by* Rps. sphaeroides *reaction centers*

Like PS II particles, bacterial reaction centers can use TMPD as an artificial donor and TMPD$^+$ as an artificial acceptor. The rate constant for the reaction of P^+ with TMPD is ~800 $mM^{-1} \cdot s^{-1}$ (not shown). We measured Q^- oxidation at 430 nm, a shoulder of one of the semiquinone absorbance bands [12]. Absorbance of the dye is negligible at this wavelength.

With Q_B-less reaction centers, Q_A^- became reoxidized with a rate constant of ~4 $mM^{-1} \cdot s^{-1}$ (fig.3A). In contrast to PS II particles, this reaction was nearly insensitive to herbicide (fig.3). About the same rate of TMPD$^+$ reduction was obtained with Q_B-containing reaction centers when used at pH 11 (fig.3B). At this pH, electron transfer to Q_B does not occur [13]. The same rate was also found with reaction centers at pH 8 when Q_B was displaced by ametryn (fig.4, ●).

When Q_B was present at pH 8, semiquinone ox-

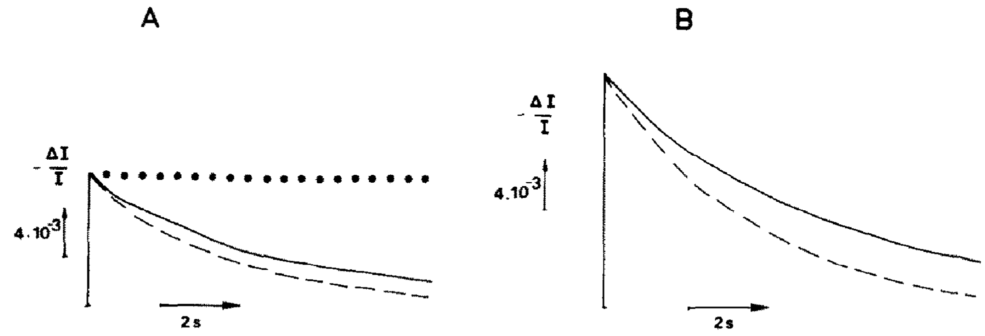

Fig.3. Semiquinone oxidation by TMPD$^+$ after illumination by a single flash. (A) Q_B-less reaction centers of *Rps. sphaeroides* (2 μM). Tris-HCl, 50 mM (pH 8); LDAO, 0.03%. (——) Without addition, (– – –) with 500 μM ametryn; (···) with 40 μM Q_6. (B) Q_B-containing (non-depleted) reaction centers (3.6 μM). Caps-NaOH (pH 11), 50 mM; LDAO, 0.03%. (——) Without addition, (– – –) with 500 μM ametryn; measuring wavelength, 430 nm; optical path length, 1 cm.

idation was greatly retarded (fig.4, ○). Clearly, Q_B^- is less reactive towards TMPD$^+$ than is Q_A^-. The ratio of the rate constants for oxidation of Q_A^- and Q_B^- was 12. This same value was found for the ratio of the charge-recombination rates of P$^+$ with Q_A^- and Q_B^-, respectively (in the absence of TMPD; not shown). We conclude that essentially all Q_B^- oxidation by TMPD$^+$ takes place via back-flow of electrons to Q_A. In other words, TMPD$^+$ reduction is mainly controlled by the electron transfer equilibrium between Q_A and Q_B. Shopes and Wraight [14] recently reached a similar conclusion for oxidation of Q_B^- by the strongly hydrophilic acceptor Fe(CN)$_6^{3-}$. It thus seems that Q_B^- is much less accessible, i.e. much more extensively covered by protein, than is Q_A^-. This conclusion contradicts an earlier suggestion by Debus et al. [15].

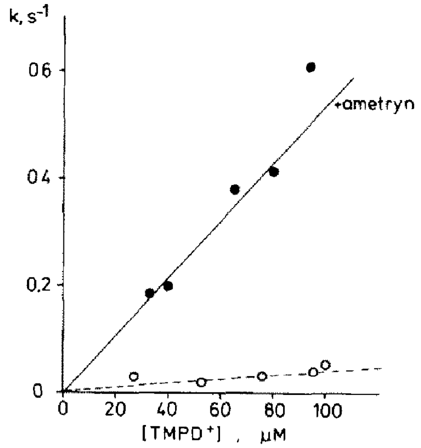

Fig.4. Rate of Q_A^- oxidation after a flash, as a function of the concentration of TMPD$^+$. Q_B-containing (non-depleted) reaction centers of *Rps. sphaeroides* (2 μM). TMPD, 200 μM; $K_3Fe(CN)_6$, 0–100 μM; ametryn, 0 μM (○) or 500 μM (●). Other conditions as for fig.3.

Unlike PS II particles, the bacterial centers never seem to reduce TMPD$^+$ via the Q_B site. This apparent difference between the two types of particle may be an artefact. Tentatively, we suppose that when the Q_B site is not occupied by Q_B or by herbicide, it is occupied by the detergent (LDAO) that is used to keep the bacterial centers in solution.

ACKNOWLEDGEMENT

We thank Mrs Clerot for her assistance in preparing bacterial reaction centers.

REFERENCES

[1] Okamura, M.Y., Debus, R.J., Kleinfeld, D. and Feher, G. (1982) in: Function of Quinones in Energy-Conserving Systems (Trumpower, B.L. ed.) pp.299–317, Academic Press, New York.
[2] Goldfeld, M.G., Khangulov, S.V. and Blumenfeld, L.A. (1978) Photosynthetica 12, 21–34.
[3] Crofts, A.R. and Wraight, C.A. (1983) Biochim. Biophys. Acta 726, 149–185.
[4] Velthuys, B.R. (1982) in: Function of Quinones in Energy-Conserving Systems (Trumpower, B.L. ed.) pp.401–408, Academic Press, New York.
[5] Wraight, C.A. (1981) Isr. J. Chem. 21, 348–354.

[6] Jursinic, P. and Stemler, A. (1983) Plant Physiol. 73, 703–708.
[7] Diner, B.A., Schenck, C.C. and De Vitry, C. (1984) Biochim. Biophys. Acta 766, 9–20.
[8] Reiss-Husson, F. and Jolchine, G. (1974) FEBS Lett. 40, 5–8.
[9] Okamura, M.Y., Isaacson, R.A. and Feher, G. (1975) Proc. Natl. Acad. Sci. USA 72, 3491–3495.
[10] Straley, S.C., Parson, W.W., Mauzerall, D.C. and Clayton, R.K. (1973) Biochim. Biophys. Acta 305, 597–609.
[11] Miyao, M. and Murata, N. (1984) Biochim. Biophys. Acta 765, 253–257.
[12] Vermeglio, A. and Clayton, R.K. (1977) Biochim. Biophys. Acta 461, 159–165.
[13] Wraight, C.A. (1979) Biochim. Biophys. Acta 546, 309–327.
[14] Shopes, R.J. and Wraight, C.A. (1986) Biochim. Biophys. Acta, in press.
[15] Debus, R.J., Valkirs, G.E., Okamura, M.Y. and Feher, G. (1982) Biochim. Biophys. Acta 682, 500–503.