Evidence for resistance of the microenvironment of the primary plastoquinone acceptor ($Q_A^- \cdot Fe^{2+}$) to mild trypsinization in PS II particles

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Mild trypsinization of PS II particles at pH 6 0, which almost completely blocks reoxidation of the primary plastoquinone acceptor, $Q_A^- Fe^{2+}$, by endogenous plastoquinone (PQ) as well as by exogenous (*p*-BQ) quinones, does not affect the shape or the amplitude of the EPR signal attributed to $Q_A^- Fe^{2+}$ The effect of DCMU on the EPR signal [(1984) FEBS Lett 105, 156–162] is completely eliminated in mildly trypsinized PS II particles The lack of effect of mild trypsin treatment on the $Q_A^- Fe^{2+}$ microenvironment is briefly discussed in relation to the functional and structural organization of the PS II acceptor side

Photosystem II *Semiquinone-iron complex* *Trypsin* *Herbicide* *Photosythesis*

1. INTRODUCTION

There are two membrane-bound quinone molecules with different functional properties which are essential for the formation of bound hydrogen (in the form of quinols) in photosynthetic purple bacteria as well as in PS II of oxygen-evolving organisms (review [1]). The primary quinone acceptor, referred to as Q_A, is indispensable for stabilization of the light-induced charge separation within the reaction center and acts under normal conditions as a one-electron redox component only Regardless of its redox state (either quinone or semiquinone), Q_A is tightly bound to its apoprotein. The secondary quinone, Q_B, on the other hand, exhibits the two-electron redox chemistry more commonly associated with quinones. Q_B is thought to be a quinone molecule of the mobile pool of quinones which becomes firmly attached to its protein binding site only in the semiquinone form. This bound semiquinone is highly stabilized. Many herbicides are inferred to compete with the endogenous quinones for the Q_B binding site either directly [2] or in an allosteric manner [3].

EPR and Mossbauer spectroscopy have shown that Q_A and Q_B are associated with a ferrous iron center [4–9]. The situation in PS II is interpreted to be the same as in purple bacteria [7,9]. Removal of the iron alters the redox properties of Q_A to that of an $n = 2$ component [8]. Accordingly, the iron center may play a crucial functional role for determining the peculiar univalent reactivity of Q_A while it does not prevent the quinol formation at the Q_B site. As the magnetic interaction between Q_A^- and Fe^{2+} is very similar to that with Q_B^- [10] the functional difference between Q_A and Q_B might be related to different microenvironments of both quinones In PS II it has been shown that the EPR signal arising from $Q_A^- \cdot Fe^{2+}$ is affected by changes

Abbreviations· PS, photosystem, DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea, $Q_A \cdot Fe^{2+}$, primary plastoquinone acceptor of PS II, Mes, 4-morpholinoethanesulfonic acid, *p*-BQ, *p*-benzoquinone

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in external pH [11], by herbicide binding [12–14] and by HCO_3^- depletion [15] Mild trypsinization is another treatment which affects the acceptor side of PS II It blocks forward electron flow at the level of Q_A^- oxidation, drastically enhances the accessibility of Q_A to $K_3[Fe(CN)_6]$ and markedly modifies herbicide binding [16–19] In this report we have treated PS II preparations with trypsin and have monitored the effects of this treatment upon the $Q_A^- Fe^{2+}$ signal in the absence and presence of the PS II herbicide DCMU.

2 MATERIALS AND METHODS

Oxygen-evolving PS II-enriched particles were prepared from spinach according to the procedure in [20] with modifications as in [10]. The freshly prepared thylakoid fragments were treated with trypsin in the dark in an incubation medium containing 20 mM Mes/NaOH, pH 6.0, 10 mM NaCl and 5 mM $MgCl_2$ The same trypsin treatments were carried out at low concentrations of chlorophyll, however the dilution and subsequent centrifugation resulted in loss of activity in control samples Thus the data presented are taken from experiments where PS II particles at 5 mg chlorophyll/ml were treated with 2.5 mg trypsin/ml After a defined incubation time the samples were transferred to quartz EPR tubes, mixed with EDTA to a concentration of 1 mM and frozen in liquid nitrogen.

EPR measurements were made using a Bruker ER-200t X-band spectrometer fitted with an Oxford Instrument liquid He temperature cryostat and control system [5]. Samples were illuminated at 77 K for 20 min in an unsilvered dewar containing liquid nitrogen, using an 800 W projector light source. Other additions were as described in the figure legends

Oxygen measurements were performed with a Clark-type electrode as outlined in [21]. For the sake of comparability with EPR measurements, the trypsin treatment was performed in concentrated suspensions as described above After a defined incubation time the trypsinized samples were injected by syringe into the cuvette, to a final chlorophyll concentration of 50 μg/ml. To reduce additional effects due to sample dilution the measurements were started at the earliest possible time after injection of the thylakoid fragments (normally 1 min after injection) In both the EPR and O_2-evolution experiments control samples were incubated under conditions identical to those used for the treated samples except for the omission of trypsin

3. RESULTS AND DISCUSSION

To ensure that our trypsinization procedure (at pH 6.0) of PS II particles modifies the acceptor side without affecting the donor side, oxygen yield measurements were performed with different exogenous electron acceptors The data presented in fig 1 reveal that oxygen evolution after 15 min trypsinization is markedly suppressed if *p*-BQ is used as the electron acceptor In contrast, when $K_3[Fe(CN)_6]$ is used as the electron acceptor the oxygen-evolving capacity remains almost fully intact, even after 30 min trypsin treatment Furthermore, the $K_3[Fe(CN)_6]$-mediated oxygen evolution is highly insensitive to DCMU (not shown) These results show that under our experimental conditions trypsin selectively modifies the polypeptides that are essential for electron transfer in the acceptor side of PS II without inhibiting the donor side. This is in agreement with recent results using trypsin at this pH [22].

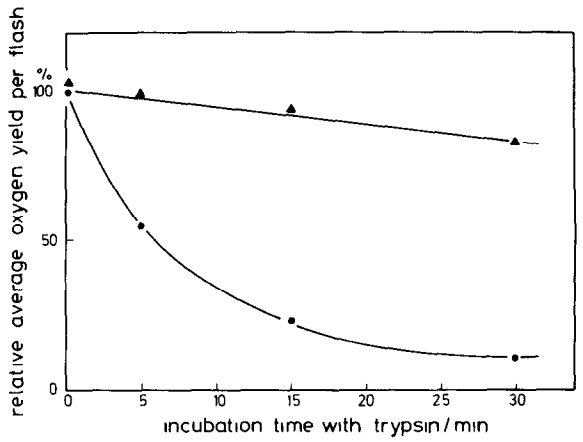

Fig 1 Average oxygen yield per flash as a function of dark incubation of PS II particles with trypsin The assay medium contained 2 mM $MgCl_2$, 10 mM $CaCl_2$, 10 mM KCl, 20 mM Mes, pH 6 0, 2 mM *p*-BQ (●) or 1 mM $K_3[Fe(CN)_6]$ (▲) as electron acceptor Time between the flashes, t_d = 250 ms Control values 7 4 × 10^{-4} mol O_2/mol Chl per flash (▲), 8 6 × 10^{-4} mol O_2/mol Chl per flash (●)

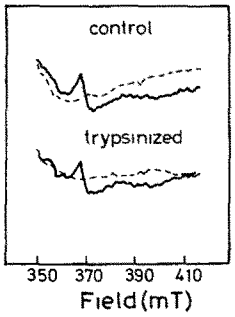

Fig 2 EPR spectra of the photoinduced Q_A^- Fe^{2+} signal in PS II particles (– – –) Dark, (——) after illumination for 20 min at 77 K. Instrument settings T = 4.8 K; microwave power, 32 mW (8 dB down from 200 mW), frequency, 9 44 GHz, modulation amplitude, 20 G; gain, 1 25 × 10^5

If this specific proteolytic attack on the apoprotein(s) in the Q_A-Q_B region induces significant changes in the microenvironment of Q_A^- Fe^{2+}, then marked effects on the $Q_A^- \cdot Fe^{2+}$ EPR signal might be expected. The experimental data presented in fig.2, however, reveal that 15 min trypsin treatment at pH 6.0 does not affect the amplitude or the shape of the Fe^{2+}-Q_A^- EPR signal at g = 1.82 This shows that the microenvironment of the Q_A^--Fe^{2+} group (as monitored by the g = 1.82 EPR signal) is rather resistant to proteolytic modification by trypsin of polypeptides at the PS II acceptor side. Indirect lines of evidence corroborate this conclusion. For example, it has been shown that the kinetics of Q_A^- reoxidation by the PS II donor side (i.e. by S_2 of the water-oxidizing enzyme system) in chloroplasts are almost the same when electron transfer from Q_A^- to Q_B is blocked either by trypsin treatment or by DCMU addition [23] Furthermore, the local electrochromic absorption changes around 550 nm (C550), which are probably due to a local electrochromic effect of Q_A^- on pheophytin [24], remain nearly unaffected by mild trypsinization (Weiss and Renger, unpublished).

It has been demonstrated previously that certain herbicides modify the $Q_A^- \cdot Fe^{2+}$ EPR signal [12,13]. Since trypsin treatment markedly decreases the binding of the herbicide DCMU on the acceptor side of PS II [25], it was therefore expected that the DCMU-induced effect on the EPR signal would be lost in trypsin-treated membranes. The data in fig.3 show that in the control sample,

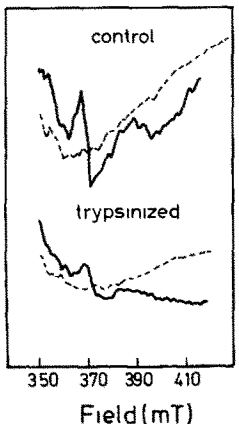

Fig.3 EPR spectra of photoinduced $Q_A^- \cdot Fe^{2+}$ signal in PS II Particles in the presence of 100 μM DCMU (– – –) Dark, (——) after illumination for 20 min at 77 K Experimental conditions as in fig.2

DCMU at a concentration of 1 DCMU molecule per 50 chlorophylls markedly increases the amplitude of the EPR signal (upper trace). After trypsin treatment the DCMU effect on the EPR signal is lost, as revealed by the lower trace in fig 3.

Trypsin severely attacks the 32-kDa atrazine binding polypeptide (ABP) (for recent data about the proteolytic cleavage pattern [26]) and another lysine-containing polypeptide, which is functionally related to the PS II acceptor side [3,27]. It is seen from the present study that the structural integrity of these polypeptides is not required for establishing the normal microenvironment of the $Q_A \cdot Fe^{2+}$ center. This finding implies important consequences about the nature of the binding sites of Q_A and Q_B which can be summarized as follows:

(a) The 32-kDa–ABP does not contribute either to the first ligand sphere of the iron center or to the binding of Q_A.

(b) If the 32-kDa–ABP contributes essentially to the Q_B-binding site [3,28], this site must be positioned in such a way that it permits contact of Q_B with the Fe^{2+} which would be located on another polypeptide

(c) If blockage of electron transfer from Q_A^- to Q_B by trypsin is due to complete loss of Q_B binding [2] rather than due to a drastic decrease of the matrix for electron tunneling by disorientation of a still bound plastoquinone [16,29], then Q_B binding does not influence the interaction between Q_A^-

and Fe^{2+}, in contrast to the effect of PS II herbicide binding [12,13]. This might favor an allosteric type mechanism of PS II herbicide blockage of Q_A to Q_B electron transfer [16]; alternatively it could indicate differences between Q_B and DCMU (or other herbicides) in the interaction with the $Q_A^- \cdot Fe^{2+}$ group.

Another possibility which could satisfactorily explain our results is the assumption that the 32-kDa–ABP is not an essential constituent for the Q_B-binding site. This idea raises questions about the functional role of the 32-kDa–ABP for Q_B binding. In this respect it is of interest to compare PS II with purple bacteria. The reaction center of purple bacteria contains 3 polypeptides (L, M, H-subunit) where only the L- and M-subunits are indispensable for primary photochemical activity (recent review [30]). A complete dissociation of the H-subunit from reaction centers of *Rhodopseudomonas sphaeroides* R-26 does not affect the EPR signal of the $Q_A^- \cdot Fe^{2+}$ complex, but seriously inhibits the electron transfer between Q_A and Q_B, and reduces its sensitivity to inhibitors [30]. Similar effects are observed after trypsinization of thylakoids, which leads to destruction of the 32-kDa–ABP. Accordingly, despite the structural homologies between the L- and M-subunits of bacteria (that bind Q_A, Q_B and Fe) and the 32-kDa–ABP [28,31], it seems attractive to consider the idea that the latter component plays an indirect regulatory role which could be comparable to that of the H-unit in bacteria, rather than being directly involved in quinone binding at PS II. In this respect it is interesting to note, that the L-, M- and H-subunit of purple bacteria and the 32-kDa–ABP are assumed to be evolutionarily derived from a common precursor [28]. Therefore, during the development of the PS II reaction center, the 32-kDa–ABP might have become a regulatory polypeptide.

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