AMINE-INDUCED INHIBITION OF PHOTOSYNTHETIC OXYGEN EVOLUTION

A correlation between the microwave power saturation properties of signal IIf and photosystem II-associated manganese

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1. Introduction

Signals IIvf and IIf are light-generated free radicals detected by EPR in chloroplast thylakoid membranes [1,2]. Both signals arise from the same species, $Z^{\cdot+}$, which is an intermediate electron carrier between the photosystem II reaction center, P680, and the site of water oxidation. The difference in nomenclature, IIvf *vs* IIf, reflects the fact that the light-generated $Z^{\cdot+}$ reduction halftime can be 'very fast' or 'fast' depending on the state of the oxygen-evolving complex. In oxygen-evolving chloroplasts, $Z^{\cdot+}$ reduction occurs in the sub-ms time range and, under these conditions, its EPR signal is denoted as IIvf; in chloroplasts in which the oxygen evolving capability has been inhibited, $Z^{\cdot+}$ is stable well into the ms-range and its EPR signal is denoted as signal IIf. The microwave power saturation properties of IIvf in O_2 evolving thylakoid membranes were compared with those of IIf in membranes in which the O_2 evolving capability had been inhibited with Tris [3]. They found that the IIvf could not be saturated at microwave powers of up to 200 mW; in contrast, the IIf species was homogeneously broadened with saturation at ~25 mW. Addition of $NiCl_2$ to Tris-inhibited membranes increased the level of power required to saturate the IIf signal. These findings were interpreted to indicate that IIvf was in the vicinity of a strongly relaxing species, perhaps the Mn^{2+} associated with photosynthetic oxygen evolution. Inhibition of oxygen evolution to produce IIf was suggested to perturb the association between $Z^{\cdot+}$ and Mn^{2+} and result in lower microwave power saturation properties for the free radical.

As part of our research into the properties of signal IIf and the role of Mn^{2+} in photosynthetic oxygen evolution we have developed procedures for controlling the extent to which Mn^{2+} is removed from the oxygen-evolving complex. We report here that we can inactivate oxygen evolution with NH_3 and induce the formation of signal IIf without loss of Mn^{2+} from the oxygen-evolving complex. By the use of NH_3 or of Tris we have been able to control Mn^{2+} liberation from photosystem II so that we can now demonstrate that signal IIf microwave power saturation properties are correlated with the integrity of the Mn^{2+} associated with the oxygen-evolving complex.

2. Materials and methods

Procedures for the isolation of thylakoid membranes freed of contaminating non-functional Mn^{2+} and the detection of signal IIf by EPR have been reported in [5,6]. Tris-inactivation was carried out by exposing membranes to 0.8 M Tris (pH 8) in room light at 4°C for 20 min [7]; for NH_3 inhibition, 120 mM NH_4Cl (pH 8) was used. The inhibited membranes (routinely 3 mg chl/ml) were transferred directly to an EPR flat cell (Scanco S-813) for measurements in a Bruker ER200D X-band spectrometer. Instrument settings are denoted in the figure legends.

Abbreviations: EPR, electron paramagnetic resonance; Z, primary donor to photosystem II; PS, photosystem; Chl, chlorophyll

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Methylviologen (100 μM) was the electron acceptor; catalase (1 mg/ml) was also present to regenerate oxygen and prevent a steady-state accumulation of the methylviologen free radical. Saturating white light was provided by a microscope illuminator.

3. Results

Table 1 presents data on the amounts of EPR-detectable Mn^{2+} released from thylakoid membranes by exposure to Ca^{2+} alone [8] and to Tris or NH_3 in the presence and absence of Ca^{2+}. A small amount of Mn^{2+} is detected by addition of Ca^{2+} alone to these membranes; with Tris and Tris + Ca^{2+}, 1 and 2 Mn^{2+}/photosystem II trap are released (after correction for Mn^{2+} released by Ca^{2+} alone) into an EPR-detectable form, consistent with the action of Tris as an inhibitor of oxygen evolution [9]. For NH_3, on the other hand, no Mn^{2+} is released in excess of that observed with Ca^{2+} alone, a finding coincident with the observation that NH_3 produces a freely reversible attack on the S_2 and S_3 states of the oxygen-evolving system [10]. Fig.1 presents EPR spectra of illuminated chloroplast thylakoid membranes in the presence of 120 mM NH_3. Comparison of the spectra obtained on illumination clearly show the presence of signal IIf. Note, however, that if the microwave power is increased (to 200 mW) the amplitude of the light-induced IIf change is increased, whereas the amplitude of the signal I change is decreased. This finding suggests that although signal IIf is present in NH_3-inhibited thylakoid membranes, its power saturation

Table 1
Effect of Tris, NH_3 and Ca^{2+} on EPR-detectable Mn^{2+} released from chloroplast thylakoid membranes

Addition	EPR-detectable Mn^{2+} [a] (per 400 chl)
40 mM Ca^{2+}	0.4–0.6
0.8 M Tris/light	1.2
0.8 M Tris/light, 40 mM Ca^{2+}	2.6
0.12 M NH_3	0
0.12 M NH_3/40 mM Ca^{2+}	0.4

[a] Determined by a comparison of the amplitudes of line 3 (low field side of g = 2.00) to standard Mn^{2+} solutions

Acidification in the presence of 40 mM Ca^{2+} yielded 4.6–4.8 Mn^{2+}/400 chl. EPR conditions were: power = 100 mW; modulation amplitude, 9.5 Gpp; center field = 3385 G

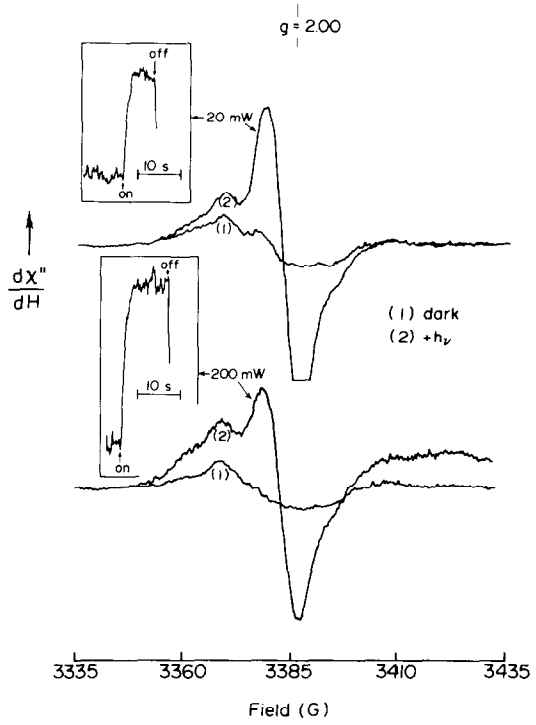

Fig.1. Signal IIf induction by exposure of thylakoid membranes to NH_3. Conditions: modulation amplitude, 4 Gpp; center field, 3385 G; scan rate, 60 G/min; time constant, 0.2 s; gain, 1 × 10^6. Insets: field locked on IIf (3370 G); gain, 2 × 10^6. Conditions of illumination as indicated.

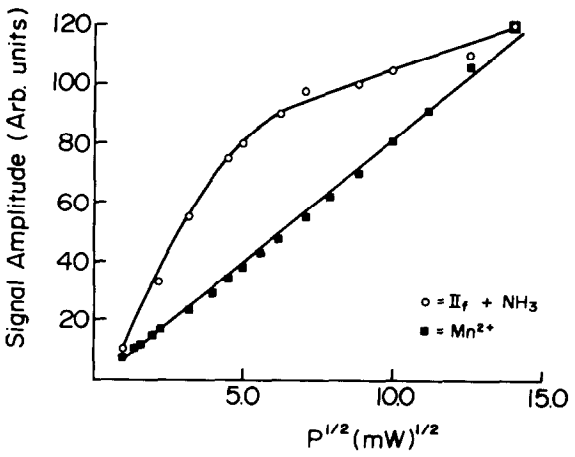

Fig.2. Microwave power saturation properties of signal IIf in NH_3-inhibited thylakoid membranes and of $Mn(H_2O)_6^{2+}$. EPR conditions as in fig.1.

properties remain similar to those observed for signal IIvf in oxygen-evolving chloroplasts [3].

A further examination of the properties of signal IIf in NH_3-inhibited thylakoid membranes is presented in fig.2, where the light-induced change is not saturated at the highest microwave power we have available. In view of the fact that this phenomenon is observed under conditions where the Mn^{2+} required for O_2 evolution activity is retained by the membranes, we have also presented in this figure the results of a power saturation experiment on $Mn(H_2O)_6^{2+}$; as can be seen from this figure, the hexaquo ion is not power saturated. A comparison of the two curves in fig.2 shows that while the amplitude of the Mn^{2+} EPR signal is linear with the square root of applied microwave power, that of signal IIf shows deviations from linearity and indicates that its relaxation properties are complex. These results on IIf in NH_3 inhibited chloroplasts are substantially different from those observed in [3] for this species, in Tris-washed preparations, i.e., we show here that IIf retains a power saturation property reminiscent of that observed for signal IIvf.

If the result obtained in fig.2 is due to coupling of IIf to a strongly relaxing paramagnetic species, namely Mn^{2+}, then perturbation of Mn^{2+} associated with thylakoid membranes should produce a corresponding change in the microwave power saturation properties of signal IIf. That this is in fact the case is demonstrated by the data of fig.3, where power saturation data on signal IIf in Tris- or Tris, Ca^{2+}-inhibited membranes is presented. A comparison of these data with those in fig.2 shows that Mn^{2+} release from the oxygen-evolving complex produces a profound effect on the power saturation properties of signal IIf, and further that sequential liberation of 1 or 2 Mn^{2+} from the oxygen-evolving complex (by Tris or by Tris + Ca^{2+}) produces a perceptible difference in IIf power saturation. These data and those of fig.2 are collected in summary in table 2, where it is clear that the sequential loss of Mn^{2+} from the oxygen-evolving complex correlates with decreases in the microwave power levels necessary to saturate signal IIf.

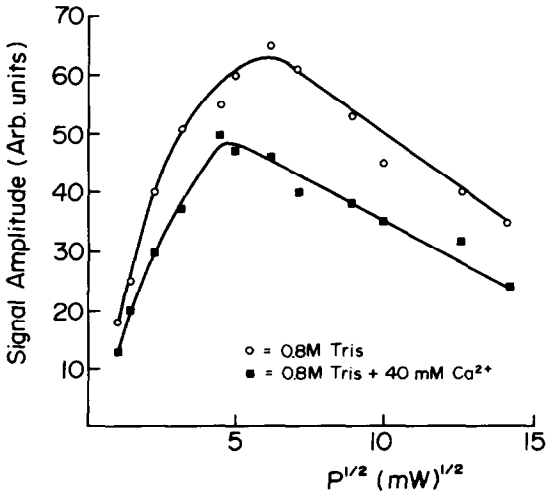

Fig.3. Microwave power saturation properties of signal IIf in Tris-inhibited thylakoid membranes. EPR conditions as in fig.1.

Table 2
Effect of Mn^{2+} release from thylakoid membranes on the power saturation properties of signal IIf

Treatment	Mn^{2+} released[a]/ 400 chl	Signal IIf power saturation[b] (mw)
NH_3	0	>200
Tris/light	1.2	39
Tris light + 40 mM Ca^{2+}	2.0	20

[a] Corrected for Mn^{2+} released by Ca^{2+} alone
[b] Estimated values obtained from the data in fig.2 and 3

4. Discussion

Signal IIf is induced in chloroplast thylakoid membranes where oxygen evolution has been inactivated by exposure to Tris [2], a treatment which also releases thylakoid bound Mn^{2+} [7]. As we show here, the block at S_2 or S_3 states of the oxygen-evolving system by NH_3 [10] is sufficient to induce signal IIf, and more importantly, has enabled us to demonstrate that retention of Mn^{2+} in the oxygen-evolving complex produces a condition whereby signal IIf cannot be microwave power saturated. By substituting Tris or Tris + Ca^{2+} for NH_3, we have been able to correlate changes in the power saturation properties of signal IIf with the liberation of Mn^{2+} from the oxygen-evolving complex. The data we have obtained for NH_3-inhibited membranes are qualitatively similar to the data on signal IIvf in [3] and provide further evidence that IIf and IIvf are the same species (see [11]) and that this component is in close association with Mn^{2+} presumed to be an integral component of the oxygen-evolving complex.

Our data suggest that the presence of NH_3 in the oxygen-evolving complex does not induce dramatic perturbations to either Mn^{2+} or to the signal IIf species. This may be a consequence of the possibility [10] that NH_3 replaces water as a substrate for the oxygen-evolving reaction sequence. In pursuing this question further, our preliminary work shows that other amines may be used to block oxygen evolution without extraction of Mn^{2+} but that in certain instances, they also lower the power required to saturate signal IIf.

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