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Hydroxylamine, hydrazine and methylamine donate electrons to the photooxidizing side of Photosystem II in leaves inhibited in oxygen evolution due to water stress

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When leaf discs are water stressed, they lose the capacity for photosynthetic oxygen evolution and variable (chlorophyll *a*) fluorescence. Such a loss of variable fluorescence was previously reported by Govindjee et al. (Plant Sci. Lett. 20 (1981) 191–194). The later activity is not lost if prior to the water-stress treatment the leaf is incubated with typical water analogs known to act as electron donors to Photosystem II, such as hydroxylamine and hydrazine. Methylamine also acts in the same fashion. These results indicate that one of the sites of drought damage is the oxidizing side of Photosystem II, and that electron donors can restore electron transport, at least to the plastoquinone pool, similar to their effect in Tris treatment of isolated chloroplasts.

Photosynthetic water cleavage requires the cycling of a donor complex of Photosystem II (PS II) between five oxidation states, S_0–S_4 [1]. Hydroxylamine (NH_2OH) and hydrazine (NH_2NH_2) which are both analogs of two H_2O, can act as artificial electron donors in Tris-treated chloroplasts devoid of oxygen evolution. At low concentrations, these donors can also act in O_2-evolving competent chloroplasts as competitive inhibitors of water oxidation without destroying the O_2-evolving complex [2–5].

Water stress in leaves was shown to inhibit CO_2 fixation, which partly could be traced to the closure of stomata, as well as an increased diffusion resistance of mesophyll cells [6–8]. Chloroplasts, which were isolated from such stressed leaves, exhibited reduced PS II activities [9]. However, this could be due to non-specific improper conditions of the biological material during preparation. On the other hand, changes in the typical (chlorophyll *a*) fluorescence transients observed from stressed leaves indicated changes in the photochemical apparatus, and it was suggested that water stress caused damage specifically to Photosystem II [10].

We investigated water stress in leaves by a combined photoacoustic and fluorimetic methods [11]. The photoacoustic method has an advantage that it is largely independent of the state of the stomata, and hence it is capable of giving a more direct indication on the photosynthetic apparatus (unpublished results).

During an extensive investigation on water stress we came upon the observation that in stressed leaves, in which oxygen evolution was inhibited and the variable fluorescence decreased significantly, the latter could be restored by incubating the leaves, prior to the stress treatment with the water analog donors. The oxygen evolution itself was not affected by this incubation. In

Abbreviation: PS II, Photosystem II.

this report we focus attention on the effect of the electron donors, while a more comprehensive account of water stress will be reported elsewhere. The sum of all observations suggests that there is a site for water stress damage in the oxidizing side of PS II and that water analog compounds can penetrate into the leaves' internal structures and act as donors to PS II.

A photoacoustic apparatus as described in Refs. 11 and 12 was used to monitor O_2 evolution yield from intact tobacco leaves. The photoacoustic signal arises from the release of modulated heat and modulated photosynthetic oxygen evolution as a result of absorption of modulated light by a leaf. The two contributions can be separated and the photoacoustic oxygen signal (O) divided by the photothermal signal (T) is taken to be proportional to the quantum yield of oxygen evolution [12]. Photochemical energy storage was measured at high chopping frequency by the extent of increase of the photothermal signal in the presence of photosynthetically saturating light, as described in Refs. 11 and 12. Fluorescence rise curves from the upper surface of the same leaf disc, inserted in the photoacoustic cell, were monitored [13] after 1 h of dark adaptation time. Excitation light of broad wavelength band was obtained from a d.c. projector equipped with a Corning 4-96 filter (400–600 nm). The exciting light, of intensity 40 W/m^2, was passed on to the leaf through a branched fiber optics which also conducted fluorescence from the same surface of the leaf to the photodetector. Fluorescence emission was filtered through a 680 nm transmitting plus 700 nm short pass interference filters (Ditric Optics) and was detected by a photodiode (EG and G). The resulting signal was recorded on a Tektronix 564B storage oscilloscope. Normalized fluorescence area was determined by dividing the area by a ratio equal to the variable fluorescence yield as described in detail in ref. 13.

Water stress was imposed on tobacco (*Nicotiana tabacum* L., var Xanthi) leaf discs by exposing them to air of 53% relative humidity, for several hours at low light intensity (4 W/m^2) or darkness with equal consequences as described in Ref. 14. Relative water content was measured according to the ratio: (Fresh weight − dry weight)/(water-saturated weight − dry weight), as

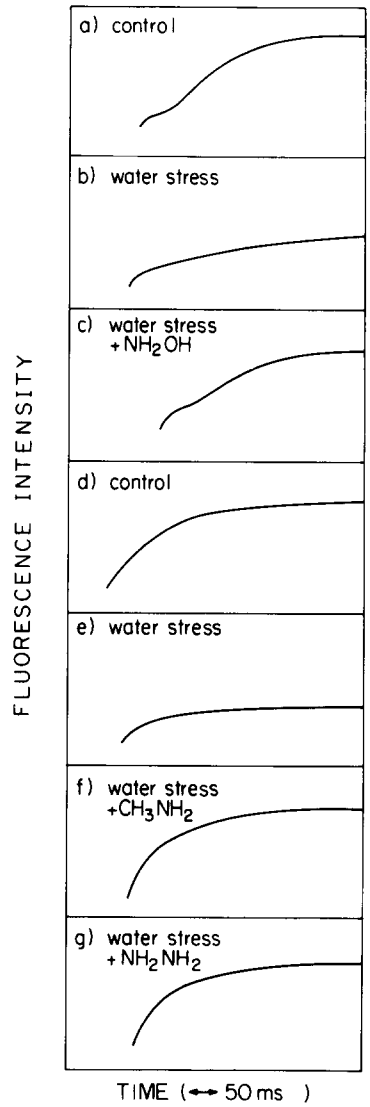

Fig. 1. Fluorescence induction curves of tobacco leaves. Full scale on oscilloscope traces is 500 ms. (a)–(c) is one series of experiments, (d)–(g) is another series of experiments. (a) Control: relative water content, W, (c.f. Materials and Methods) 85%; oxygen-evolution yield O/T; 2.5 (100%); relative variable fluorescence F_v 3.8 (100%); relative normalized area above fluorescence induction A 100%. (b) Leaf exposed to 4 h water stress; W 32%l O/T 0.7 (28%); F_v 1.8 (47%); A 94.5%. (c) leaf incubated in 10 μM hydroxylamine for 1 h and then exposed to 4 h water stress W 32%; O/T 0.98 (39%); F_v 3.4 (89%); A 94.1%; (d) control: W 85%; O/T 2.5 (100%); F_v 3.7 (100%); A 100%; (e) leaf exposed to 4 h water stress: W 32%; O/T 0.72 (29%); F_v 1.7 (46%); A 92%; (f) leaf incubated in 5 mM methylamine and then exposed to water stress: W 32%; O/T 0.77 (31%); F_v 3.7 (100%); A 83%; (g) leaf incubated in 10 mM hydrazine and exposed to water stress: W 32%; O/T 0.77 (31%): F_v 3.6 (97%): A 83%.

in ref. 15. As suggested by Sinclair and Ludlow [15], relative water content is a major determinant of metabolic activity and leaf survival and should be used instead of the less reliable leaf water potential. Moreover, leaf water potential cannot be easily measured in leaf discs. For assaying the effect of electron donors, leaf discs were incubated by floating them in a solution of either hydroxylamine-HCl (Fluka, Switzerland), hydrazine (Fluka) or methylamine (Merck, Germany) for 1 h, and were directly tested by fluorescence induction and oxygen evolution yield, or further exposed to water stress for 4 h after 1 h incubation in the electron donor solution, and tested as above.

Fig. 1a depicts the primary phase of the fluorescence induction curve of a dark-adapted tobacco leaf disc. Similar to variable fluorescence kinetics in isolated chloroplasts [13], there is usually an initial rise from the non-variable level F_0 to a somewhat higher plateau level which is followed by a large increase to the maximal level of fluorescence yield, F_{max}. The large increase in fluorescence yield reflects the photoreduction of the plastoquinone pool and the accumulation of reductive equivalents on Q, the primary electron acceptor of PS II.

After exposing the leaf disc to water stress for 4 h, there was a drastic change in the fluorescence induction (Fig. 1b) as also reported by Govindjee et al. [10]. The final level F_{max} decreased by a large extent, although the initial level F_0 remained essentially the same. Thus the yield F_v of the variable fluorescence $F_v = (F_m - F_0)/F_0$ decreased to about 47% of the control, untreated leaf. The oxygen evolution quantum yield decreased to about 28% of the control. The extent of the water stress was measured by relative water content which decreased from 85% to 32%. The inhibition of oxygen evolution was reversible when the stressed leaf disc was immersed in water for about 2 h. Recovery of O_2 evolution and fluorescence maximum were 88% and 100% of the control (Table I). When a leaf disc was initially incubated in low NH_2OH (10 μM) concentration for 1 h and then exposed to 4 h of water stress (Fig. 1c) the maximal fluorescence yield F_m remarkably remained at a high level and the fluorescence induction transient was similar to that of the control. At the same time, however, the inhibition degree of oxygen evolution remained almost the same. Incubation of a control leaf disc in low concentration (10 μM) of hydroxylamine for 1 h resulted in no significant change in either oxygen evolution or variable fluorescence yields, each of which was 100% of the control. We looked at the concentration dependence of the hydroxylamine effect and found that quite a low concentration (10 μM) was sufficient to obtain a donor activity, without affecting the oxygen-evolution activity of the control. At a much higher concentration (500 μM) NH_2OH was inhibitory to oxygen evolution, and oxygen evolution yield decreased to 47% of the control.

Similar experiments as above were conducted with hydrazine and methylamine. Hydrazine (10 mM) and methylamine (5 mM) had no effect on either oxygen evolution or fluorescence yields in control leaves. Again, hydrazine and methylamine restored fluorescence in water-stressed leaves with similar results (Fig. 1d–g), however higher con-

TABLE I

RECOVERY OF OXYGEN EVOLUTION AND VARIABLE FLUORESCENCE FROM WATER STRESS IN INTACT LEAVES

Treatment	Relative water content (%)	Oxygen yield (O/T) (%)	F_v (%)	Normalized area above fluorescence induction (% of control)
Control intact leaf	85	2.5 (100)	3.84 (100)	100
Water-stressed leaf (4 h)	32	0.7 (28)	1.80 (47)	94.5
Water-stressed leaf (4 h) rehydrated for 2 h	85	2.2 (88)	3.84 (100)	100

centration (10 mM) could be used with no inhibitory effect. In all the above-mentioned experiments the treatment by the water analogs was performed prior to the exposure of the same leaf to water stress, since a reversed experimental procedure would result in a complete recovery of desiccated leaves within the one hour of floating in the water as found for control leaves.

We also compared the normalized area above the fluorescence induction curves which is proportional to the total pool of electron acceptors – per active reaction centers (mostly the plastoquinone pool) between the two photosystems [13]. Upon exposure of the leaves to dehydration, the normalized fluorescence induction area diminished only by a small extent. Exposure of leaves to dehydration in the presence of hydroxylamine resulted in a somewhat smaller area (legend of Fig. 1) indicating only marginal change of the yield and extent of reduction of the total electron-acceptor pool compared to the control.

Studying photosynthesis in intact leaves is hampered by the complexity of the internal structure which makes the interpretation of physiological effects due to stress difficult and multifactorial. In addition, it is considered difficult to administer chemicals and study their effects on the photosynthetic apparatus, since a double barrier of leaf cell membrane and chloroplast membrane has to be crossed. In this paper, we have shown three new aspects. (1) Water analogs such as hydroxylamine, hydrazine and methylamine diffuse through both the cell membrane and the chloroplast membrane of an intact leaf in order to exert their effect. (2) They can serve as electron donors to PS II in intact leaves which were exposed to water stress. (3) Recovery of maximal fluorescence yield by these water analogs with the persistence of inhibition in oxygen-evolution yield suggests that one site of damage by water stress resides in the oxidizing side of PS II.

In the case of hydroxylamine these results were obtained at a very low concentration (10 μM), which by itself did not inhibit oxygen evolution, unless water stress has occurred. Comparing the donor activity of hydroxylamine for Tris treatment of chloroplasts (about 0.5 mM for half recovery of the variable fluorescence), it is remarkable that for water-stressed leaf it is at least 50 times stronger (about 10 μM are sufficient for full effect). This difference in the concentration range of hydroxylamine is even more remarkable, since usually 10-fold increase of a substance is needed in leaves to get the same effects as in chloroplasts [16]. It is possible that the damage inflected by water stress to the oxygen-evolving apparatus is much less severe than in Tris treatment. In the latter treatment there are indications that essential factor(s) are extracted [1]. In water-stressed leaf, one may consider the possibility that a conformational change has occurred in which the O_2-evolving complex protein cannot undergo the S-state changes, but apparently water analogs can still bind to the protein cleft and undergo photooxidation. Methylamine is a known uncoupler in chloroplasts, at concentrations higher than 60 μM [17]. In intact leaves, however, we did not observe any uncoupling effect at our concentration of 5 mM, as reflected by the preservation of the yield of oxygen evolution as well as the extent of photochemical energy storage measured photoacoustically (not shown). The effect of methylamine as a donor is probably documented here for the first time, in addition to the other well-known donors in chloroplasts.

It appears from the effect of electron donors, that the oxidizing side of PS II, possibly the oxygen-evolving complex, is an important primary target for the effect of leaf dehydration. Our suggestion is in agreement with Govindjee et al. [10] who attributed their observed fluorescence changes in water-stressed leaves to specific damage in PS II. The overall effects of water stress on photosynthesis have been studied extensively and will be reported elsewhere.

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