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HYDRAZINE AS AN ELECTRON DONOR TO THE WATER-OXIDATION SITE IN PHOTOSYNTHESIS

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SUMMARY

Hydrazine is an electron donor to the oxidizing side of Photosystem II in photosynthesis. Hydrazine supports a light-dependent electron flow in chloroplasts inhibited at the water-oxidation site. This reactivated electron flow requires 10–20 mM hydrazine for maximal activity and is inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea. In addition, hydrazine restores the fluorescence yield of the chlorophyll previously lowered by water-oxidation inhibitors and does not alter the oxidation or reduction of cytochrome *f*.

The sites of electron transfer on the oxidizing side of the photo-act of Photosystem II in photosynthesis can be reduced by several compounds. These compounds by-pass the inhibition of the water-oxidation step and allow a light-dependent electron flow from the donor compound to electron acceptor. YAMASHITA AND BUTLER[1] found that phenylenediamine (+ ascorbate) restored electron flow which had been inhibited by washing the chloroplasts in high concentrations of Tris. IZAWA *et al.*[2] showed that hydroxylamine functioned as an electron donor by eliminating the inhibition of electron flow due to Cl^- deficiency. Furthermore, hydroxylamine also can overcome the inhibition induced by manganese deficiency[3]. VERNON AND SHAW[4] have used semicarbazide as an electron donor to the oxidizing side of Photosystem II in order to study the fractionation of that photosystem by Triton. Ascorbic acid[2] and Mn^{2+} (ref. 5) among other compounds[6] have been used as electron donors. This paper reports that hydrazine can also serve as an electron donor.

Two types of chloroplasts preparations were made from commercial spinach — uncoupled chloroplasts prepared by an EDTA wash[2] and coupled chloroplasts prepared in sucrose[7]. Hydrazine sulfate, from Mathison, Coleman and Bell (Chicago, Ill.), was neutralized to the correct pH prior to the experiments. The *o*-chlorophenolindo-2,6-dichlorophenol (TCIP) reduction was performed as described by HIND *et al.*[7]. Ferredoxin was prepared according to SAN PIETRO AND LANG[8] and not purified further. $NADP^+$ reduction was measured at 340 nm with a reference set at 370 nm at 16° with an intensity of actinic light (645 nm ± 5 nm) of 52 kergs/cm²·sec using an Amino-Chance Double Beam Spectrophotometer. The cytochrome spectra were obtained using the Double Beam Spectrophotometer. The intensity of actinic illumination

Abbreviations: TCIP, *o*-chlorophenolindo-2,6-dichlorophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

(715 nm ± 5 nm) in these experiments was 2.0 kergs/cm^2·sec. Three separate methods were used to inhibit the water-oxidation step of electron transport: Cl^- deficiency[7], high concentrations of ammonia[9], and heating at 40° for 15 min (ref. 2).

Chemical similiarities between hydroxylamine and hydrazine suggested that hydrazine could serve as a donor. When water oxidation is inhibited by Cl^- deficiency, added hydrazine increases the electron flow to TCIP (Fig. 1A). The rate of dye reduction in the dark is sizable, especially at high concentration of hydrazine. However, the figure shows only the light-supported electron flow (Light–Dark). The control with added Cl^- shows a slight inhibition of water oxidation by hydrazine. For chloroplasts without added Cl^-, the electron flow rate with the addition of a high concentration of hydrazine never quite reaches the control rate obtained with added Cl^-. The maximum stimulation of electron flow (without Cl^-) by hydrazine is reached at concentration of about 10 mM.

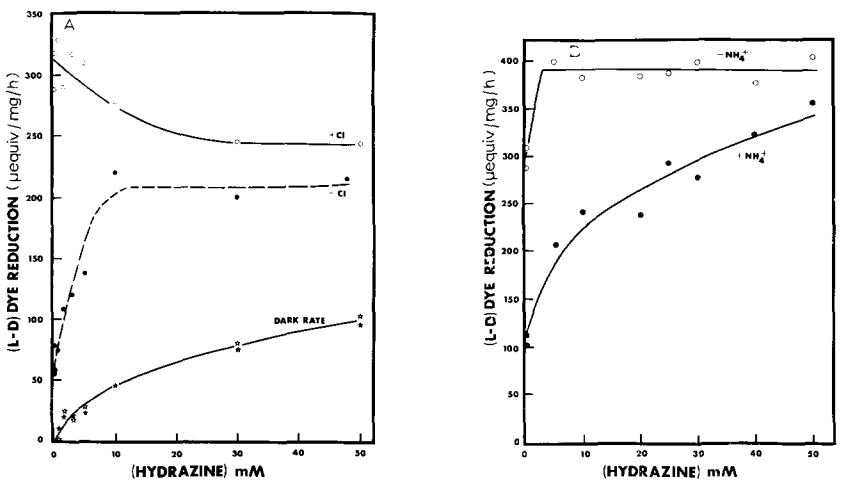

Fig. 1. Hydrazine as an electron donor with an inhibition of the oxidation of water. A. Cl^- deficiency as an inhibitor. Chloroplasts (10 μg/ml), prepared by EDTA wash, were suspended in 0.1 M sucrose–0.03 M *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid buffer (pH 7.9)–5 mM $MgSO_4$–50 mM SO_4^{2-} (as Na^+ or neutralized hydrazine)–25 μM TCIP and, where indicated, 10 mM NaCl and hydrazine, neutralized to pH 7.9. Rate expressed as per mg chlorophyll. B. Ammonia as an inhibitor. Chloroplasts (10 μg/ml), prepared by sucrose wash, were suspended as above at pH 7.7 with 20 mM NaCl–100 mM SO_4^{2-} as (Na^+, NH_4^+ or neutralized hydrazine) and, where indicated, 100 mM NH_4^+. Rate expressed as per mg chlorophyll.

Hydrazine also by-passes the ammonia inhibition of water-oxidation, as shown in Fig. 1B. Hydrazine apparently uncouples these chloroplasts. This uncoupling is observed by the stimulation of electron flow for the $-NH_4^+$ case. Again even with 50 mM hydrazine, the electron flow rate does not quite equal that obtained without ammonium ion present. Although not shown here, the electron flow rate obtained with high concentrations of hydrazine is nearly totally inhibited (95 %) by 3-(3,4 dichlorophenyl)-1,1-dimethylurea (DCMU) for figs. 1A and B.

Hydrazine can also donate electrons photosynthetically to $NADP^+$ through ferredoxin and $NADP^+$ reductase (Table I). In this case, the electron flow from water oxidation has been inhibited by a mild heating. The rates of electron transfer are relatively low due to the high pH and heating. Again the rate of electron flow reaches

TABLE I

HYDRAZINE AS AN ELECTRON DONOR TO FERREDOXIN–$NADP^+$

Chloroplasts (20 μg chlorophyll per ml), prepared by an EDTA wash and heated for 15 min at 40°, were suspended in 125 mM sucrose–19 mM NaCl–3 mM $MgSO_4$–19 mM Tricine buffer (pH 8.4)–50 mM SO_4^{2-} (as either neutralized hydrazine or Na^+)–167 μM $NADP^+$ and, where indicated 21 μM ferredoxin, and 7 μM DCMU. The $NADP^+$ reduction was measured as in the text (340–370 nm), expressed as per mg chlorophyll.

Hydrazine (mM)		*$NADP^+$ reduction (μequiv/mg·h)*
0		0.3
3.3		11
6.7		13
10		14
20		18.5
30		21.5
50		20
100		20
100	+ DCMU	1
100	— Ferredoxin	0

a maximum when the concentration of hydrazine is about 20 mM. The hydrazine-supported rate is nearly totally inhibited by DCMU or by a lack of ferredoxin. In addition, no reduction of $NADP^+$ in the dark is observed with $NADP^+$/ferredoxin as an acceptor.

Hydrazine restores the high fluorescence yield induced by actinic illumination if the yield is lowered by the lack of Cl^- (see ref. 10), as shown in Table II. The inital yield or the yield due to excitation by the measuring beam (given by ϕ_0) is also increased by hydrazine. This would indicate that hydrazine increases the electron flow from Photosystem II, leading to an increase in the reduction of the primary acceptor of the photo-act of Photosystem II (Q) by both the measuring beam and the actinic light. For a more complete explanation, the reference by DUYSENS[11] should be consulted. The increased reduction of Q by the actinic light (or ϕ_F) with added hydrazine is

TABLE II

FLUORESCENCE YIELD OF ISOLATED CHLOROPLASTS WITH HYDRAZINE AS AN ELECTRON DONOR

Chloroplasts (20 μg/ml chlorophyll), prepared by an EDTA wash, were suspended in 0.1 M sucrose–0.03 M Tricine (pH 8.0)–5 mM $MgSO_4$–50 mM SO_4^{2-} (as Na^+ or neutralized hydrazine) and, where indicated, 25 mM hydrazine and 10 mM NaCl. Actinic illumination was blue light (480–540 nm) at an intensity of 5 kergs/cm²·sec. The relative fluorescence yield was measured by signal averaging techniques as described in ref. 10, with Φ_0 (measuring beam) for – Cl set to 1.00. The variation in the measured fluorescence yield was less than 2 %.

Conditions		*Relative fluorescence yield*	
		ϕ_0 (measuring beam)	*ϕ_F (+ actinic)*
+Cl		1.09	4.04
	$+NH_2NH_2$	1.54	4.60
–Cl		1.00	3.28
	$+NH_2NH_2$	1.33	4.78

consistent with hydrazine by-passing the —Cl^- inhibition site. Hydrazine increases electron flow and, likewise, the fluorescence yield. The fluorescence yield measurements show that hydrazine acts as a donor to System II, similar to hydroxylamine.

Hydrazine does not seem to reduce the components of System I, as shown in Fig. 2. Cytochrome *f* is oxidized by a System I-activating light of 715 nm (on response) and, if water is the electron donor, remains oxidized when the illumination is extinguished (off-response). This same response is observed when hydrazine is the electron donor. Reduced hydroquinone and ascorbate (see ref. 1) interact strongly with the components of system I, causing a reduction of the oxidized cytochrome *f* when the actinic light is extinguished. Thus, hydrazine apparently acts as a donor to only components in Photosystem II.

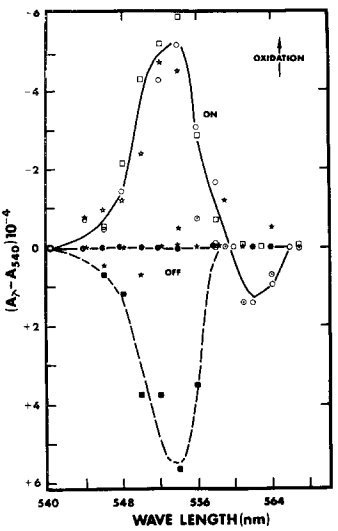

Fig. 2. Spectrum of cytochrome *f* reduction with electron donors. Chloroplasts (50 μg/ml), prepared by EDTA wash, were suspended in 0.03 M NaCl–0.04 M Tricine buffer (pH 8.4)–5 mM $MgSO_4$–167 μM methyl viologen–133 mM SO_4^{2-} (as neutralized hydrazine or Na^+) and either with water as a donor (○) using normally prepared chloroplasts, or with 33 mM hydrazine (✲) as a donor or with 100 μM hydroquinone and 300 μM sodium ascorbate (□) as a donor, using heated chloroplasts (40° for 15 min). On = initial response with 715 nm actinic light (open symbols). Off = initial response after extinguishing the actinic light (closed symbols). Reference wavelength was set at 540 nm. Slit width was 2 nm.

Studies of oxygen evolution, using methyl viologen as the electron acceptor, indicate that hydrazine also acts as an competitive inhibitor of water oxidation. The rates of electron flow supported by hydrazine and measured by this method can be as high as 2500 μequiv/mg chlorophyll·h. The flash yield of oxygen, investigated by K. MANTAI AND G. HIND[12], is too high for normal photosynthesis when hydrazine is the donor. Hydrazine or the products of hydrazine oxidation seem to alter the apparent oxygen in the solution. The uptake of oxygen through autooxidation of methyl viologen, therefore, should not be used to indicate electron flow with hydrazine present.

Hydrazine is an electron donor to site(s) on the oxidizing side of Photosystem II since hydrazine supports a light-dependent electron flow to dye or $NADP^+$, which is

inhibited by DCMU; eliminates the inhibition of electron flow due to Cl^- deficiency, ammonia, and mild-heating; and increases the fluorescence yield. Hydrazine may prove to be a valuable donor to Photosystem II due to its lack of interaction with the components of Photosystem I and its lack of inhibition at sites near the water oxidation mechanisms (compare with hydroxylamine, ref. 2).

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