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THE MECHANISM OF THE OXIDATION OF ASCORBATE AND Mn^{2+} BY CHLOROPLASTS

THE ROLE OF THE RADICAL SUPEROXIDE

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SUMMARY

1. The mechanism of the photooxidation of ascorbate and of Mn^{2+} by isolated chloroplasts was reinvestigated.

2. Our results suggest that ascorbate or Mn^{2+} oxidation is the result of the Photosystem I-mediated production of the radical superoxide, and that neither ascorbate nor Mn^{2+} compete with water as electron donors to Photosystem II nor affect the rate of electron transport through the two photosystems: The radical superoxide is formed as a result of the autooxidation of the reduced forms of low potential electron acceptors, such as methylviologen, diquat, napthaquinone, or ferredoxin.

3. In the absence of ascorbate or Mn^{2+} the superoxide formed dismutases either spontaneously or enzymatically producing O_2 and H_2O_2. In the presence of ascorbate or Mn^{2+}, however, the superoxide is reduced to H_2O_2 with no formation of O_2. Consequently, in the absence of reducing compounds, in the reaction H_2O to low potential acceptor one O_2 (net) is taken up per four electrons transported where as in the presence of ascorbate, Mn^{2+} or other suitable reductants up to three molecules O_2 can be taken up per four electrons transported.

4. This interpretation is supported by the following observations: (a) in a chloroplast-free model system containing NADPH and ferredoxin–NADP reductase, methylviologen can be reduced to a free radical which is autooxidizable in the presence of O_2; the addition of ascorbate or Mn^{2+} to this system results in a two fold stimulation of O_2 uptake, with no stimulation of NADPH oxidation. The stimulation of O_2 uptake is inhibited by the enzyme superoxide dismutase; (b) the stimulation of light-dependent O_2 uptake in the system $H_2O \rightarrow$ methylviologen in chloroplasts is likewise inhibited by the enzyme superoxide dismutase.

5. In Class II chloroplasts in the system $H_2O \rightarrow$ NADP upon the addition of ascorbate or Mn^{2+} an apparent inhibition of O_2 evolution is observed. This is

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Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; HEPES, hydroxyethylpiperazineethanesulfonic acid; MES, (*N*-morpholino)ethanesulfonic acid; DCIP, 2,4-dichlorophenolindophenol.

explained by the interaction of these reductants with the superoxide formed by the autooxidation of ferredoxin, a reaction which proceeds simultaneously with the photoreduction of NADP. Such an effect usually does not occur in Class I chloroplasts in which the enzyme superoxide dismutase is presumably more active than in Class II chloroplasts.

6. It is proposed that since in the Photosystem I-mediated reaction from reduced 2,4-dichlorophenolindophenol to such low potential electron acceptor as methylviologen, superoxide is formed and results in the oxidation of the ascorbate present in the system, the ratio ATP/2*e* in this system (when the rate of electron flow is based on the rate of O_2 uptake) should be revised in the upward direction.

INTRODUCTION

In recent years it has been suggested that in isolated chloroplasts ascorbate and Mn^{2+} can competitively replace water as electron donors to Photosystem II[1-4] This interpretation has been based on the observation that O_2 uptake is stimulated 2–3-fold upon the addition of ascorbate (or Mn^{2+}) to chloroplasts in the presence of a low potential, autooxidizable electron acceptor[1-4]. The stimulation of O_2 uptake was shown to be sensitive to 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU)[1-4] and it exhibited a red drop in quantum efficiency[3,4] implicating the involvement of Photosystem II. When NADP served as the electron acceptor, conflicting results have been reported[2-4] One group reported that in the presence of NADP the addition of ascorbate or Mn^{2+} caused an inhibition of O_2 evolution which they assumed was due to the replacement of water by ascorbate or Mn^{2+} as the electron donor since the rate of NADP reduction was not affected[3,4]. On the other hand, according to Bohme and Trebst[2], when NADP serves as the electron acceptor the addition of ascorbate is without effect on O_2 evolution and thus does not appear to compete with water as an electron donor to Photosystem II[2]. It remained a troublesome point, however, why the nature of the terminal acceptor (whether methylviologen or NADP) should affect the competition between ascorbate or H_2O as electron donors to System II.

Our results are not in agreement with the assumption that ascorbate or Mn^{2+} can compete with water as electron donors to Photosystem II. We suggest alternatively that the stimulation of O_2 uptake by ascorbate in the presence of methylviologen or diquat and the occasional inhibition of O_2 evolution by ascorbate in the presence of NADP are the result of the oxidation of ascorbate by superoxide. Superoxide is produced as the results of the autooxidation of the reduced forms of low potential electron acceptors such as methylviologen, diquat, napthoquinone, flavin mononucleotide and ferredoxin. A preliminary report of part of this work has been presented previously[15]

MATERIALS AND METHODS

Broken Class II chloroplasts were prepared from fresh lettuce bought in the market (*Latuca sativa* var. romaine) by a modification of the procedure of Avron[5]. 50 g of leaves were blended in a 220-V Waring blender for 15 s at 120 V in 120 ml

of medium, pH 8.0, containing 0.4 M sucrose, 0.01 M NaCl, 0.01 M Tricine, 0.05 M ascorbate and 0.1 % human serum albumin. The homogenate was filtered through 4 layers of gauze and centrifuged at $500 \times g$ for 2 min. The supernatant was then centrifuged at $2000 \times g$ for 7 min and the pellet was resuspended in 0.001 M Tricine, pH 8.0, and centrifuged at $7300 \times g$ for 5 min. The final pellet was resuspended in a minimal amount of 1 mM Tricine buffer, pH 8.0, containing 0.5 % human serum albumin and gently homogenized with a teflon homogenizer.

Whole Class I chloroplasts[6] were prepared by a modification of the procedure of Jensen and Bassham[7] from fresh lettuce leaves. 60 g of leaves which were preilluminated for 1 h with white light from incandescent lamps at an intensity of 1000 ft-candles, were blended in a 220-V Waring blender for 5 s at 120-V in 150 ml of medium, brought to pH 6.1, which contained 0.33 M sorbitol, 2 mM $NaNO_3$, 2 mM EDTA, 2 mM sodium isoascorbate, 1 mM $MnCl_2$, 0.5 mM KH_2PO_4, 50 mM (*N*-morpholino)ethanesulfonic acid (MES) and 20 mM NaCl. The homogenate was filtered through 20 layers of gauze and centrifuged at $2000 \times g$; the total time of centrifugation from start to stop was 40 s. The supernatant was decanted and the liquid removed by suction. The pellet was gently resuspended with the aid of a cotton-tipped glass rod in 0.5 ml of a medium containing 0.33 M sorbitol, 2 mM $NaNO_3$, 2 mM EDTA, 1 mM $MnCl_2$, 1 mM $MgCl_2$, 0.5 mM KH_2PO_4, 20 mM NaCl, and 50 mM hydroxyethylpiperazineethanesulfonic acid (HEPES) buffer, pH 6.7 (resuspension medium).

Swollen Class I chloroplasts were obtained by preincubating 0.08 ml Class I chloroplasts in 1.5 ml distilled water for 60 s to which was then added 1.5 ml 2 times concentrated reaction mixture. The reaction mixture was similar to the resuspension medium with the exception that NaCl was deleted, 5 mM pyrophosphate was added, and the pH of the HEPES medium was 7.6

Chlorophyll was determined according to the procedure of Arnon[8]. O_2 production or uptake was measured polarographically with a Clark-type electrode. NADP reduction was measured in a Cary 15 spectrophotometer modified to allow actinic illumination at right angles to the measuring beam; the phototube was protected from the actinic beam by a Corning filter 5840. The actinic beam was provided by a 500-W projector passing through a red filter (Corning 2403).

Superoxide dismutase was isolated from human erythrocytes by the procedure of McCord and Fridovich[9]

RESULTS

The addition of ascorbate to Class II chloroplasts of lettuce in the presence of a low potential acceptor such as methylviologen was found, in agreement with the results of others, to result in a 2–3-fold stimulation of O_2 uptake[1-3]. Similar results were obtained with swollen Class I chloroplasts either in the presence or absence of the uncoupler NH_4Cl when methylviologen served as the electron acceptor

However, when NADP served as the electron acceptor differing results were obtained upon the addition of ascorbate depending on whether Class I or Class II chloroplasts were used in the photoreaction

With hypotonically swollen Class I chloroplast with NADP serving as the electron acceptor, we found no inhibition of O_2 evolution upon the addition of

TABLE I

LACK OF EFFECT OF ASCORBATE ON OXYGEN EVOLUTION AND ON NADP PHOTOREDUCTION IN SWOLLEN CLASS I CHLOROPLASTS

All reactions were carried out in a 3-ml reaction mixture containing in μmoles sorbitol, 1000; $NaNO_3$, 6; EDTA, 6, $MnCl_2$, 3, $MgCl_2$, 3; KH_2PO_4, 1 5, HEPES buffer (ph 7 6), 150; pyrophosphate, 15, NADP, 15, saturating amount of ferredoxin; the indicated concentration of sodium ascorbate; and swollen Class I chloroplasts with 30 μg chlorophyll for the measurement of NADP photoreduction, or 60 μg for the measurement of O_2 evolution

Addition of ascorbate (mM)	*Rate/mg chlorophyll per h*			
	μmoles NADP reduced		*μatoms O_2 evolved*	
	Control	*+NH_4Cl (2 mM)*	*Control*	*+NH_4Cl (2 mM)*
0	62	169	44	144
0 033	64	180	62	140
0.1	67	169	68	148
1 7	67	174	42	132
3 3	67	174	62	150
10 0	64	174	56	134

ascorbate either in the absence or presence of uncoupler (Table I). However, in identical experiments with Class II chloroplasts, variable results were obtained from preparation to preparation. In Table II are presented two examples of experiments performed on different days exemplifying the extreme variability of the results. The chloroplasts in Expt I behaved like swollen Class I chloroplasts with ascorbate having little or no inhibitory effect on O_2 evolution either in the presence or absence of the uncoupler NH_4Cl. In contrast, the chloroplast in Expt 2 behaved quite differently. The addition of ascorbate to these chloroplasts in the absence of uncoupler resulted in complete inhibition of O_2 evolution. Paradoxically, the addition of

TABLE II

EFFECT OF ASCORBATE ON O_2 EVOLUTION IN CLASS II CHLOROPLAST

All reactions were carried out in a 3-ml reaction mixture containing in μmoles Tricine buffer (pH 8.0) 60, NaCl, 105; saturating amount of ferredoxin; broken Class II chloroplast with 60 g chlorophyll. Experiments were performed on different days.

	Addition	*H_2O to NADP (μmoles O_2 evolved/mg chlorophyll per h)*		*H_2O to ferredoxin (μmoles O_2 taken up/mg chlorophyll per h)*	
		Control	*NH_4Cl (2 mM)*	*Control*	*NH_4Cl (2 mM)*
Expt 1	None	68	102	10	6
	Ascorbate (1 mM)	86	100	16	32
Expt 2	None	34	0	50	86
	Ascorbate (1 mM)	0	−140	138	214

uncoupler to these chloroplasts (in the absence of ascorbate) also inhibited O_2 evolution. Addition of ascorbate in the presence of uncoupler caused a strong Mehler-type reaction, *i.e* O_2 uptake.

If NADP served as the sole electron acceptor in this system no O_2 uptake should have been observable even if the water-splitting reactions were completely inhibited. It thus seemed obvious that a Mehler-type reaction of some sort was occurring in these chloroplasts preparations where a net O_2 uptake takes place. As can be seen in Table II when NADP was omitted from the reaction mixture, those preparations which showed an inhibition of O_2 production upon the addition of ascorbate also showed much higher rates of O_2 uptake when ferredoxin served as the terminal electron acceptor These data suggested that ascorbate rather than serving as an electron donor at a site prior to Photosystem II is possibly interacting chemically with some oxidant produced as a result of the Mehler-type reaction.

TABLE III

EFFECT OF ASCORBATE ON O_2 UPTAKE IN CHLOROPLAST-FREE MODEL SYSTEM

All reactions were carried out in a 3-ml reaction mixture containing except as noted: Tricine buffer (pH 8 0), 10 mM, glucose 6-phosphate, 3 3 mM; glucose-6-phosphate dehydrogenase, 2 units, methylviologen, 1 mM, ferredoxin–NADP reductase, 0 00022 mM, NADP, 0 08 mM

Addition	*nmoles O_2 taken up/min*	
	Control	*Ascorbate (1 mM)*
Complete	31	57
Complete + 2 FTR	66	115
– Methylviologen	0	11
– Methylviologen, – ferredoxin	15	29

To test this hypothesis, the effect of ascorbate on a simulated Mehler-type reaction in a chloroplast-free model system was measured. In the presence of NADPH and NADP–ferredoxin reductase, methylviologen can be reduced to a free radical which is then autooxidized in the presence of O_2. Table III shows the results of the addition of ascorbate on O_2 uptake by the model system where the concentration of NADPH was kept constant by the presence of an NADPH-generating system. The addition of ascorbate results in a 2-fold stimulation of O_2 uptake Similar results were obtained with Mn^{2+} and dithiothreitol. However, other reducing compounds such as glutathione, cysteine, hydroquinone or diaminobenzidine were ineffective (Table IV) Mg^{2+} in contrast to Mn^{2+} gave no stimulation

It is conceivable that the observed ascorbate induced stimulation of O_2 uptake was the result of a stimulation in the rate of NADPH oxidation (either by stimulating the activity of the diaphorase or by stimulating the activity of the generating system). This possibility, however, can be ruled out on the basis of the results presented in Fig. 1 In this experiment limiting amounts of $NADPH_2$ were added to the chloroplast-free model system either in the presence or absence of ascorbate and the rate and extent of O_2 uptake measured By adding ascorbate, both the rate of O_2 uptake and the extent of O_2 uptake were approximately doubled Since the amount of

TABLE IV

EFFECT OF VARIOUS REDUCTANTS ON OXYGEN UPTAKE IN CHLOROPLAST-FREE MODEL SYSTEM

Conditions as in Table III.

Addition	*Concentration (mM)*	*nmoles O_2 taken up per min*	
		Control	*+ Addition*
Ascorbate	1	16	30
Dithiothreitol	1	23	55
Cysteine	1	23	27
Glutathione	1	19	19
Hydroquinone	1	25	25
Hydroxylamine	3 3	16	16
Diaminobenzidine	1	19	19
DCIP/ascorbate	0.1/3.3	18	34
$MnCl_2$	0 7	18	28
$MgCl_2$	0.7	19	16
$MgCl_2$	1 4	17	14

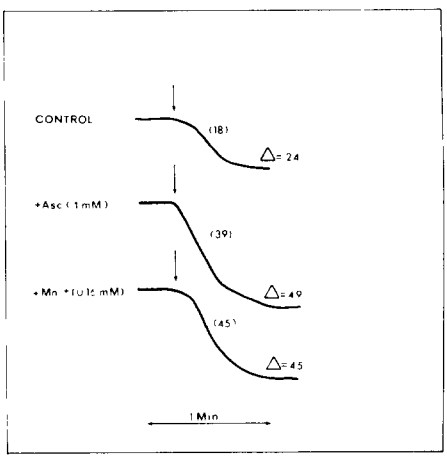

Fig 1 Stimulation of O_2 uptake in a chloroplast-free reaction by ascorbate or Mn^{2+} The basic reaction mixture in a total volume of 3 ml contained the following in μmoles: Tricine buffer (pH 8 0), 30; methylviologen, 3, $NADPH_2$, 0 036, ferredoxin–NADP reductase, 0 00022. The enzyme ferredoxin–NADP reductase was added at the arrow to start the reaction. Numbers in parenthesis are initial rate of O_2 consumption Numbers after Δ = represent the total amount of O_2 consumed in nmoles

$NADPH_2$ oxidized in both experiments was the same and limiting, it is obvious that the addition of ascorbate resulted only in a change in the stochiometry of O_2 taken up per $NADPH_2$ oxidized.

Role of superoxide

Recently, it has been demonstrated that the radical superoxide is formed upon the aerobic oxidation of the reduced forms of low potential dyes such as FMN,

FAD, ferredoxin, menadione, or diquat[9-11]. Superoxide was shown to be decomposed either in a dismutation reaction or in the presence of suitable electron donors or acceptors it can act either as an oxidant or as a reductant, respectively[10]. In the presence of the enzyme superoxide dismutase the dismutation reaction is greatly accelerated with the result that the oxidizing (or reducing) activity of the superoxide radical is inhibited[10].

TABLE V

EFFECT OF SUPEROXIDE DISMUTASE ON O_2 UPTAKE IN CHLOROPLAST-FREE MODEL SYSTEM

Conditions as in Table III 300 μg/ml superoxide dismutase added where indicated.

Compounds added	*Concentration (mM)*	*Control*	*nmoles O_2 taken up per min*	
			+ Compound added	*+ Compound + superoxide dismutase*
None	—	17	—	18
Ascorbate	1	17	35	23.5
Dithiothreitol	1	21	37	19
Mn^{2+}	1	19	30	17
Mn^{2+}	0.33	22	37	21

To test whether the stimulation of O_2 uptake observed upon the addition of such components as ascorbate or Mn^{2+} was due to the production of superoxide, the effect of the enzyme superoxide dismutase on these reactions was measured. The presence of the enzyme nearly completely inhibited the ascorbate or Mn^{2+} induced stimulation of O_2 uptake when tested either with the chloroplast-free model system or with chloroplasts where methylviologen served as the terminal electron acceptor (Tables V, VI). Similar results were obtained in the presence of other low potential acceptors such as Diquat, anthraquinone 2-sulfonic acid, ferredoxin, or phenozine methosulfate.

TABLE VI

EFFECT OF SUPEROXIDE DISMUTASE ON O_2 UPTAKE IN CHLOROPLASTS

All reactions were carried out in a 3-ml reaction mixture containing Class II chloroplast with 60 μg chlorophyll and in μmoles: Tricine buffer (pH 8.0), 60; NaCl, 105; methylviologen 0.3; NaN_3,3; and where indicated dichlorophenolindolphenol, 0.3, DCMU, 0.003, sodium ascorbate, 3; and 300 μg/ml superoxide dismutase.

Reaction	*Control*	*μmoles O_2 taken up per mg/chlorophyll per h*	
		+ Ascorbate	*+ Ascorbate + superoxide dismutase*
H_2O → Methylviologen	157	—	150*
H_2O → Methylviologen	157	420	225
DCIP/ascorbate/DCMU → Methylviologen	—	1087	720

* —Ascorbate.

DISCUSSION

We suggest that the stimulation of O_2 uptake of photosynthetic electron transport from H_2O to methylviologen (MV) to O_2 by ascorbate or Mn^{2+} is due to the oxidation of the latter by superoxide.

These reactions are depicted in Eqns 1 to 3B.

$$2\,H_2O + 4\,MV \xrightarrow{light} 4\,MVH + O_2 \quad (1)$$

$$4\,MVH + 4\,O_2 \longrightarrow 4\,MV + 4\,O_2^{\cdot -} + 4\,H^+ \quad (2)$$

$$2\,O_2^{\cdot -} + 2\,O_2^{\cdot -} + 4\,H^+ \longrightarrow 2\,H_2O_2 + 2\,O_2 \quad (3A)$$

Sum 1, 2 and 3A; $2\,H_2O + O_2 \rightarrow 2\,H_2O_2$

Ascorbate (H_2A) present:

$$4\,O_2^{\cdot -} + 2\,H_2A + 4\,H^+ \longrightarrow 4\,H_2O_2 + 2\,A \quad (3B)$$

Sum 1, 2, 3B; $2\,H_2O + 2\,H_2A + 3\,O_2 \rightarrow 4\,H_2O_2 +$ 2 dehydroascorbate.

Thus, in the system $H_2O \rightarrow$ methylviologen, 1 molecule of O_2 (net) is taken up per transfer of 4 electrons. Upon addition of a reductant (like ascorbate or Mn^{2+}) up to 3 molecules of O_2 can be taken up per transfer of 4 electrons through the electron transport chain. This is a maximum value which can be obtained when all the superoxide formed, oxidizes the added reductant. The actual stimulation in the presence of the reductant will depend on the relative rates of the oxidation of the reductant by the superoxide *vs* the rate of the dismutation reaction. The latter will depend on the amount of the enzyme superoxide dismutase present in the chloroplast preparation. Upon addition of sufficient amounts of exogenous superoxide dismutase, the stimulation of O_2 uptake by ascorbate can be entirely prevented (Table V).

Since electron transport from H_2O to methylviologen (both in the absence and presence of ascorbate or Mn^{2+}) involves both photosystems, sensitivity to DCMU[1-4] and the red drop of the quantum yield[3,4] should take place. The variable effect of ascorbate on electron flow from H_2O to NADP as reported previously (compare ref. 2 with ref. 3) and as obtained in our work (Tables I and II) would depend on the amount of superoxide which is formed and not dismutated, and thus available for oxidation of an added reductant such as ascorbate or Mn^{2+}. It is interesting that the level of the superoxide radical is lower (indicating perhaps a higher activity of superoxide dismutase) in Class I chloroplasts which resemble to a larger extent the chloroplasts *in situ* than Class II chloroplasts.

Although the involvement of superoxide in ascorbate oxidation by chloroplasts was previously suggested by Elstner *et al.*[12] it went unappreciated that this reaction could sufficiently account for the observed ascorbate induced stimulation of O_2 uptake. Elstner *et al.*[12] made the unnecessary *ad hoc* suggestion that monodehydroascorbate is formed as an intermediate and then acts as a donor to Photosystem II in place of water. However, as Eqns 1 through 3B show there is no need for such an assumption since the observed stimulation of O_2 uptake fits that predicted by these equations.

Avron and Ben-Hayyim[13] in studies on the quantum requirement of photoinduced electron transport in lettuce chloroplasts reported that the absorbed quanta

were distributed differently between the two photosystems when using 640-nm light depending on the electron acceptors employed. When NADP served as the electron acceptor, the value of quantum requirement, extrapolated to zero intensity was 2.5 quanta per electron when either water or the electron couple DCIP–ascorbate served as the electron donor. They interpreted this as indicating that the absorbed quanta were equally divided between the two photosystems. However, when low potential dyes such as diquat or FMN served as electron acceptors a quantum requirement of 1 was observed when DCIP–ascorbate served as the electron donor. The latter result is surprising since 640-nm light should be absorbed mainly by Photosystem II and the reaction $DCIPH_2 \rightarrow$ methylviologen is a Photosystem I reaction. Avron and Ben-Hayyim[13] suggested that under these experimental conditions almost all the quanta absorbed were directed from Photosystem II to Photosystem I. However, in light of our findings the interpretation of these data should be reconsidered. In calculating the quantum requirement, Avron and Ben-Hayyim[13] assumed a stoichiometry of 2 electrons per O_2 uptake. However, in the presence of superoxide and ascorbate the stoichiometry between O_2 uptake and electron transport would be different

$$DCIPH_2 + 2\ MV \xrightarrow{light} DCIP + 2\ MVH \tag{4}$$

$$2\ MVH + 2\ O_2 \longrightarrow 2\ MV + 2\ O_2^{\cdot -} + 2\ H^+ \tag{5}$$

$$2\ O_2^{\cdot -} + H_2A + 2\ H^+ \longrightarrow 2\ H_2O_2 + 2\ A \tag{6}$$

$$\text{Sum}\quad DCIPH_2 + H_2A + 2\ O_2 \longrightarrow DCIP + 2\ H_2O_2 + 2A \tag{7}$$

or one O_2 taken up per electron transferred through the chain. Using this stoichiometry the quantum yield should be revised to 2 quanta required per transfer of one electron. This result fits the notion that the reaction $DCIPH_2 \rightarrow$ methylviologen is a System I reaction and that at 640 nm approximately half the quanta are absorbed by Photosystem I.

Bohme and Trebst[2] in studies on the properties of ascorbate photooxidation in isolated chloroplasts interpret their data as indicating that two ATP sites exist in non-cyclic photophosphorylation, that one of these sites is between water and Photosystem II, and that during ascorbate photooxidation this latter site is not operating. The interpretation that one of the two phosphorylating sites exists between water and Photosystem II and that this site is inoperative during ascorbate photooxidation was based on the assumption that ascorbate replaces water as the electron donor to Photosystem II and on the observation that ATP formation remained constant while electron transport was apparently stimulated 2-fold. However, in light of our interpretation on the mechanism of ascorbate photooxidation and the stoichiometry presented in Eqn 3B which would account for the apparent stimulation in electron transport there is no need to postulate the existence of a phosphorylating site between water and Photosystem II.

In the Photosystem I mediated electron transport from the electron couple DCIP-ascorbate to low potential electron acceptors, such as methylviologen, ATP is formed. Generally a low ATP/2*e* ratio has been observed for this reaction. It need be noted, however, that the rate of electron transport was calculated assuming a stoichiometry of 2 electrons transported per molecule O_2 taken-up. However, since superoxide is produced in this reaction and ascorbate is present in the reaction mixture the

stoichiometry shown in Eqn 7 should be employed *i e.* one electron transferred per O_2 consumed. Thus the ATP/2*e* ratios reported should be increased by a factor of two. Using glutathione or cysteine, which do not interact with superoxide, (Table IV), in place of ascorbate, we have found that indeed the ATP/2*e* ratio increases nearly 2-fold (Yannai, Y. and Epel, B., unpublished data).

The low stoichiometry of ATP/2*e* in the system $DPIPH_2$ to methylviologen was used as one argument against the notion that in this system ATP is coupled to non-cyclic electron flow[14] Our data very much weaken this argument.

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