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The Competition between Methyl Viologen and Monodehydroascorbate Radical as Electron Acceptors in Spinach Thylakoids and Intact Chloroplasts

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In spinach thylakoids prepared from intact chloroplasts by shocking in the presence of ascorbate to preserve the operation of ascorbate peroxidase, the rate of oxygen uptake with methyl viologen as acceptor decreased in response to the addition of H_2O_2. Such a decrease was not observed in the presence of KCN or when the thylakoids lost ascorbate peroxidase activity. Illumination of intact chloroplasts in the presence of H_2O_2 and methyl viologen showed an initial rate of oxygen exchange, which is intermediate between the initial rate of oxygen evolution in the presence of H_2O_2 alone and steady-state oxygen uptake in the presence of methyl viologen. The data showed that monodehydroascorbate radical generated in ascorbate peroxidase reaction could compete with methyl viologen for electrons supplied by the electron transport chain in both thylakoids and intact chloroplasts. During the illumination of intact chloroplasts the rate of oxygen uptake increased. The presence of nigericin swiftly led to steady-state oxygen uptake, and to a clear-cut 1 : 1 relationship between the electron transport rate estimated from fluorescence assay and the electron transport rate determined from oxygen uptake, taking the stoichiometry $1O_2 : 4e$. The increase in oxygen uptake was attributed to the cessation of monodehydroascorbate radical generation brought about by consumption of intrachloroplast ascorbate in the peroxidase reactions, and the effects of nigericin were explained by acceleration of such consumption. The competition between methyl viologen and monodehydroascorbate radical in the intact chloroplasts was estimated under various conditions.

Keywords: Chloroplasts, oxygen reduction, ascorbate peroxidase reaction, monodehydroascorbate radical

Abbreviations: APX, ascorbate peroxidase; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; DHA, dehydroascorbate; ETC, electron transport chain; F, steady-state fluorescence yield; F'_m, maximal fluorescence yield under illumination; ΔF, $F'_m - F$; MDHA, monodehydroascorbate; Mv, methyl viologen; Nig, nigericin; PS1 and PS2, photosystems 1 and 2; SOD, superoxide dismutase; tAPX, thylakoid membrane bound APX

INTRODUCTION

Illumination of chloroplasts under aerobic conditions leads to H_2O_2 production as a result of oxygen reduction with electrons from electron transport chain (ETC) (Mehler reaction) (for

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review see Refs. [1,2]). Detoxification of this product, which is dangerous for Calvin cycle enzymes, is performed by stromal[3] and thylakoid bound (tAPX)[4] ascorbate peroxidases. Ascorbate concentration in chloroplasts has been estimated to be in a range of 10–50 mM,[5–7] and ascorbate was predominately found in the reduced form in plant tissues.[8] In the ascorbate peroxidase (APX) reaction monodehydroascorbate (MDHA) radical is produced as the primary oxidation product of ascorbate by H_2O_2. E_0' of this radical is 320 mV and pK is −0.45.[9] It was established that regeneration of ascorbate from MDHA could be achieved at the expense of NADPH either through direct reduction of MDHA or through reduction of dehydroascorbate (DHA) after MDHA dismutation, with participation of the respective reductases.[1,2] Later, it was found that MDHA could be reduced directly by the carriers of the ETC.[4,10] Miyake and Asada[11] showed that MDHA is reduced by reduced ferredoxin 30 times higher rate than $NADP^+$ is. In their experiments MDHA was generated in the suspension by added ascorbate oxidase, i.e. in the bulk solution, while MDHA, which appears in the course of the reaction catalyzed by thylakoid membrane bound APX (tAPX), localizes on the membrane surface. Therefore, the ability of MDHA to compete as an acceptor might be underestimated.

For electron flow through the ascorbate-peroxidase cycle, which is the combination of O_2 reduction, peroxidase reactions, and regeneration of ascorbate at the expense of electrons derived from water, the oxygen balance is equal to zero. So, these electrons cannot be observed as a change of oxygen concentration in cells or suspensions. The measurement of chlorophyll fluorescence to characterize ETC operation has been highly improved thanks to possibility to calculate the rate of electron transport as the product of the quantum yield of photosystem 2 (PS2) ($\Delta F/F_m'$ where F is steady-state fluorescence yield and F_m' is maximal fluorescence yield under illumination) and the intensity of absorbed light.[12] This permits the estimation of total electron transport along ETC, which may include the electron transport not displayed as O_2 uptake or O_2 evolution.

The goal of the present work was to characterize the reduction of MDHA by the photosynthetic ETC in spinach thylakoids and chloroplasts with respect to its potency as an acceptor. For this purpose the competition of MDHA reduction with the reduction of an effective artificial electron acceptor, methyl viologen (Mv), was studied.

MATERIALS AND METHODS

The intact chloroplasts were isolated from spinach using the stage of Percoll purification.[13] Ascorbate at 10 mM was present in the grinding, Percoll, and suspension media. The intactness was determined according to Ref. [14] and was not less than 95%. In experiments with intact chloroplasts the reaction medium contained 0.3 M sorbitol, 50 mM Hepes (pH 7.6), 30 mM KCl and 0.25 mM KH_2PO_4; 10 mM DL-glyceraldehyde was added to inhibit CO_2 fixation. The thylakoids for experiments presented in Figure 1 were obtained by placing the intact chloroplasts in 1 ml of either distilled water or 3.75 mM ascorbate up to chlorophyll concentration 3–3.5 μg ml^{-1} (that corresponded to an addition of 2.5–3.5 μl of stock suspension), followed in 1–1.5 min by addition of 0.5 ml of the above reaction medium. After 7 min of incubation 2.5 mM ascorbate (if it was not present), 0.3 μM nigericin (Nig), and Mv at indicated concentrations were added. An oxygen exchange in experiments presented in Figures 1–3 was measured in a Hansatech chamber. A slide projector provided actinic illumination through red cut-off filter RG-610 and neutral filters. The simultaneous measurements of chlorophyll fluorescence parameters, using the PAM-101, and oxygen exchange, using Clark type O_2-electrode, were conducted in square quartz cuvette which was thermostated at 20°C.[15] The data obtained using saturating pulses were corrected by the addition of 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) for all experimental conditions. Illumination

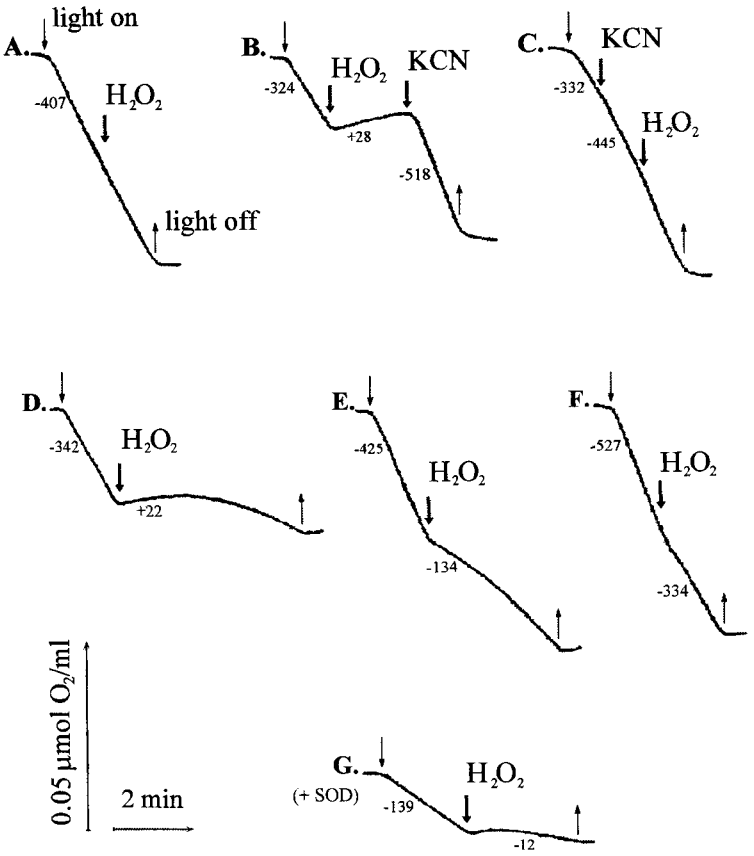

FIGURE 1 Effects of H_2O_2 addition (275 µM) on the rate of oxygen uptake with Mv as acceptor in illuminated spinach thylakoid. Thylakoids were obtained from intact chloroplasts by shocking in the absence (A) and in the presence of ascorbate (B–G) (see Methods). The concentration of Mv were: 5 µM in A–D; 35µM in E and G; 200 µM in F. The other additions in the reaction mixture: SOD, 2000 units; KCN, 1 mM. An intensity of red light (passed through RG 610 and neutral filter) was 90 µmol quanta $m^{-2} s^{-1}$. The numbers near the traces are the rates of oxygen concentration changes in µmol O_2 $(mg/Chl)^{-1} h^{-1}$ measured 1 min after H_2O_2 or KCN additions (plus sign denotes an oxygen evolution, and minus sign denotes an oxygen uptake). ↓ and ↑ – switching on and off the light. Chlorophyll, 3.2 µg ml^{-1}.

provided by a 250 W halogen lamp the light of which was filtered through a RG 610 cut-off filter and 675 nm interference filter (halfbandwidth 11.1 nm) of Schott and a heat absorbing filter Calflex X of Balzers. The light intensity was measured with a Li-Cor Quantum meter.

RESULTS

Thylakoids

Figure 1 shows the influence of H_2O_2 addition on oxygen exchange in the presence of Mv in the suspension of thylakoids prepared by shocking intact chloroplasts in the reaction vessel. Before the H_2O_2 addition, dioxygen is photoreduced to H_2O_2 due to Mv-mediated reduction of O_2 followed either by dismutation of the primary product superoxide radicals, $O_2^{\bullet -}$,

$$O_2^{\bullet -} + O_2^{\bullet -} \rightarrow O_2^{2-} + O_2 \qquad (1)$$

or by their reaction with ascorbate,

$$O_2^{\bullet -} + \text{Asc} \rightarrow \text{MDHA} + O_2^{2-} \qquad (2)$$

When chloroplasts were shocked in the absence of ascorbate and APX was inactivated, the rate

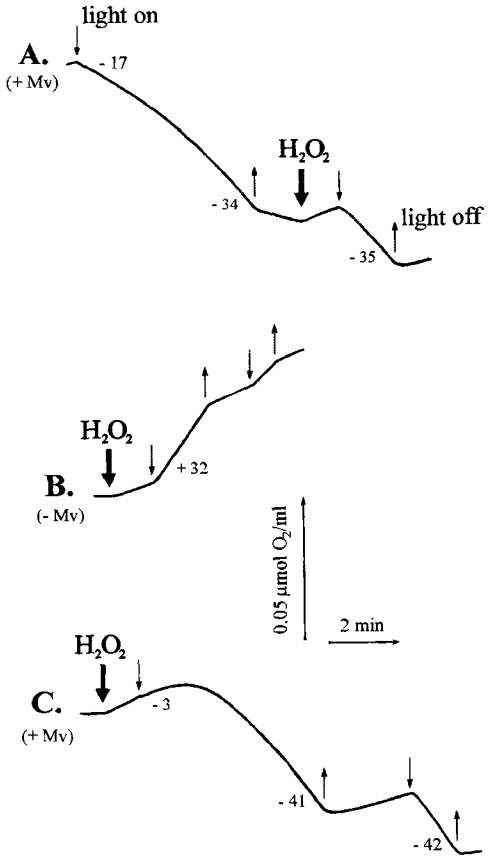

FIGURE 2 Influence of H_2O_2 addition on light-induced oxygen exchange in intact spinach chloroplasts. (A) and (C) – 75 μM Mv was present in the reaction mixture; 500 μM H_2O_2 was added where indicated. The red light intensity was 750 μmol quanta m^{-2} s^{-1}. The numbers near the traces mean the same as in Figure 1; the rates in the presence of H_2O_2 were calculated taking into account the dark oxygen evolution due to H_2O_2 decomposition presumably by catalase contaminating the chloroplasts preparations. Chlorophyll, 26 μg ml^{-1}.

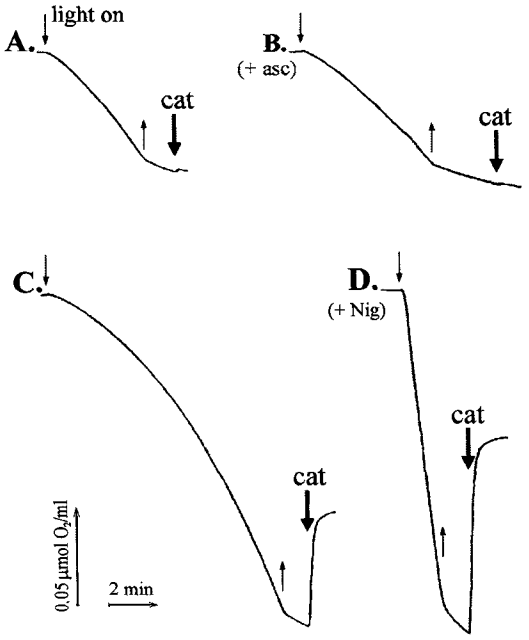

FIGURE 3 The appearance of H_2O_2 in the reaction medium during the illumination of the intact chloroplasts of spinach in the presence of 75 μM Mv. (A) and (C) – no additions into the reaction mixture; (B) – 10 mM ascorbate-Na was present; (D) – 0.5 μM Nig was present. Catalase where indicated (cat) was added at 800 units ml^{-1}. Other conditions as in Figure 2.

of oxygen uptake decreased only slightly if at all on addition of H_2O_2 (Figure 1A). However, when shocking was made in the presence of ascorbate and thus APX activity was retained, the H_2O_2 addition induced a notable decrease of oxygen uptake (Figure 1B and D–F).

It was established[4] that APX activity of thylakoids disappeared very quickly ($t_{1/2} = 15$ s) during thylakoids incubation in the absence of ascorbate. When this activity was preserved, an H_2O_2 addition (in the absence of any added acceptors) induced an oxygen evolution.[4,10] This oxygen evolution has proved to be a result of the reduction of MDHA production by APX reaction. Thus, the H_2O_2 induced decrease of the rate of oxygen uptake in Figure 1 means that MDHA can be reduced concurrently with Mv.

The addition of KCN, which is a potent inhibitor of APX, after H_2O_2 addition led promptly to higher oxygen uptake (Figure 1B), and KCN addition before H_2O_2 addition prevented the decrease of oxygen uptake (Figure 1C). In both cases the result coincided with that that just the MDHA production in APX reaction provided the decrease of oxygen uptake rate after H_2O_2 addition. Figure 1D–F indicate that the extent of decrease in oxygen uptake after H_2O_2 addition is higher at lower Mv concentration.

This dependence implies that the competition occurs at the stage of acceptors, Mv and MDHA, reductions but not between MDHA and oxygen at the stage of Mv oxidation.

The fact that in Figure 1C the rate of oxygen uptake after KCN addition increases implies that H_2O_2 generated in the reaction mixture in reactions (1) and/or (2) is also used in the APX reaction to produce MDHA, which is able to compete with Mv. The higher rate of oxygen uptake at higher Mv concentrations before H_2O_2 addition (see Figure 1D–F) supports this conclusion, as the rate of oxygen uptake in thylakoids isolated without ascorbate saturates at lower Mv concentrations (5–7 μM).[16]

The stoichiometry between Mv-mediated oxygen uptake and electrons supplied from ETC is indefinite in the presence of ascorbate, and depends on the ratio between rates of reactions (1) and (2). Taking into account the oxygen evolution accompanying the extraction of electrons from water, the stoichiometry of oxygen uptake is $1O_2 : 4e^-$ when reaction (1) proceeds exclusively, and the stoichiometry is $3O_2 : 4e^-$ when reaction (2) proceeds exclusively. So, at the same electron transport rate the oxygen uptake rate has to be three times more in the second case. In the experiments in Figure 1 we used the limiting light intensity, and it is seen that in the presence of SOD (when the stoichiometry had to be shifted to classic one, $1O_2 : 4e^-$, due to an increase of $O_2^{\bullet -}$ disproportionation rate[17]) the rate of oxygen uptake before H_2O_2 addition is really lower approximately three times (compare Figure 1E with G at the same Mv concentration). In response to H_2O_2 addition an absolute decrease in the oxygen uptake rate in the presence of superoxide dismutase (SOD), from 139 to 12 μmol O_2 $(mg\,Chl)^{-1}\,h^{-1}$ (Figure 1G), was lower than that in the absence of SOD, from 425 to 134 μmol O_2 $(mg\,Chl)^{-1}\,h^{-1}$ (Figure 1E). This had to be due to the fact that the electrons, which started to reduce MDHA after H_2O_2 addition, provided before H_2O_2 addition higher rate of oxygen uptake in the absence of SOD.

Intact Chloroplasts

A picture similar to the one observed with thylakoids was obtained with intact chloroplasts. The first illumination of the chloroplasts in the presence of Mv and H_2O_2 (Figure 2C) resulted in an oxygen exchange which was intermediate to those when these substances were separately added (Figure 2A and B). Quantitatively, the initial change in oxygen concentration in Figure 2C upon first illumination was close to the sum of the rate of light-induced oxygen evolution in the presence of H_2O_2 alone upon first illumination (Figure 2B), and the rate of oxygen uptake in the presence of Mv alone after 5-min illumination (Figure 2A).

The light-induced oxygen evolution by intact chloroplasts in the presence of H_2O_2 is the result of APX operation followed by the MDHA and DHA reduction by NADPH which in its turn is rereduced by ETC,[1,2] or by direct reduction of MDHA by carriers of ETC (see Introduction). The initial oxygen exchange in Figure 2C represents the summation of the rates of oxygen evolution and oxygen uptake, and is the expected result of the ability of the product of APX reaction, MDHA, to be reduced by ETC simultaneously with Mv. However, in the presence of Mv the $NADP^+$ reduction by ETC is hardly possible. Rather, it is the direct MDHA reduction by ETC which competes with Mv reduction in intact chloroplasts, just as it took place in the thylakoids in Figure 1.

The APX reaction can proceed inside the intact chloroplasts while ascorbate preserved during chloroplast isolation remained. Figure 2C shows that during illumination the rate of oxygen uptake increased and the initial balance disappeared, implying the disappearance of the competition between Mv and MDHA with prolonged illumination. The addition of catalase showed that almost all of added H_2O_2 remained in the suspension up to this time (not shown). So, the disappearance of competition may be explained from results by Law *et al.*[5] and Foyer *et al.*[6] who

found that the concentration of ascorbate inside the intact chloroplasts decreased due to reaction with H_2O_2; in which case the rate of MDHA generation should become low.

The rate of Mv-dependent oxygen uptake also increased during illumination when H_2O_2 was not added to the suspension (Figure 2A) that was observed also by Nakano and Asada.[18] This means that MDHA, which could be produced inside chloroplasts via the APX-reaction between intrachloroplast ascorbate and H_2O_2 (the last-mentioned is produced as the result of oxygen reduction through Mv), initially competed with Mv, but the competition gradually ceased over time as the APX-reaction consumed ascorbate. The exhaustion of ascorbate during illumination in the presence of Mv has been shown.[5,6] In this context it is important to note that the addition of H_2O_2 to preilluminated chloroplasts did not cause a decrease of the oxygen uptake rate at the second illumination (Figure 2A). This is the opposite of what is observed in Figure 2C when H_2O_2 was added to dark-adapted chloroplasts.

The fact that the time of the generation of MDHA depends on the availability of the second substrate of APX, ascorbate, within the intact chloroplasts was confirmed by the data presented in Figure 3. As indicated by oxygen evolution upon catalase addition, H_2O_2 could not be found in the medium after a short illumination (Figure 3A), but appeared after longer illumination (Figure 3C). The appearance of H_2O_2 is an indication of a cessation of APX reactions, which effectively use H_2O_2 until ascorbate is present.

In the presence of Nig, H_2O_2 appeared in the medium after a short illumination (Figure 3D). This means that Nig accelerates the process of ascorbate oxidation evidently due to an increase of the rate of H_2O_2 generation. The addition of ascorbate in the reaction mixture led to a longer preservation of a lower rate of oxygen uptake (Figure 3B); this was more pronounced in experiments with Nig, and the preservation time was dependent on the concentration of added ascorbate (not shown).

Thus, from Figures 2 and 3 it follows that just the exhaustion of intrachloroplast ascorbate, which could not be completely rereduced by ETC in isolated chloroplasts, led to a switching off of MDHA generation, and that this process turned out promptly in the presence of Nig.

To obtain quantitative estimations of competition between MDHA and Mv, the rate of oxygen exchange and the rate of electron flow through ETC were compared. What is a possible theoretical relationship between the oxygen uptake rate in the intact chloroplasts and the rate of electron transport? In intact chloroplasts SOD works effectively, and then the reaction of $O^{\bullet -}$ with ascorbate (reaction (2)) is highly impossible. This is supported by the fact that an addition of ascorbate to the reaction mixture with intact chloroplasts did not bring any increase in the oxygen uptake rate (that might be expected owing to probable stimulation of reaction (2)) but on the contrary provided maintenance of the low initial rate (compare Figure 3A and B).

Thus, in intact chloroplasts the stoichiometry $1O_2 : 4e$ between oxygen consumption and electron flow to Mv may be changed only when MDHA is produced in APX reaction and can be reduced concurrently with Mv. It can be shown (see Appendix) that in this case the rate of oxygen concentration change, V^*, is

$$V^* = -\frac{1}{4} V_e \frac{1-(1-x)(1-y)}{1+(1-x)(1-y)} \qquad (3)$$

where V_e is the rate of electron flow from water along the ETC, "x" is the part of H_2O_2 which does not participate in APX reaction, and "y" is the part of MDHA which is not reduced by ETC. If all of H_2O_2 and MDHA are used in the above mentioned reactions, i.e. both "x" and "y" are zero, the rate of oxygen uptake will be zero, and if "x" or "y" is equal to 1 the stoichiometry should be $1O_2 : 4e$.

Thus, any oxygen uptake in the presence of Mv when the APXs, stromal and thylakoid bound, operate, indicate an escape of H_2O_2 and/or

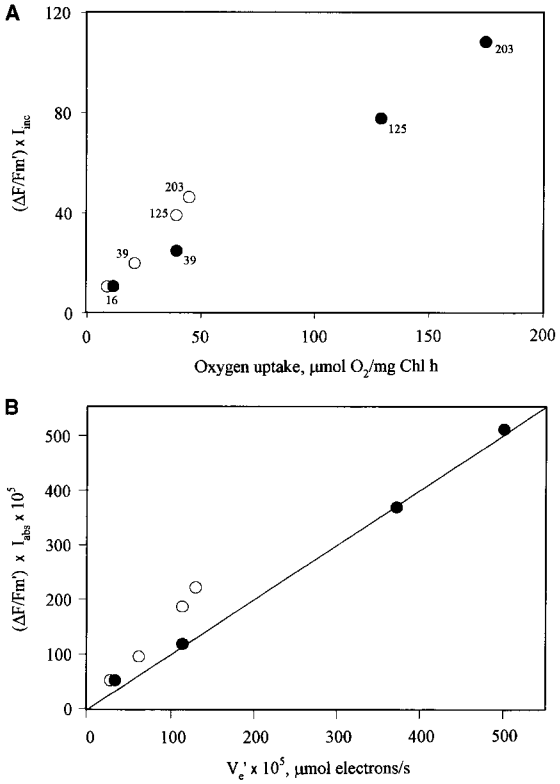

FIGURE 4 (A) The relationship between the apparent rate of electron transport, $(\Delta F/F'_m) \times I_{inc}$, where I_{inc} is the incident light intensity, and the rate of oxygen uptake in intact chloroplasts in the presence of 0.2 mM Mv. The data were obtained at various light intensities as indicated (in μmol quanta $m^{-2} s^{-1}$) near data points; light passed through RG 610 and interference filter IF 675. Open symbols, the results in the absence of Nig; closed symbols, in the presence of 0.5 μM Nig. The rates of oxygen uptake to the second minute of illumination were used in both cases. (B) The data from A presented as relationship between the electron transport rate, $(\Delta F/F'_m) \times I_{abs}$, where I_{abs} is the intensity of absorbed light (see text), and the rate of electron transport, V'_e, calculated from oxygen uptake assuming the stoichiometry of oxygen uptake to be $1O_2$:4e. The straight line is a theoretical relationship for $1O_2$:4e stoichiometry between oxygen uptake and electron flow.

MDHA from participation in the APX cycle, and the higher oxygen uptake rate indicates a lesser rate of MDHA reduction.

Figure 4A shows that at the same relative rate of electron flow through ETC (obtained as a product of $\Delta F/F'_m$ and incident light intensity) the rate of oxygen uptake was higher in the presence of Nig (the rates achieved to the second minute of illumination were considered in the absence and in the presence of Nig). This might result from cessation of MDHA generation due to quick ascorbate exhaustion in the presence of Nig (see above).

If this proposition is correct and the cessation of MDHA reduction in the presence of Nig is the actual reason for the observed difference in the above relationships, an oxygen uptake with Mv as acceptor in the presence of Nig should be related to the electron transport in accordance with stoichiometry of $1O_2$:4e. To check this, it is necessary to know the absolute rate of electron flow through ETC. The determination of the latter from fluorescence parameters requires knowing the absorbed light intensity.[12] At chlorophyll (Chl) concentration of 20–30 μg Chl ml^{-1} approximately 95% of the incident 675 nm red light is absorbed along the 1 cm pathway, and approximately 50% of absorbed quanta of this light is delivered to PS2.[19] Thus, I_{abs} (μmol quanta s^{-1}) = $I_{incident}$ (μmol quanta $m^{-2} s^{-1}$) × 0.95 × 0.5 × $10^{-4} \times S$ (cm^2), where S is the illuminated area of cuvette wall (in our experiments S was approximately 1 cm^2). Assuming the stoichiometry of $1O_2$:4e the rate of electron transport found from oxygen exchange in the corresponding volume of a cuvette should be V'_e(μeqv s^{-1}) = 4 × V_{O_2}(μmol O_2 ml^{-1} s^{-1}) × S (cm^2) × 1 cm, where 1 cm is the thickness of cuvette. The data of Figure 4A plotted in such a way show that only in the presence of Nig the relationship between $(\Delta F/F'_m \times I_{abs})$ vs. V'_e is very close to the theoretical one (Figure 4B). So, the fact that correspondence between experimental and theoretical data only occurred when ascorbate was promptly consumed and the appearance of MDHA ceased supports that in the absence of Nig MDHA was reduced concurrently with Mv.

It is possible to calculate the competition between MDHA and Mv in intact chloroplasts as a percent of electrons, which go to reduce MDHA. If we suppose as for Equation (3) that all electrons for reduction of Mv and MDHA are supplied from water, then the rate of electron flow

to MDHA, V_{MDHA}, which occurs simultaneously with Mv reduction is (see Appendix)

$$V_{MDHA} = 1/2(V_e + 4V^*) \quad (4)$$

V_e can be estimated from fluorescence data as described above. If Mv and MDHA are the only acceptors, then from Figure 4A at incident light intensity of 675 nm light 125 μmol quanta $m^{-2} s^{-1}$, it may be found that MDHA in the absence of Nig is reduced at a rate of 50 μeqv $(mg\ Chl)^{-1} h^{-1}$ that is 19% of the total electron transport estimated from fluorescence measurements, 260 μeqv $(mg\ Chl)^{-1} h^{-1}$

DISCUSSION

From results presented it may be concluded that the reduction of MDHA by the thylakoid ETC can compete with the reduction of Mv. What is the primary reason that MDHA is able to compete? In the experiments with isolated thylakoids (Figure 1) MDHA might be reduced even at relatively high concentration, 200 μM, of Mv. The maximal rate of MDHA production in the solution in the course of APX reaction with added H_2O_2 may be estimated as 0.5 μM s^{-1}, taking into account the APX activity at used pH (approximately 500 μmol $(mg\ Chl)^{-1} h^{-1}$ [4]). Then the steady-state MDHA concentration, taking into account only the dismutation, would be approximately 2 μM at pH 7.6. This concentration is much less than Mv concentration, and the best explanation of the observed competition is the prompt arrival of MDHA to the site of its reduction before dismutation. This conclusion is corroborated by data regarding the localization of APX near PS1[4] where MDHA reduction seems to occur.[10]

The data obtained with intact chloroplasts showed that MDHA can also compete with Mv in intact chloroplasts. In this case the MDHA would be generated at two sites, near the thylakoid membrane and in the stroma (see Figure 5). The generation of MDHA in reaction (2), shown in this figure for thoroughness, is insignificant in intact chloroplasts due to SOD operation providing the usage of superoxide radicals almost exclusively in reaction (1). The tAPX and stromal APX reduce H_2O_2 very effectively, as is seen in Figure 3A: in the absence of Nig H_2O_2 did not appear in the reaction mixture during the first minutes of illumination (see also Ref. [18]). Taking into account the ability of H_2O_2 to permeate through biological membranes, its absence in the medium and the observed oxygen uptake (pointing to incompleteness of APX cycle, see above) suggest that just the MDHA molecules escape reduction. It is reasonable to suppose that these escaping molecules mostly appear in the reaction catalyzed by a soluble APX, i.e. relatively far from MDHA reduction site in the thylakoid membrane. An escape may be a result of their dismutation followed by the DHA degradation before the latter may be reduced by the glutathione system.[5] This system in the presence of Mv cannot operate during long time as this artificial acceptor prevents the NADPH generation. Thus, as in naked thylakoids, the MDHA molecules, which can compete with Mv in the intact chloroplasts, are most probably the ones generated in the reaction, which is catalyzed by tAPX.

Competition between MDHA and $NADP^+$ as acceptors was shown in Refs. [10,11]. However, the competition between MDHA and $NADP^+$ just for ferredoxin can be considered to fit only the conditions of work.[11] It is important, that the stimulation of MDHA reduction by ferredoxin was only found in the latter, while in work[10] such stimulation was not observed. In[10] H_2O_2 was added to thylakoids as in our experiments in Figure 1, while in Ref. [11] the generation of MDHA was conducted with ascorbate oxidase in the bulk of solution. The decrease of $NADP^+$ reduction when APX-reaction operated, in Ref. [10] might result from a decrease of the electron flow *to* ferredoxin, i.e. from the competition between MDHA and ferredoxin as acceptors of PS1. The competition between MDHA and Mv

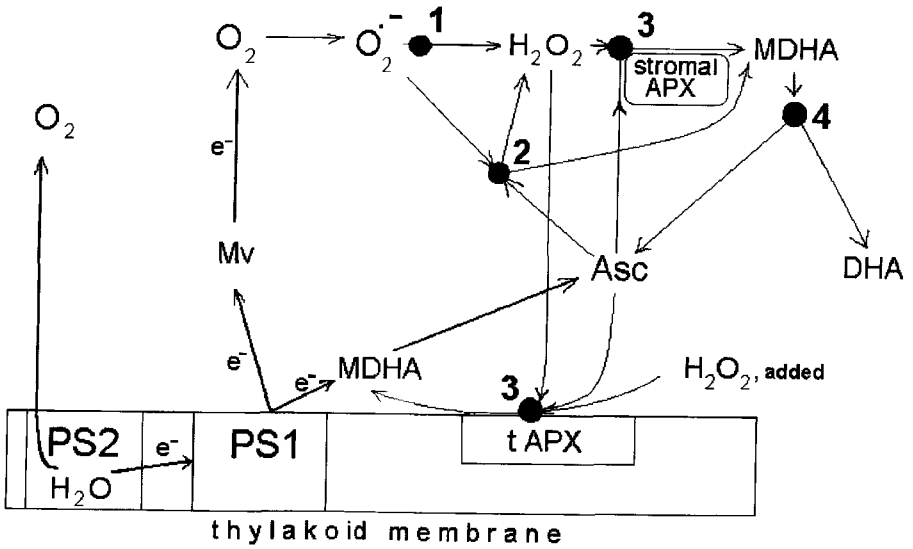

FIGURE 5 Schematic representation of the reactions which take place in the presence of Mv in chloroplast stroma in vicinity of acceptor side of PS1. 1 – Reaction of superoxide radicals dismutation, which may be spontaneous, but in intact chloroplasts it is catalyzed almost exclusively by SOD (reaction (1) in text); 2 – spontaneous reaction between superoxide radicals and ascorbate (reaction (2) in text), which is insignificant in intact chloroplasts, but can be essential in experiments with thylakoids; 3 – ascorbate peroxidase reactions catalyzed by indicated ascorbate peroxidases; 4 – spontaneous reaction of MDHA dismutation.

that is followed from the present work suggests such a possibility.

An important role which the ascorbate-peroxidase cycle (frequently named as Mehler-peroxidase reaction) can play in chloroplasts, was stressed in the literature.[20–23] The potent ability of MDHA to accept electrons from ETC implies that the reactions of this cycle can operate even under low level of MDHA production.

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APPENDIX

If we accept that all MDHA molecules in chloroplasts originate in the reaction

$$2\text{Asc} + H_2O_2 \rightarrow 2\text{MDHA} + H_2O$$

where H_2O_2 appears only during O_2 reduction through reduced Mv, then the rate of MDHA generation, V'_{MDHA}, is

$$V'_{\text{MDHA}} = 2V_{H_2O_2}(1 - x) \quad (1)$$

where $V_{H_2O_2}$ is the rate of H_2O_2 production, and "x" is the part of H_2O_2 molecules which does not participate in APX reaction.

If "y" denotes the part of MDHA molecules that is not reduced by ETC, then the rate of MDHA reduction, V''_{MDHA}, is

$$V''_{\text{MDHA}} = 2V_{H_2O_2}(1 - x)(1 - y) \quad (2)$$

The rate of H_2O_2 production is equal to a half of the univalent reduction rate of O_2, V_{O_2}, (expressed in mol electrons per time unit per Chl amount unit), when $O_2^{\bullet -}$ is completely dismutates according to the reaction (1) in the text

$$V_{H_2O_2} = 1/2V_{O_2} = 1/2\,(V_e - V''_{\text{MDHA}}) \quad (3)$$

where V_e is the rate of the total electron flow along the ETC.

After combination of Equations (2) and (3) we have

$$V_{O_2} = \frac{V_e}{1 + (1 - x)(1 - y)} \quad (4)$$

The oxygen balance rate, V^* (expressed in mol O_2 per time unit per Chl amount unit) during simultaneous reduction of MDHA and O_2 is (taking the rate of O_2 uptake with minus sign, and taking into account that the stoichiometry of oxygen uptake during oxygen reduction followed by complete dismutation is $4e : 1O_2\downarrow$, and stoichiometry of oxygen evolution during MDHA reduction is $4e : 1O_2\uparrow$)

$$V^* = -1/4V_{O_2} + 1/4V''_{\text{MDHA}} \quad (5)$$

Substituting V_{O_2} and V''_{MDHA} from Equations (4) and (2) and making the simplifications

we obtain

$$V^* = -\frac{1}{4} V_e \frac{1-(1-x)(1-y)}{1+(1-x)(1-y)} \qquad (6)$$

which is Equation (3) in the text.

From Equation (6) it is possible to write V''_{MDHA} through V^* and V_e. Equation (6) after transformation may be written as

$$V^* = -\frac{1}{4} V_e \left(1 - \frac{2(1-x)(1-y)}{1+(1-x)(1-y)}\right) \qquad (7)$$

The fraction in the brackets is equal to $2V''_{MDHA}/V_e$ that may be easy obtained after combination of Equations (2)–(4). Then Equation (7) may be transformed to

$$V^* = -1/4\,(V_e - 2V''_{MDHA}). \qquad (8)$$

From Equation (8) we have

$$V''_{MDHA} = 1/2\,(V_e + 4V^*), \qquad (9)$$

which is Equation (4) in the text.