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Superoxide as an Obligatory, Catalytic Intermediate in Photosynthetic Reduction of Oxygen by Adrenaline and Dopamine

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ABSTRACT

The superoxide anion radical is known to be the first product of photosynthetic reduction of oxygen mediated by a variety of electron carriers. The effectiveness of many of these electron carriers as herbicides, and the toxicity of the superoxide they produce, have been suggested to rule out oxygen reduction as a physiological component of normal photosynthesis. Here results with isolated spinach chloroplasts are presented that demonstrate that the related catecholamines adrenaline and dopamine mediate photosynthetic reduction of oxygen. Complete inhibition by added superoxide dismutase of light-dependent oxygen uptake by isolated chloroplasts and of the electron transport it supports indicates that superoxide is an obligatory catalytic intermediate, not a product, in adrenaline- and dopamine-mediated oxygen reduction. These compounds might function as chemical analogues of a proposed natural mediator, or oxygen-reducing factor, that allows oxygen reduction to participate in energy transduction in photosynthesis. The identity of the putative natural mediator and the role of oxygen reduction in photosynthesis are discussed. The fully oxidized form of adrenaline, adrenochrome, also acts as a mediator of photosynthetic oxygen uptake, but only by reducing oxygen to superoxide. *Antioxid. Redox Signal.* 5, 7–14.

INTRODUCTION

PHOTOSYNTHESIS is light-driven redox (reduction–oxidation) chemistry (electron or hydrogen atom transfer) that is coupled to ATP synthesis and to a variety of assimilatory and biosynthetic processes, chief amongst which is fixation of atmospheric carbon dioxide in photoautotrophs (12). Photosynthesis as redox chemistry was indicated by the work of Hill (21), who showed that chloroplasts isolated from leaves carry out light-dependent evolution of oxygen if provided with a suitable electron acceptor, or "Hill oxidant" (A), which accepts electrons (becomes reduced) from water (which becomes oxidized, liberating molecular oxygen). The Hill reaction is summarized in Eq. 1.

$$2H_2O + 2A \rightarrow O_2 + 2AH_2 \quad (1)$$

Certain Hill oxidants, such as quinones and flavins, have the property of "autoxidation," that is, they readily transfer the electron or hydrogen atoms to molecular oxygen (Eq. 2), mediating a variant of the Hill reaction, the "Mehler reaction" (24), in which the Hill oxidant is molecular oxygen itself (Eq. 3, simplifying to Eq. 4).

$$2H_2O + 2A \rightarrow O_2 + 2AH_2 \quad (1)$$

$$2AH_2 + 2O_2 \rightarrow 2H_2O_2 + 2A \quad (2)$$

Sum:
$$2H_2O + 2O_2 \rightarrow O_2 + 2H_2O_2 \quad (3)$$

Or:
$$2H_2O + O_2 \rightarrow 2H_2O_2 \quad (4)$$

In the Mehler reaction (Eq. 4), photosynthetic electron transport can be measured as light-dependent oxygen uptake. Oxygen uptake in the Mehler reaction has the same stoichiometry as oxygen production in the Hill reaction (Eq. 1), one oxygen molecule per four electrons transferred through the photosynthetic chain.

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The role of the superoxide anion radical ($O_2^{\cdot -}$) as an intermediate in the Mehler reaction was first indicated by work of Allen and Hall (6).The initial step in oxygen reduction is transfer of a single electron to each molecule of oxygen (Eq. 5). Four-electron transfer from water through the chain therefore results in one-electron reduction of four oxygen molecules (Eq. 5). Subsequent dismutation of superoxide (Eq. 6) may proceed spontaneously or by catalysis by the enzyme superoxide dismutase (SOD) (23). The sum of superoxide production (Eq. 5) and dismutation (Eq. 6) gives the same overall reaction as transfer of electrons from the mediator to oxygen, yielding hydrogen peroxide (H_2O_2) (Eq. 2).

$$2AH_2 + 4O_2 \rightarrow 4O_2^{\cdot -} + 4H^+ + 2A \quad (5)$$

$$2O_2^{\cdot -} + 2O_2^{\cdot -} + 4H^+ \rightarrow 2H_2O_2 + 2O_2 \quad (6)$$

Sum: $$2AH_2 + 2O_2 \rightarrow 2H_2O_2 + 2A \quad (2)$$

The role of superoxide in the Mehler reaction precisely explains the effect of ascorbate in stimulating photosynthetic oxygen uptake, because ascorbate is able to reduce superoxide, replacing Eq. 6 (which yields oxygen) with Eq. 7 (which yields no oxygen).

$$2AH_2 + 4O_2 \rightarrow 4O_2^{\cdot -} + 4H^+ + 2A \quad (5)$$

$$4O_2^{\cdot -} + 2AscH_2 + 4H^+ \rightarrow 4H_2O_2 + 2Asc \quad (7)$$

Sum: $$2AH_2 + 4O_2 + 2AscH_2 \rightarrow 4H_2O_2 + 2Asc + 2A \quad (8)$$

If the effect of ascorbate is to replace Eq. 2 of the Mehler reaction with Eq. 8, then the overall reaction becomes that shown in Eq. 9, rather than Eq. 4, and the stoichiometry of oxygen uptake to electron transfer is increased threefold.

$$2H_2O + 2A \rightarrow O_2 + 2AH_2 \quad (1)$$

$$2AH_2 + 4O_2 + 2AscH_2 \rightarrow 4H_2O_2 + 2Asc + 2A \quad (8)$$

Sum: $$2H_2O + 3O_2 + 2AscH_2 \rightarrow 4H_2O_2 + 2Asc \quad (9)$$

Thus, the first evidence for superoxide in photosynthesis was the observed threefold increased in oxygen uptake upon addition of ascorbate to chloroplasts carrying out the Mehler reaction, and the reversal of this effect by SOD, whereupon the catalyzed reaction 6 replaces reaction 7. Thus Eq. 2 replaces Eq. 8, and Eq. 4 is restored in place of Eq. 9. In the absence of SOD, reaction 4 is dependent on a spontaneous reaction 6 (superoxide dismutation) that is unable to complete with reaction 7 (reduction of ascorbate by superoxide).

The role of superoxide as an intermediate in these reactions solves a problem in estimates of quantum yield of photosystem I where these were based on an incorrect assumption of the relation of oxygen uptake to electron transport (7). The production of superoxide by chloroplasts and other photosynthetic systems *in vitro* is confirmed by a variety of techniques (3), but the well documented toxicity of superoxide suggests that cellular constituents react destructively with superoxide, making the Mehler reaction unsuitable as a contributory reaction to photosynthesis. Nevertheless, a variety of natural components of chloroplasts, including flavin and quinone cofactors (26) as well as the iron-sulfur protein ferredoxin (2), readily transfer single electrons to oxygen, working as A in Eq. 5. Furthermore, there is abundant evidence that oxygen may indeed work as the Hill oxidant in chloroplasts (10). Despite a number of antioxidant defenses in photosynthetic systems, including ascorbate, glutathione, SOD, and ascorbate peroxidase (27), it would seem preferable for the initial steps of any physiological oxygen reduction in photosynthesis to proceed without superoxide as an end product, and to work instead either by concerted two- or four-electron reduction of oxygen, or by means of simultaneous consumption of superoxide so that it exists only as a short-lived intermediate.

The chemical feasibility of the option of rapid consumption of superoxide is suggested by the work of Misra and Fridovich on the pathways of autoxidation of adrenaline (25) and by Heikkila and Cohen for dopamine (20). Both adrenaline and dopamine are catecholamines that cycle initially between three redox states, RH_3^-, RH_3, and RH_2. Initial oxidation of RH_3^- by superoxide (Eq. 10) yields superoxide that is produced by the product, RH_3 (Eq. 11).

$$RH_3^- + 2H^+ + O_2^{\cdot -} \rightarrow RH_3 + H_2O_2 \quad (10)$$

$$RH_3 + O_2 \rightarrow RH_2 + O_2^{\cdot -} + H^+ \quad (11)$$

If the oxidized form of adrenaline and dopamine, RH_2, is able to function as a Hill oxidant (A in Eq. 1), then the outcome will be a variant of the Mehler reaction in which superoxide production (Eq. 11) is dependent on its prior consumption (Eq. 10). Thus, the steady-state concentration would be likely to be small even in the absence of SOD and other antioxidant defenses. This investigation suggests that RH_2 can act as a Hill oxidant in the case of both adrenaline and dopamine.

MATERIALS AND METHODS

Chloroplasts were isolated from commercially grown spinach (*Spinacea oleracea* L.) leaves using the method of Reeves and Hall (30) and as previously described (6). Chlorophyll was estimated by the method of Arnon (8), and values are given without the corrections shown to be necessary by Porra *et al.* (29). SOD was isolated from human blood by the method of McCord and Fridovich (23) and assayed using the methionine–riboflavin–nitro blue tetrazolium assay of Beauchamp and Fridovich (11).

Oxygen uptake was measured in a Rank Bros. (Bottisham, U.K.) oxygen electrode with 2 ml final volume for the hypotonic 0.1 *M* sorbitol-based reaction medium, as described (6). Reactions were carried out at 15°C, and illumination was provided by a 250-W slide projector filtered with an orange filter. Chlorophyll concentration was 50 μg of chlorophyll (chl) ml^{-1} unless stated otherwise.

Bovine catalase was purchased from Boehringer GmbH and added as a small volume of crystalline suspension. Adrenaline, dopamine, and adrenochrome were purchased from Sigma Chemical Co. (Poole, U.K.) and added as small volumes of freshly made-up, aqueous solution.

RESULTS

Table 1A shows that both adrenaline and dopamine can function as mediators of oxygen uptake by isolated spinach chloroplasts. The reactions are fully light-dependent and sensitive to inhibition by 3-(3′,4′-dichlorophenyl)-1,1′-dimethyl urea (DCMU; Diuron), an inhibitor of electron transfer through chloroplast photosystem II. Table 1B shows that this oxygen uptake is eliminated by catalase, which catalyzes the disproportionation of hydrogen peroxide.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

The effect of catalase shows that the photosynthetic oxygen uptake in the absence of catalase (Table 1A) conforms to the overall equation for the Mehler reaction (Eq. 4).

Figure 1A shows that adrenaline and dopamine support light-dependent oxygen uptake at catalytic concentrations of less than 0.1 m*M*. The oxygen uptake is therefore driven by photosynthetic electron transport, and is not a purely chemical oxidation initiated by the action of light on chloroplasts. In further support of this conclusion, the light-dependent oxygen uptake mediated by adrenaline and dopamine is proportional to chlorophyll concentration in the chloroplast suspension carrying out the reaction, as shown in Fig. 1B.

Table 2 shows that chloroplast oxygen uptake mediated by adrenaline or dopamine is inhibited by addition of the enzyme SOD. At pH 7.5, inhibition is more than 90% (adrenaline) and 85% (dopamine). The inhibition is reversed by KCN, an inhibitor of SOD. An appreciable lag phase occurred before the maximal rate of oxygen uptake was obtained, suggesting a requirement for accumulation of a catalytic intermediate. The simplest explanation of the kinetics at pH 7.5 is that the catalytic intermediate is superoxide.

It is suggested here that the intermediate RH_2 functions as an electron acceptor, that is, as a Hill oxidant, for photosynthetic electron transport (Eq. 12). Regeneration of RH_2 depends on sequential, single-electron transfers to superoxide (Eq. 10) and to oxygen, generating the required superoxide (Eq. 11). Adrenaline and dopamine therefore act as two-electron mediators of a new class of Mehler reaction, as follows.

$$2H_2O + 2RH_2 \rightarrow O_2 + 2RH_3^- + 2H^+ \quad (12)$$

$$2RH_3^- + 4H^+ + 2O_2^{\cdot -} \rightarrow 2RH_3 + 2H_2O_2 \quad (10)$$

$$2RH_3 + 2O_2 \rightarrow 2RH_2 + 2O_2^{\cdot -} + 2H^+ \quad (11)$$

$$\text{Sum:} \quad 2H_2O + O_2 \rightarrow 2H_2O_2 \quad (4)$$

Once initiated, these reactions would be self-maintaining, requiring only an input of light and oxygen, and with hydrogen peroxide as the product. The competitive inhibition by added SOD (Table 2) will then result from its displacement of reaction 10 by reaction 6, and regeneration of the electron acceptor, RH_2, would be prevented. Furthermore, the lag phase in oxygen consumption (Table 2) would be the time required for the build-up of a steady-state superoxide concentration sufficient for production of RH_3 (Eq. 10). The overall Mehler reaction would need to be primed by superoxide from endogenous electron acceptors, but would subsequently become independent of superoxide production by any reaction other than univalent oxidation of adrenaline or dopamine (Eq. 10).

At pH values higher that 7.5, both the duration of the lag phase and the extent of inhibition by SOD are decreased (Table 2). For adrenaline, these effects might result from the operation of further steps in adrenaline oxidation leading to the formation of adrenochrome (R).

TABLE 1. OXYGEN UPTAKE IN ISOLATED CHLOROPLASTS MEDIATED BY ADRENALINE AND DOPAMINE

*(**A**) Light dependency and DCMU sensitivity*

	Rate of oxygen uptake, μmol (mg chl)⁻¹ h⁻¹				
Mediator	*Initial*	*Plus mediator*	*Light off*	*Light on*	*Plus DCMU*
Adrenaline	14.4	146	0.01	59	0.0
Dopamine	13.0	39.6	1.8	40.7	5.4

DCMU was added where indicated to a final concentration of 5 μ*M*. Adrenaline and dopamine were present at 0.5 m*M* final concentration with 50 m*M* HEPES, pH 7.5 (adrenaline) or 50 m*M* Tricine, pH 8.0 (dopamine).

*(**B**) Effect of catalase and sodium azide (NaN_3; inhibitor of catalase activity)*

	Rate of oxygen uptake, μmol (mg chl)⁻¹ h⁻¹		
Mediator	*Plus mediator*	*Plus catalase*	*Plus catalase, NaN_3*
Adrenaline	113	29.4	92.8
Dopamine	59.4	9.8	59.0

Catalase (4,000 units) and NaN_3 (to 10 m*M* final concentration) were added where indicated. The buffer was 50 m*M* Tricine, pH 8.0.

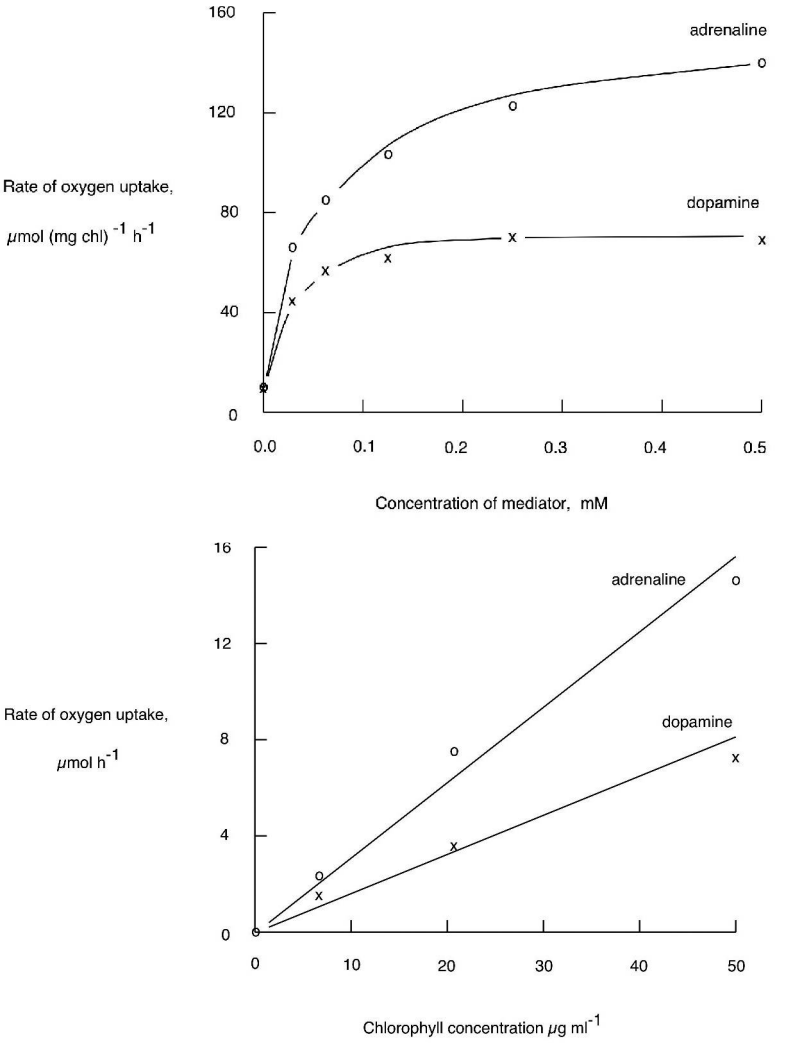

FIG. 1. **(A)** Rate of light-dependent oxygen uptake in isolated chloroplasts as a function of the concentration of either adrenaline or dopamine. Reaction medium was at pH 8.0 maintained by Tricine buffer (at 50 m*M* final concentration). **(B)** Rate of light-dependent oxygen uptake by isolated chloroplasts as a function of chloroplast density measured as chlorophyll concentration. Adrenaline was present at 0.5 m*M* in a reaction medium containing HEPES buffer (50 m*M* final concentration) and at pH 7.5. Dopamine was present at 0.25 m*M* with Tricine buffer (50 m*M* final concentration) at pH 8.0.

TABLE 2. EFFECTS OF pH AND ADDITION OF SOD AND POTASSIUM CYANIDE (KCN), AN INHIBITOR OF SOD, ON OXYGEN UPTAKE MEDIATED BY ADRENALINE AND DOPAMINE

Rate of oxygen uptake, μmol (mg chl)$^{-1}$ h^{-1}				
	Initial	*Plus SOD*	*Plus SOD, KCN*	*Lag phase, s*
Adrenaline				
pH 7.5	90.8	6.8	81.8	80
pH 8.0	123	68.4	96.2	20
pH 8.5	117	56.6	89.0	12
Dopamine				
pH 7.5	34.9	5.0	36.7	360
pH 8.0	77.0	10.4	83.5	90
pH 8.5	73.8	57.6	70.2	40

Adrenaline and dopamine were present where indicated to a final concentration of 250 μ*M*. For pH 7.5, the buffer was HEPES (50 m*M*); otherwise the buffer was Tricine (50 m*M*). SOD (200 units) and KCN (to 5 m*M*) were present where indicated, and were added after addition of the mediator. The lag phase is the time taken, after addition of the mediator, for the rate of oxygen evolution to reach the given, constant rate.

Figure 2 shows that adrenochrome, too, is effective at catalytic concentrations as a mediator of oxygen uptake by isolated chloroplasts. Table 3 shows that adrenochrome-mediated oxygen uptake is light-dependent and DCMU-sensitive. Table 4A shows that adrenochrome-mediated oxygen uptake is prompt (has little or no lag phase) and is insensitive to SOD alone. Table 4B shows that adrenochrome-mediated oxygen uptake is stimulated by ascorbate, whereas the ascorbate stimulation is reversed by addition of SOD. It is concluded that adrenochrome, too, accepts electron from the photosynthetic chain (reaction 13), but, unlike adrenaline and dopamine, reduced adrenochrome passes single electrons to oxygen, giving superoxide (reaction 14). This superoxide is a product of oxygen reduction, not a catalytic intermediate, and may subsequently react with itself (by spontaneous or catalytic dismutation; Eq. 6) or with reductants such as ascorbate (Eq. 7).

$$2H_2O + 4R \rightarrow O_2 + 4RH \quad (13)$$

$$4RH + 4O_2 \rightarrow 4R + 4O_2^{\cdot -} + 4H^+ \quad (14)$$

$$2O_2^{\cdot -} + 2O_2^{\cdot -} + 4H^+ \rightarrow 2H_2O_2 + 2O_2 \quad (6)$$

$$\text{Sum:} \quad 2H_2O + O_2 \rightarrow 2H_2O_2 \quad (4)$$

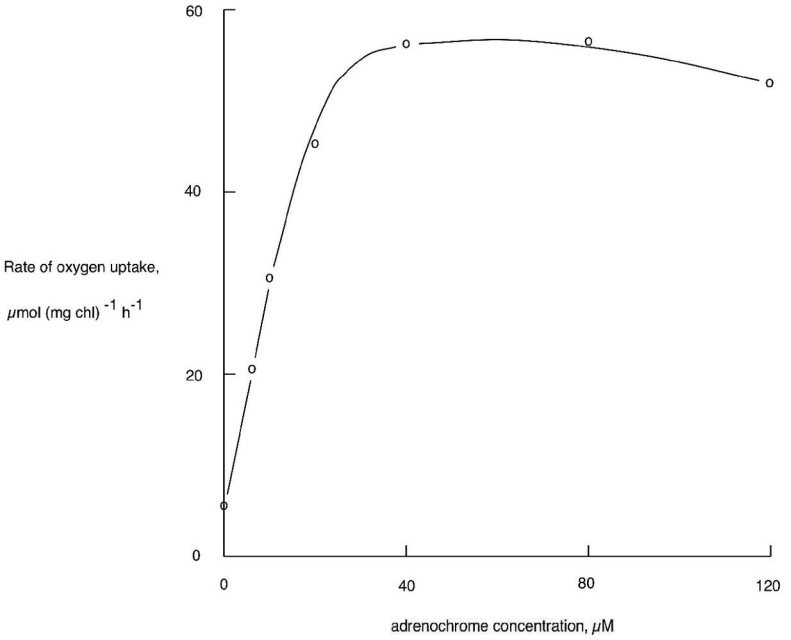

FIG. 2. Rate of light-dependent oxygen uptake in isolated chloroplasts as a function of the concentration of adrenochrome. Reaction medium was at pH 7.5 with HEPES buffer (50 m*M* final concentration).

Stimulation of oxygen uptake in the Mehler reaction has already been shown to occur when superoxide dismutation (Eq. 6) is replaced by superoxide reduction by ascorbate (Eq. 7). This mechanism may account for the effect of ascorbate on the adrenochrome-mediated Mehler reaction (Table 4B).

In the case of adrenaline and dopamine, addition of ascorbate was also found to stimulate oxygen uptake, and the stimulation was reversed by addition of SOD, as shown in Table 5A. However, if the reaction of ascorbate with superoxide (Eq. 7) is responsible for this effect, then addition of ascorbate to the reaction medium should suppress the initiation of

TABLE 3. OXYGEN UPTAKE IN ISOLATED CHLOROPLASTS MEDIATED BY ADRENOCHROME

*(**A**) Light dependency and DCMU sensitivity*

	Rate of oxygen uptake, μmol (mg chl)⁻¹ h⁻¹			
Initial	*Plus adrenochrome*	*Light off*	*Light on*	*Plus DCMU*
7.2	56.2	0.0	50.4	0.0

DCMU was added where indicated to a final concentration of 5 μ*M*. Adrenochrome was present where indicated at 0.5 m*M* final concentration with 50 m*M* HEPES, pH 7.5.

*(**B**) Effect of catalase and KCN (inhibitor of catalase activity)*

Rate of oxygen uptake, μmol (mg chl)⁻¹ h⁻¹		
Plus adrenochrome	*Plus catalase*	*Plus catalase, KCN*
64.1	0.0	64.8

Adrenochrome was present at 0.1 m*M* final concentration with 50 m*M* HEPES, pH 7.5. Catalase (4,000 units) and KCN (to 10 m*M* final concentration) were added where indicated.

TABLE 4. OXYGEN UPTAKE IN ISOLATED CHLOROPLASTS MEDIATED BY ADRENOCHROME

*(**A**) Effects of pH and addition of SOD and KCN, an inhibitor of SOD, on oxygen uptake mediated by adrenochrome*

	Rate of oxygen uptake, μmol (mg chl)⁻¹ h⁻¹			
	Initial	*Plus SOD*	*Plus SOD, KCN*	*Lag phase, s*
pH 7.5	60.1	54.0	64.4	8
pH 8.0	55.8	54.7	51.8	6
pH 8.5	52.6	49.4	45.7	4

Adrenochrome was present at a final concentration of 0.5 m*M*. For pH 7.5, the buffer was HEPES (50 m*M*); otherwise the buffer was Tricine (50 m*M*). SOD (200 units) and KCN (to 5 m*M*) were present where indicated, and were added after addition of the mediator. The lag phase is the time taken, after addition of the mediator, for the rate of oxygen evolution to reach the given, constant rate.

*(**B**) Effects of pretreatment with ascorbate, SOD, and KCN*

Conditions	*Rate of oxygen uptake, μmol (mg chl)⁻¹ h⁻¹*
No addition	56.9
Plus ascorbate	124
Plus SOD	55.4
Plus SOD, ascorbate	90.0
Plus SOD, ascorbate, KCN	168

Adrenochrome was present at a final concentration of 0.5 m*M*. The buffer was HEPES (50 m*M*), pH 7.5. SOD (200 units), ascorbate (to 1 m*M*), and KCN (to 5 m*M*) were present where indicated.

TABLE 5. OXYGEN UPTAKE MEDIATED BY ADRENALINE AND DOPAMINE AND EFFECTS OF ASCORBATE, SOD, AND KCN

(A) Effects of addition of ascorbate, SOD, and KCN to the reaction already initiated and proceeding at the rate indicated (initial)

	Rate of oxygen uptake, μmol (mg chl)$^{-1}$ h^{-1}			
Mediator	*Initial*	*Plus ascorbate*	*Plus ascorbate, SOD*	*Plus ascorbate, SOD, KCN*
Adrenaline	118	164	90.0	144
Dopamine	62.0	112	28.8	58.3

Adrenaline and dopamine were present where indicated at a final concentration of 250 μ*M*. The buffer was Tricine (50 m*M*), pH 8.0. SOD (200 units), ascorbate (to 1 m*M*), and KCN (to 5 m*M*) were added where indicated.

(B) Effects of pretreatment with ascorbate, SOD, and KCN

	Rate of oxygen uptake, μmol (mg chl)$^{-1}$ h^{-1}	
Conditions	*Adrenaline*	*Dopamine*
No addition	99.0	62.0
Plus ascorbate	6.8	22.3
Plus SOD	8.3	23.0
Plus SOD, ascorbate	5.0	—
Plus SOD, KCN	64.4	59.4
Plus ascorbate, KCN	15.8	—
Plus KCN	104	—

Adrenaline was present where indicated at a final concentration of 0.5 m*M* in HEPES buffer (50 m*M*), pH 7.5. Dopamine was present where indicated at a final concentration of 250 μ*M* in Tricine buffer (50 m*M*), pH 8.0. SOD (200 units), ascorbate (to 1 m*M*), and KCN (to 5 m*M*) were present where indicated. Blank (—) means value was not determined.

the adrenaline- and dopamine-mediated Mehler reactions, essentially extending the lag phase indefinitely. Table 5B confirms that this is the case. When added before the mediator, both SOD and ascorbate suppressed the adrenaline- and dopamine-mediated Mehler reactions (Table 5B). This effect confirms the requirement for a build-up of superoxide (Eq. 10) in order for adrenaline or dopamine to be oxidized to a state (RH_2) that is able to act as an electron acceptor from the photosynthetic chain (Eq. 12).

For adrenaline, also termed epinephrine, (RH_3^-), five oxidation states (RH_3^-, RH_3, RH_2, RH, R) were proposed by Misra and Fridovich (25). From the results presented here, it may be concluded that two of these states (RH_2 and R) accept electrons from the photosynthetic chain. Photosynthetic reduction of RH_2 to RH_3^- allows oxygen uptake that is dependent on superoxide as an obligatory, catalytic intermediate in regeneration of the electron acceptor, which also seems to be the case with dopamine. In contrast, photosynthetic reduction of R (adrenochrome) produces an autoxidizable species (RH or $R^{\cdot-}$) that produces superoxide, without electron transport then becoming dependent on superoxide as an intermediate in regeneration of the electron acceptor.

DISCUSSION

The Mehler reaction with artificial mediators of photosynthetic electron transport to oxygen usually proceeds by means of univalent reduction of oxygen to superoxide (6), as shown in Fig. 3. Examples of mediators that work by this route now include adrenochrome, as indicated in Fig. 2 and Tables 3 and 4. In addition, a two-step reduction of oxygen can be concluded for the mediators adrenaline and dopamine, as shown in Fig. 4. In this two-step reduction, superoxide serves as an obligatory, catalytic intermediate in reduction of oxygen.

This process (Fig. 4) may serve as an analogue of a physiological Mehler reaction, because there is no net production of superoxide. A similar mechanism for physiological oxygen reduction in photosynthesis was suggested by Elstner and Heupel (14), but with an "oxygen reducing factor" (16) as the intermediate (15). One argument against this mechanism (Fig. 4) for photosynthetic oxygen reduction under physiological conditions is that the suppression of superoxide production by endogenous ascorbate or SOD will prevent oxygen reduction from occurring in the first place, as seen experimentally in Tables 2 and 5.

The remaining pathway of oxygen reduction on photosynthesis is the two-step oxygen reduction by ferredoxin, originally proposed by Allen (2), and depicted here in Fig. 5. In this reaction, superoxide is both produced and consumed (2, 13), but there is no obligatory requirement for superoxide production in order for the reaction to be initiated, and so oxygen reduction may proceed even in the presence of ascor-

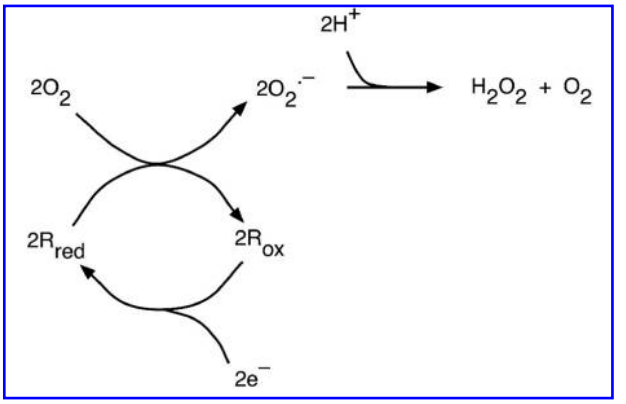

FIG. 3. Single-step reduction of oxygen by electrons (2e$^-$) from the photosynthetic chain. Mediators (R) may be bipyridyl compounds (*e.g.,* methyl viologen or paraquat), quinones, flavins, or adrenochrome (as shown here). R is a one-electron carrier and has two redox states. SOD has no effect although it may competitively replace reactions of superoxide, *e.g.,* with ascorbate. Such effects are partial and noncompetitive. Production of superoxide is the basis of paraquat toxicity. Because of the toxicity of superoxide, the reaction is unlikely to proceed under normal physiological conditions *in vivo*.

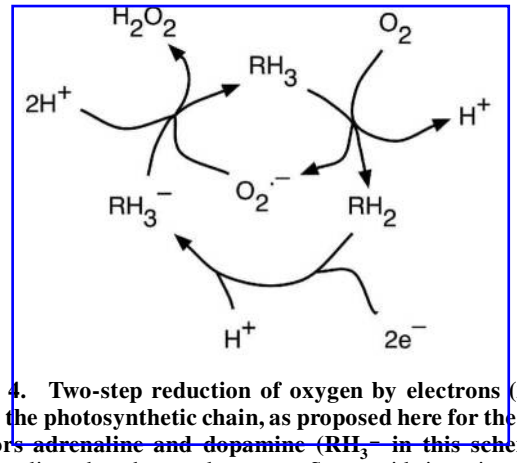

FIG. 4. Two-step reduction of oxygen by electrons ($2e^-$) from the photosynthetic chain, as proposed here for the mediators adrenaline and dopamine (RH_3^- in this scheme). The mediator has three redox states. Superoxide is an intermediate that is necessary for regeneration of the fully oxidized state, and hence for the operation of the cycle and for the effectiveness of the electron acceptor. Addition of SOD therefore causes complete, competitive inhibition of oxygen uptake and photosynthetic electron transport. Ascorbate may also cause competitive inhibition by reacting with superoxide and preventing regeneration of the electron acceptor, although transient stimulation of oxygen uptake may occur if the cycle is established before ascorbate is added.

bate and SOD. Furthermore, ferredoxin is a well characterized electron carrier in photosynthesis, and allows a relatively safe leakage of a variable proportion of electrons to oxygen (1) rather than to $NADP^+$, or to plastoquinone in a photosystem I-mediated cyclic pathway. For these reasons, I suggest that ferredoxin remains the most likely candidate for a physiological mediator of oxygen reduction in photosynthesis.

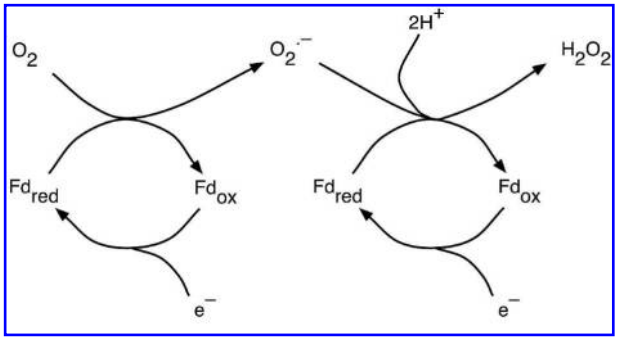

FIG. 5. Two-step reduction of oxygen by ferredoxin (Fd), a one-electron carrier with two redox states. Superoxide is an intermediate that is both generated and consumed by reduced ferredoxin. Addition of SOD suppresses the superoxide-mediated oxidation of ferredoxin, but has no effect on oxidation of ferredoxin by oxygen itself, so SOD caused partial and competitive inhibition of oxygen uptake and electron transport. Ascorbate has no effect. This mechanism may allow photosynthetic electron transfer to oxygen without production of superoxide, and without complete inhibition of electron transport by SOD. Of the three mechanisms (Figs. 3–5) for photosynthetic reduction of oxygen, two-step reduction of oxygen by ferredoxin is therefore the most feasible for any Mehler reaction operating under physiological conditions *in vivo*.

Although noncyclic electron flow to oxygen is known to be coupled to ATP synthesis in ferredoxin-catalyzed pseudocyclic photophosphorylation (31), the role of supplying additional ATP for assimilatory reactions is equally well taken by cyclic photophosphorylation, and more recent evidence suggests that cyclic electron flow may be important in a variety of physiological circumstances (4, 5). Far from eliminating need for photosynthetic reduction of oxygen, the operation of cyclic photophosphorylation is crucially dependent on maintenance of correct redox poise, and seems to require the availability of oxygen as an electron sink or "safety valve" to compensate for overreduction of the cyclic electron transfer chain, as proposed originally by Grant and Whatley (17). Subsequent experiments underline the importance of oxygen reduction in maintaining redox poise (9), and more recent evidence suggests that the protonation of the thylakoid lumen plays a role in energy dissipation by nonphotochemical quenching of chlorophyll excited states (18, 19).

The mechanism by which electrons may be passed safely to oxygen under physiological conditions in photosynthesis continues to be of relevance to understanding energy coupling and the ways in which plants, algae, and cyanobacteria adapt to meet changing demands for ATP relative to reducing power. The obligatory role of superoxide poses a problem that may also be addressed by signaling functions for intermediates in oxygen reduction in photosynthesis (22, 27, 28).

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ABBREVIATIONS

chl, chlorophyll; DCMU, 3-(3′,4′-dichlorophenyl)-1,1′-dimethylurea; H_2O_2, hydrogen peroxide; $O_2^{\cdot-}$, superoxide anion; SOD, superoxide dismutase.

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