On the Mechanism of Photosynthetic Oxygen Reduction by Isolated Chloroplast Lamellae

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Ferredoxin-stimulated photosynthetic oxygen reduction and concommitant decarboxylation of glyoxylate by chloroplast lamellar systems is inhibited by ascorbate but ferredoxin dependent $NADP^+$-reduction is not. In the presence of low potential electron acceptors (AQ or MV) this influence of ascorbate on glyoxylate decarboxylation by chloroplast lamellar systems is no longer observed.

Using the diaphorase activity of NADP-ferredoxin reductase either bound to the chloroplast lamellar system or as an isolated enzyme fraction glyoxylate decarboxylation in the dark can be observed in the presence of $NADPH+H^+$ and autooxidizable electron acceptors (AQ, MV, ferredoxin). The influence of ascorbate on this dark reaction, depends on whether the activity of the isolated or the lamellae-bound enzyme is measured:

1. With the isolated enzyme no influence of ascorbate on AQ-, MV- or ferredoxin-stimulated glyoxylate decarboxylation is observed.

2. The dark-reaction with $NADPH+H^+$ as electron donor, catalyzed by the bound enzyme however is inhibited by ascorbate both in the presence of either ferredoxin or AQ. This and other observations support the view that the site of inhibition by ascorbate of oxygen reduction by chloroplast lamellar systems in the presence of ferredoxin is not identical with either the reducing side of photosystem I, ferredoxin or NADP-ferredoxin reductase. The site of inhibition by ascorbate is more likely connected with an additional pathway involved in photosynthetic oxygen reduction by chloroplast lamellar systems, which is located at the reducing side of photosystem I, *i. e.* in the vicinity of the NADP-ferredoxin reductase. By heat treatment of isolated chloroplast lamellar systems a factor is released showing the activity of an ascorbate-sensitive oxygen reductant upon illumination in the presence of chloroplast lamellar systems. A model for photosynthetic oxygen reduction is proposed which includes ferredoxin and a membrane-bound oxygen reductant in series. Oxygen reduction by this pathway is only operating when the available NADP is fully reduced.

Introduction

Photosynthetic oxygen reduction by isolated chloroplast lamellar systems is quite commonly used for studying the rates of photosynthetic electron transport and photophosphorylation ("pseudocyclic photophosphorylation"). Oxygen uptake by illuminated chloroplast lamellar systems during these "Mehler"-type reactions is stimulated by autooxidizable electron acceptors such as certain quinones [8] and low potential dyes [9]. H_2O_2 is stoichiometrically formed during the autooxidation of the reduced acceptors [8].

The suggestion has been made that photosynthetic oxygen reduction may play an important role *in vivo* in providing additional ATP for CO_2 fixation [10, 11]. These proposals may be based on and substantiated by the fact that ferredoxin as the natural electron acceptor of photosystem I is also autooxidizable in its reduced form [12–14]. Again H_2O_2 has been shown as the stoichiometric product of oxygen reduction by reduced ferredoxin [13, 14]. The light-dependent peroxide production in turn is assumed to be involved in at least one possible way of initiating the complex sequence of photorespiration [15] although alternative ways have been emphasized [16].

If indeed photosynthetic oxygen reduction is involved as an initiating step in photorespiration, ferredoxin (upon the present knowledge) has been assumed to act as the predominant oxygen reductant [17].

Recently we reported about the involvement of the superoxide free radical ion ($O_2^{\cdot-}$) in an ascor-

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Abbreviations: AQ, anthraquinone-2-sulfonic acid; MV, methylviologen; TQ, I,I′-trimethylene-2,2′-bipyridylium dibromide (Triquat); DAD, diaminodurene; DCMU, 3-(3,4-dichlorphenyl-) I,I-dimethylurea.

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bate dependent photosynthetic phosphorylation [7] and of H_2O_2 in photosynthetic decarboxylation of α-keto acids [6]. We could show that in the presence of ferredoxin and NADP electron flow in chloroplast lamellar systems seems to switch from NADP to oxygen as terminal electron acceptor as soon as the available NADP is reduced initiating

a. ascorbate dependent ATP-formation by $O_2^{\cdot -}$ and

b. decarboxylation of glyoxylate by H_2O_2.

This communication will provide evidence that ferredoxin is the terminal oxygen reductant in the above case a. but not in case b. In the absence of ascorbate ferredoxin seems to act only as an electron donator for another membrane bound compound in the chloroplasts which in turn seems to function as the terminal electron acceptor and oxygen reductant in a reaction diverging from NADP reduction under certain conditions [11, 6].

Material and Methods

Ferredoxin and NADP-ferredoxin-reductase were isolated from spinach leaves as described by Tagawa and Arnon [1] and Shin *et al.* [2], respectively. Cytochrome$_{552}$ was isolated from *Euglena gracilis* [3].

Chloroplast lamellar systems were prepared from spinach or sugar beet leaves as described by Nelson *et al.* [4]. The preparation of chloroplast lamellar systems from sugar beet leaves was changed insofar as PVP (Polyvinylpyrrolidone, Serva; MV = 350 000) was included in the grinding medium in a concentration of 3 mg/ml. Digitonin fragmented chloroplast were prepared from spinach chloroplast lamellar systems [5].

The photosynthetic experiments were carried out in cubic Warburg vessels in a Photo-Warburg thermostate at 15 °C with illumination (25 000 lx) from the bottom. Decarboxylation of [I-^{14}C]glyoxylate, ATP formation, and NADP reduction were measured as recently described [6, 7]. Oxygen uptake was measured with a Gilson oxygen electrode. Spinach was grown in a greenhouse at 20 °C under a light cycle of 11 hours illumination and 13 hours dark. Sugar beet leaves (greenhouse cultures) were a gift from the Kleinwanzlebener Saatzucht AG, Einbeck/Hann. (FRG). Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and NADP were purchased from Boehringer, Mannheim; [I-^{14}C]glyoxylate from the Radiochemical Center, Amersham.

Results

Effect of ascorbate on the ferredoxin-stimulated oxygen reduction by isolated chloroplast lamellar systems

Oxygen uptake by illuminated chloroplast lamellar systems in the presence of an autooxidizable electron acceptor (like AQ) is stimulated by ascorbate, as already reported by several authors [7, 8, 18–20]. If ferredoxin is used as the "autooxidizable" electron acceptor however an inhibition by ascorbate of oxygen uptake is observed (Fig. 1).

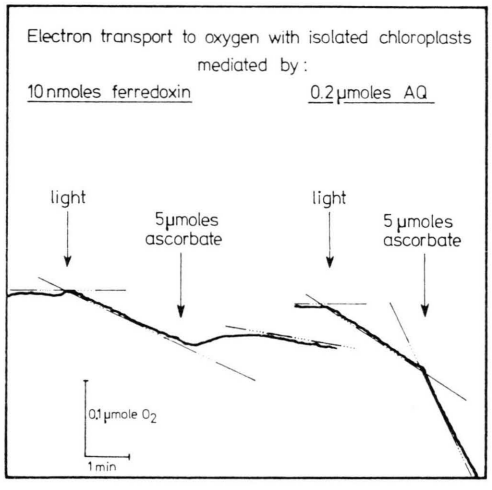

Fig. 1. Influence of ascorbate on photosynthetic oxygen reduction by chloroplast lamellar systems in the presence of either ferredoxin or AQ. The reaction mixture contained in 1.5ml: 40 µmol Tris buffer, pH 8.0, isolated chloroplast lamellar systems with 0.1 mg chlorophyll, 0.5 µmol sodium azide and the indicated additions. The reaction was conducted at 15 °C in the light (80 000 lx).

If the decarboxylation of glyoxylate is used as a test system for H_2O_2-formation by chloroplast lamellar systems with different autooxidizable electron acceptors [6] no influence of ascorbate on the decarboxylation is observed in the case of AQ or MV. The ferredoxin-stimulated decarboxylation however is inhibited by addition of ascorbate (Table I).

Another observation also demonstrates the different behaviour of ferredoxin-stimulated oxygen reduction in the presence or absence of ascorbate: As shown in Table II, NADP reduction and photophosphorylation proceed at the same rates, whether the water-splitting system or asc/DAD (in the

Table I. Decarboxylation of glyoxylate by chloroplast lamellar systems with different electron acceptors in the absence or presence of ascorbate. The reaction mixture contained in 3 ml: Chloroplast lamellar systems from sugar beet leaves with 0.1 mg chlorophyll, 80 μmol Tris buffer (pH 7.6), 5 μmol NH_4Cl, 3 μmol sodium [1-^{14}C] glyoxylate (0.07 Ci/mol), 1 μmol sodium azide, 10 μmol sodium ascorbate where indicated. The reaction was carried out for 15 min at 15 °C in the light (25 000 lx).

Electron acceptor	[μmol glyoxylate decarboxylated per mg chlorophyll/h]	
	−Ascorbate	+Ascorbate
none	1.1	0.3
ferredoxin (10 nmol) plus 1 μmol NADP	4.5	1.4
AQ (0.2 μmol)	6	8
MV ((0.2 μmol)	6	7

Table II. Comparison of photosynthetic NADP-reduction, photophosphorylation and glyoxylate decarboxylation by chloroplast lamellar systems from sugar beet leaves (μmol/mg chlorophyll/hour). The reaction mixture contained in 3 ml: Chloroplast lamellar systems from sugar beet leaves with 0.2 mg chlorophyll, 10 nmol ferredoxin, 80 μmol Tris buffer (pH 8.0), 10 μmol ADP, 10 μmol orthophosphate. In the experiments, where glyoxylate decarboxylation was measured, 3 μmol sodium [1-^{14}C] glyoxylate (0.03 Ci/mol) and 1 μmol sodium azide were present. The reactions were carried out for 15 min at 15 °C in the light (25 000 lx).

Electron transport system	NADP reduced (with 6 μmol NADP as acceptor)	ATP formed	Glyoxylate decarbox. (in the presence of 2 μmol NADP)
$H_2O \rightarrow$ NADP	75	35	4
Asc/DAD + (DCMU $2 \times 10^{-5+}$M) → NADP	70	35	0.5

presence of DCMU) are used as electron donors whereas the decarboxylation of glyoxylate is considerably lower with the artificial donor system. Identical effects are measured with chloroplast lamellar systems from either spinach or from sugar beet leaves.

The observations demonstrated in Tables I and II suggest a specific influence of ascorbate on the mechanism of ferredoxin-stimulated oxygen reduction, since the function of ferredoxin in NADP-reduction is not influenced by ascorbate.

Site of inhibition by ascorbate of oxygen reduction by chloroplast lamellar systems

Oxygen reduction and decarboxylation of glyoxylate can be observed in the dark if AQ is reduced by NADPH + H^+ in the presence of NADP-ferredoxin reductase (acting as a diaphorase). Since isolated chloroplast lamellar systems still contain the NADP-ferredoxin reductase firmly attached to the lamellae AQ reduction in the presence of NADPH + H^+ can be catalyzed by these particles in the dark. Ferredoxin can substitute for AQ in this reaction. If we compare the oxygen reduction (measured as glyoxylate decarboxylation) mediated by AQ or ferredoxin *in light* (Table I) with the one driven by NADPH + H^+ *in the dark*, a marked difference in the influence of ascorbate on the AQ system is observed. In the light-reaction ascorbate does not inhibit the AQ-stimulated oxygen reduction but does inhibit the ferredoxin-stimulated reaction. In the dark reaction with NADPH + H^+ as electron donor however both reactions are inhibited by ascorbate measured as a decrease in glyoxylate decarboxylation (Table III).

Table III. Decarboxylation of glyoxylate by isolated chloroplast lamellar systems in the dark with NADPH + H^+ as electron donor and ferredoxin or AQ as electron acceptors. The reaction mixture contained in 3 ml: Chloroplast lamellar systems from spinach-(sugar beet-)leaves with 0.2 mg chlorophyll, 80 μmol Tris buffer (pH 7.6), 10 μmol glucose-6-phosphate, 20 ng glucose-6-phosphate dehydrogenase, 2 μmol $NADP^+$, 3 μmol sodium [1-^{14}C]glyoxylate (0.07 Ci/mol), 1 μmol sodium azide, and 10 μmol ascorbate as indicated. The reaction was carried out for 15 min at 15 °C in the dark.

Acceptor	[μmol glyoxylate decarboxylated per mg chlorophyll/h]	
	−Ascorbate	+Ascorbate
none	0.9 (0.3)	0.1 (0.1)
AQ (0.2 μmol)	4.1 (4.5)	0.2 (0.1)
ferredoxin (10 μmol)	2.0 (1.5)	0.1 (0.1)

This different behaviour of AQ-stimulated oxygen reduction cannot be attributed to a specific inhibition by ascorbate of the NADP-ferredoxin reductase, since the catalytic action of the isolated enzyme is not influenced by addition of ascorbate (Table IV). As demonstrated in Table IV the rates of oxygen reduction catalyzed by the NADP-ferredoxin reductase system seem to be dependent on

a. the redox potential, and
b. on the autooxidizability of the electron acceptor,

independent of whether ascorbate is present or not. Ferredoxin seems to be less autooxidizable compared to MV in the presence of saturating amounts

Table IV. Lack of influence of ascorbate on the decarboxylation of glyoxylate by NADPH+H$^+$ and isolated NADP-ferredoxin-reductase with different electron acceptors. The reaction mixture contained in 3 ml: 80 μmol Tris buffer (pH 7.6) NADP-ferredoxin-reductase (fraction) with 0.2 mg protein, 10 μmol glucose-6-phosphate, 20 ng glucose-6-phosphate dehydrogenase, 2 μmol NADP$^+$, 3 μmole sodium [1-^{14}C] glyoxylate (0.07 Ci/mol), 1 μmol sodium azide, and 10 μmol ascorbate as indicated. The reaction was carried out for 15 min at 15 °C in the dark.

Electron acceptor —	Eo′ [mV]	[μmol glyoxylate decarboxylated per hour]	
		−Ascorbate	+Ascorbate
—	—	0.00	0.00
AQ (0.2 μmol)	−200	1.05	0.98
ferredoxin (10 nmol)	−430	0.03	0.03
MV (0.2 μmol)	−440	0.13	0.14
TQ (0.2 μmol)	−550	0.00	0.00

of NADPH + H$^+$ and NADP-ferredoxin reductase, although its redox potential is close to the one of MV. The rate of glyoxylate decarboxylation with ferredoxin and isolated NADP-ferredoxin reductase is considerably lower compared with the reaction catalyzed by chloroplast lamellar systems, although identical electron donor systems are used in both dark-reactions (*cf.* Table III). From the data presented so far it seems evident that the site of interaction of ascorbate with oxygen reduction is not due to an inhibition of 1. the lightreaction itself; 2. the mechanism of peroxide formation by autooxidation of either AQ, MV or ferredoxin; 3. NADP-ferredoxin reductase or its activity as a diaphorase.

The site of inhibition by ascorbate of oxygen reduction seems to be located close to that part of electron transport in the chloroplast membrane, which connects the reducing side of photosystem I with the final electron acceptor NADP, but does not seem to be identical with either ferredoxin or with NADP-ferredoxin reductase.

Comparison of oxygen reduction in chloroplast lamellar systems with oxygen reduction by digitonin fragmented chloroplasts

In order to further elucidate the kind of interaction of ascorbate digitonin-fragmented chloroplasts were used to study oxygen reduction. These particles have been shown to be able to decarboxylate α-keto acids in the presence of an artificial plast lamellar systems digitonin-fragmented chloroelectron donor in the light [6]. Compared with chloroplast lamellar systems digitonin fragmented chlorocentrations of ferredoxin using glyoxylate decarboxylation as a test for oxygen reduction. Fig. 2 A, shows that the decarboxylation in the dark with NADPH + H$^+$ as electron donor in chloroplast lamellar systems is stimulated by ferredoxin linearly up to 60 nmol ferredoxin added, whereas the light reaction with the intact water splitting system as electron donor shows a saturation at lower concentrations of ferredoxin. In digitonin-fragmented chloroplasts however the light reaction with an artificial electron donor system is stimulated by increasing amounts of added ferredoxin without reaching saturation at 60 nmol of added ferredoxin, while the "reverse electron transport" in the dark with NADPH + H$^+$ as electron donor is already saturated with 20 nmol of added ferredoxin (Fig. 2 B). These results may be interpreted as follows:

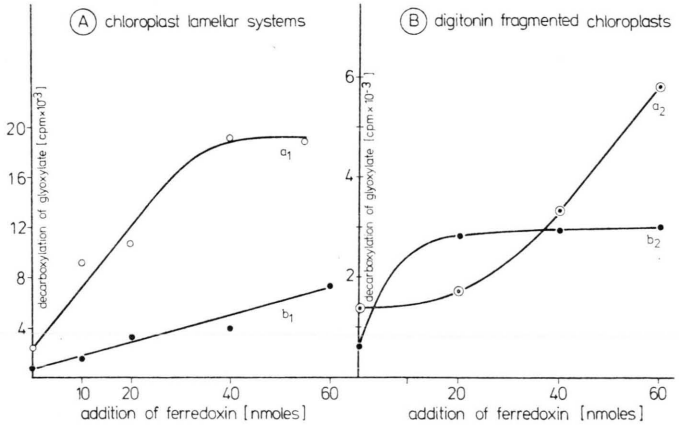

Fig. 2. Influence of increasing amounts of ferredoxin on the decarboxylation of glyoxylate by either chloroplast lamellar systems or digitonin fragmented chloroplasts. A. For experimental conditions see Table I for curve a_1 or Table III for curve b_1. cpm $^{14}CO_2$ trapped: ○—○ during the light reaction with water as electron donor; ●—● in the dark with NADPH+H$^+$ as electron donor.
B. For experimental conditions see Table V. cpm $^{14}CO_2$ trapped: ○—○ during the light reaction with NADPH+H$^+$ as electron donor in the presence of cytochrome$_{552}$; ●—● in the dark with NADPH+H$^+$ as electron donor.

1. The saturation by ferredoxin of oxygen reduction by chloroplast lamellar systems may be due to a limiting donor reaction of the donor side of photosystem I (curve a_1); if the donor side of photosystem I is "opened" by digitonin treatment, increasing amounts of ferredoxin can be reduced, resulting in increased glyoxylate decarboxylation (curve a_2).
2. In the dark reactions with $NADPH + H^+$ as electron donor apparently a factor is present in chloroplast lamellar system (curve b_1) which is limiting the ferredoxin-dependent oxygen reduction after digitonin treatment. This factor is apparently involved in ferredoxin stimulated oxygen reduction at the expense of $NADPH + H^+$ in the dark (curve b_2).

This conclusion is further substantiated by the finding (Table V A) that ascorbate as well as $NADPH + H^+$ (in the presence of $cytochrome_{552}$) can be used as electron donors for the autooxidizable photosystem I of digitonin fragmented chloroplasts in a stoichiometrically additive reaction (*cf.* Table II). The "revers electron flow" from $NADPH + H^+$ to AQ in the dark (via NADP-ferredoxin reductase which still seems to be partly present in digitonin fragmented chloroplasts) is no longer inhibited by ascorbate (Table V B). Apparently by digitonin treatment at least two changes in the vicinity of the reducing side of photosystem I have been made: 1. The autooxydizability of photosystem I is increased. This site of oxygen reduction is not influenced by ascorbate. 2. The site of inhibition by ascorbate of the "reverse electron flow" from $NADPH + H^+$ to AQ via bound NADP-ferredoxin reductase has been eliminated. These findings may be taken as evidence that the reducing side of photosystem I (primary acceptor) is not ascorbate-sensitive (*cf.* Table I, AQ and MV systems) focussing the site of inhibition by ascorbate into the vicinity of the NADP-ferredoxin reductase.

Table V. Lack of influence of ascorbate on glyoxylate decarboxylation by autooxidation of photosystem I and on AQ-reduction in the dark with $NADPH + H^+$ and lamellar-bound NADP-ferredoxin-reductase of digitonin fragmented chloroplasts. The reaction mixture contained in 3 ml: Digitonin fragmented chloroplasts with 0.1 mg chlorophyll, 80 μmol Tris buffer (pH 7.6), 3 μmol sodium [1-^{14}C] glyoxylate (0.07 Ci/mol), 1 μmol sodium azide, and an electron donor system; the electron donor system contained: 10 μmol glucose-6-phosphate, 20 ng glucose-6-phosphate dehydrogenase, 2 μmol NADP, 30 nmol $cytochrome_{552}$ were present in the light reactions.

	Reaction	Additions	[μmol glyoxylate decarboxylated per mg/chlorophyll/h]
A light	complete	—	0.9
	minus electron donor system	10 μmol asc.	2.4
	complete	10 μmol asc.	3.4
B dark	complete	—	0.1
	complete	0.2 μmol AQ	1.3
	complete	10 μmol asc.	0.3
	complete	10 μmol asc. + 0.2 μmol AQ	1.7

Extraction of a heat-stabile compound from the chloroplast lamellar systems, which exhibits the characteristics of ascorbate-sensitive oxygen reduction upon illumination together with chloroplast lamellar systems

Isolated chloroplast lamellar systems were washed twice with 2 ml (4 mg chlorophyll/ml) of resuspending medium (see Methods) and were centrifuged. The combined supernatants (wash-extracts) were stored. The pellet (containing chloroplast lamellar systems with 16 mg chlorophyll) was resuspended in 4 ml medium and heated 5 min to 80 °C. After centrifugation at 20 000 × *g* (10 min) a clear pale-green supernatant was obtained.

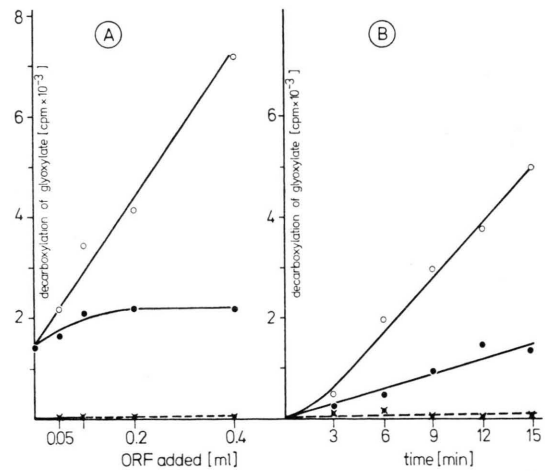

Fig. 3. Influence of a factor, released from chloroplast lamellar systems upon heat-treatment, on photosynthetic glyoxylate-decarboxylation. For experimental conditions see Table I. A. cpm $^{14}CO_2$ trapped: ○—○ with the supernatant after heat-treatment of chloroplast lamellar systems (ORF); ●—● with the wash-extract of chloroplast lamellae; ×—× with ORF in the presence of 10 μmol ascorbate. B. cpm $^{14}CO_2$ trapped: ○—○ with 0.4 ml ORF; ●—● without ORF; ×—× with ORF, but without chloroplast lamellar systems.

As shown by Fig. 3 A, the supernatant of the heat-treated chloroplast lamellar systems stimulates glyoxylate decarboxylation (as a measure for oxygen reduction) in the presence of twice washed chloroplast lamellar systems, whereas the wash-extracts do not. This stimulation is abolished by addition of ascorbate. The kinetics of glyoxylate decarboxylation (Fig. 3 B) clearly show that both chloroplast lamellar systems and an "oxygen reducing factor" (ORF), isolated by heat treatment are necessary for optimal rates and that this factor in the absence of chloroplast lamellar systems is not catalyzing the decarboxylation upon illumination.

Kinetics of glyoxylate decarboxylation by chloroplast lamellar systems in the presence or absence of ascorbate

As already shown in Table I, glyoxylate decarboxylation by chloroplast lamellar systems in the presence of ferredoxin cannot be completely inhibited by ascorbate; if the factor isolated by heat-treatment of chloroplast lamellar systems is added instead of ferredoxin however a complete inhibition is observed (Fig. 3 A).

Fig. 4 A shows the decarboxylation of glyoxylate in comparison to NADP-reduction with ferredoxin and limiting amounts of NADP (1.5 μmol). As already described in an earlier paper [6] glyoxylate decarboxylation is initiated as soon as the available NADP is reduced. In the presence of ascorbate an inhibition of glyoxylate decarboxylation is observed; a slow, linear rate of decarboxylation is retained however.

If ferredoxin is substituted for by addition of the factor isolated by heat treatment of chloroplast lamellar systems (ORF) a fast rate of decarboxylation is observed which is essentially abolished upon addition of ascorbate (Fig. 4 B). We should like to interpret these data as follows

I. In the presence of ferredoxin the allover rate of oxygen reduction consists of the two reactions, a. a slow rate representing the autooxidation of reduced ferredoxin which is not influenced by the addition of ascorbate and b. a fast ascorbate sensitive rate depending also on the presence of ferredoxin but including another reaction which is located in the chloroplast lamellae.

II. A heat stabile factor (factors?) can be isolated from spinach or sugar beet leaves which stimulates photosynthetic oxygen reduction in the presence of chloroplast lamellar systems. This stimulation of oxygen reduction is abolished upon addition of ascorbate. Since no addition of ferredoxin is necessary for the action of this factor it is most likely that this factor in its solubilized form is directly reduced by photosystem I or the primary acceptor of photosystem I. H_2O_2 is produced during the autooxidation of the reduced factor (measured as glyoxylate decarboxylation (*cf.* 6)).

Discussion

During the discussions about the nature of the primary acceptor of photosystem I a whole spec-

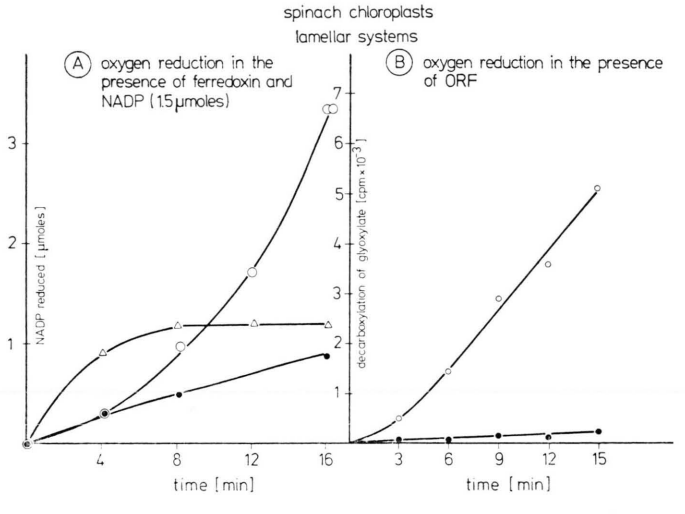

Fig. 4. Comparison of the influence of ascorbate on oxygen reduction (measured as glyoxylate decarboxylation) by chloroplast lamellar systems in the presence of either ferredoxin and NADP, or of the supernatant of heat-treated chloroplast lamellar systems (ORF). For experimental conditions see Table I.
A. △—△ NADP reduction; ○—○ glyoxylate decarboxylation (cpm $^{14}CO_2$ trapped); ●—● glyoxylate decarboxylation in the presence of 10 μmol ascorbate (cpm $^{14}CO_2$ trapped).
B. ○—○ glyoxylate decarboxylation (cpm $^{14}CO_2$ trapped); ●—● glyoxylate decarboxylation in the presence of 10 μmol ascorbate (cpm $^{14}CO_2$ trapped), 0.4 ml of the supernatant of heat-treated chloroplast lamellar systems (ORF) were added (see text!).

trum of compounds (besides "bound ferredoxin") have been presented as possible candidates for this function (*cf.* 21, 22). Many of these substances (only some have been tentatively identified) show the ability to stimulate "pseudocyclic" electron transport, *i. e.* oxygen reduction upon addition to illuminated chloroplast lamellar systems. Since the reducing side of photosystem I is rather unspecific (a variety of unphysiological compounds have been shown to act in such a manner) it is difficult to assess a physiological function for these compounds which have been isolated from green plant material. Based on the experiments described under results we should like to present a model for the function of a factor which was isolated from chloroplast lamellar systems and which stimulates oxygen reduction like many of the above mentioned compounds. Since it is certainly possible that this factor might be identical with one or more of the already described factors stimulating photosynthetic oxygen reduction [22], the main intent of this communication is not to present a "new" or another compound but a different kind of view for a possible (physiological?) function. For further discussion we should like to use the term "oxygen reducing factor" (ORF) for the described activity either isolated by heat treatment or bound (ORF_{bound}) to chloroplast lamellar systems (*cf.* 23).

The central point of the presented model (Fig. 5) is the proposal that the observed rates of oxygen reduction by chloroplast lamlelar systems in the presence of ferredoxin are not due to an autooxidation of reduced ferredoxin but to the catalysis by a membrane-bound factor (ORF_{bound}) which may be located in the vicinity of the NADP-ferredoxin reductase. The catalytic function of this factor is inhibited by ascorbate. This factor is probably not involved in NADP reduction which is supposed to be the main pathway of electron flow from photosystem I (pathway $1 \rightarrow 1a \rightarrow 1b$). As soon as the available NADP is reduced however pathway $1 \rightarrow 2 \rightarrow 2a$ involving an ascorbate-sensitive step is initiated (*cf.* 6). At least in the presence of ascorbate pathway $1 \rightarrow 3$ (autooxidation of reduced ferredoxin) is operating perhaps artificially forced by blocking pathway $2 \rightarrow 2a$ (*cf.* ref. 7).

The artificial pathways $4a \rightarrow 4c$ (light reaction) and $1b \rightarrow 1c \rightarrow 4b \rightarrow 4c$ (dark reaction) mediated by AQ have been used for determining the approximate location of the ascorbate sensitive step. Evidence for the location of the membrane-bound factor (ORF_{bound}) close to the membrane-bound NADP-ferredoxin reductase is given by the observation that AQ reduction by photosystem I or by $NADPH + H^+$ and *isolated* NADP-ferredoxin reductase is not ascorbate sensitive while AQ-reduction by $NADPH + H^+$ and chloroplast lamellar systems in the dark is ascorbate sensitive. This latter dark reaction is no longer ascorbate sensitive however if digitonin-fragmented chloroplasts are used. These particles still contain part of the membrane-bound NADP-ferredoxin reductase now apparently accessible to AQ.

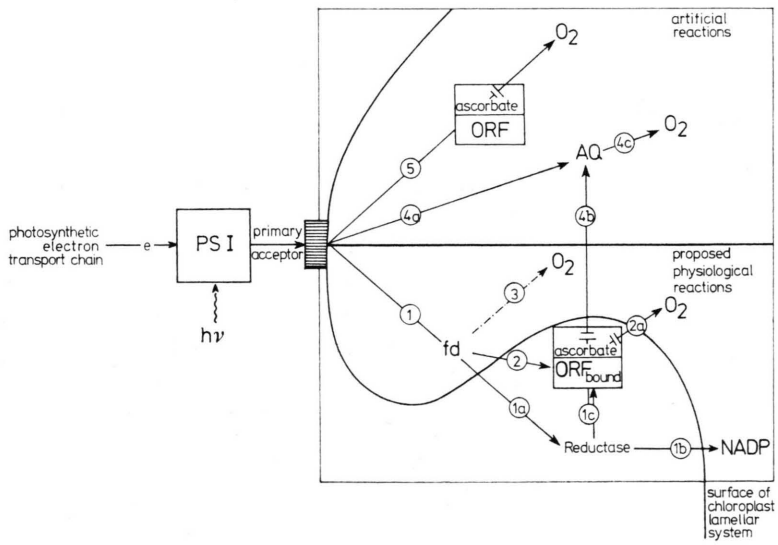

Fig. 5. Proposed model of photosynthetic oxygen reduction by chloroplast lamellar systems (for details see "discussion").

We have to assume that after heat-treatment of chloroplast lamellar systems the membrane bound factor (ORF_{bound}) is released from its binding site. Addition of the oxygen reducing factor after heat-treatment (ORF) to chloroplast lamellar systems stimulates oxygen reduction which is no longer dependent on the presence of ferredoxin but is still ascorbate sensitive (reaction 5). Besides the inhibition by ascorbate we have no evidence that ORF_{bound} and ORF are identical.

In the reactions described by the above model NADP-reduction is certainly predominant. If we however assume that in CO_2-fixation the availability of ATP might be rate-determinating (*cf.* 11) the predominant electron transport to NADP might be limiting under certain conditions, *i. e.* if NADP is fully reduced. In this case a second pathway involving oxygen as the terminal electron acceptor may take over. This pathway includes the function of an additional membrane-bound factor catalyzing the oxygen reduction. In both NADP-reduction and oxygen reduction ferredoxin is involved as an essential cofactor and center of motion. In an earlier paper we described an endogenous oxygen dependent photophosphorylation, which involves ascorbate as well as the superoxide free radical ion [7]. Under these conditions (3×10^{-3} M ascorbate), the function of ORF in oxygen reduction is obviously blocked; a small rate of oxygen reduction driven by reduced ferrodoxin is maintained, however (*cf.* Fig. 4, this paper).

In a subsequent paper (E. F. Elstner and A. Heupel, Z. Naturforsch. **29 c,** 559 [1974]) we show that a. the function of ORF demands the presence of the superoxide free radical ion and that b. ascorbate and ORF compete for the superoxide fre radical ion.

If photosynthetic oxygen reduction is considered as a physiological reaction involved in energy conservation [10, 11] and/or photorespiration [17, 15], ascorbate according to the proposed mechanism could play a role as a regulator.

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