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Photosynthetic and respiratory oxygen gas exchange measured by mass spectrometry in the filamentous cyanobacterium *Oscillatoria chalybea* in dependence on the nitrogen source in the growth medium

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Key words: Mass spectrometry; Filamentous cyanobacterium; Photosystem II; Respiration; Hydrogen peroxide; (*O. chalybea*)

Thylakoid particle preparations of the filamentous cyanobacterium *Oscillatoria chalybea* were prepared from cultures that were grown on nitrate, ammonium sulfate or arginine as the sole nitrogen source in the medium. Moreover, cultures that had no exogenous nitrogen source in the medium, hence used air as nitrogen source, were also grown. Oxygen gas exchange of these preparations was analyzed by means of mass spectrometry in an assay system that contained in the liquid phase only $H_2{}^{16}O$-containing buffer (hence no $H_2{}^{18}O$ label) and air as gas phase in which part of the $^{16}O_2$ had been substituted by $^{18}O_2$. Oxygen uptake in the dark was very low in all preparations but reacted in a subtle way already to very dim (less than 10 lx) illumination. In preparations of nitrate- and ammonium sulfate-grown cultures the dark-uptake rate was enhanced in dim light and appeared further enhanced in the dark period following this illumination. In preparations of arginine-grown cells or cells without nitrogen source in the medium dim light inhibited dark oxygen uptake. In weak light (80 lx) none of the preparations showed an O_2-uptake phenomenon but rather an O_2-evolution phenomenon. Both mass signals (mass 32 and mass 36) indicated O_2 evolution. No mixed label $^{16}O^{18}O$ was found indicating that $^{18}O_2$ evolved cannot come from water produced by respiratory activity for instance, but should come from H_2O_2. Only in strong saturating light ($2 \cdot 10^4$ lx white light) an appreciable $^{18}O_2$-uptake was observed. This uptake was sensitive to DCMU, *o*-phenanthroline, the uncoupler CCCP, KCN and SHAM. The uncoupler CCCP stimulated $^{18}O_2$ uptake in strong light, but also affected as an ADRY-reagent photosynthetic oxygen evolution. CCCP abolished also the $^{18}O_2$-evolution signal in weak light (80 lx) demonstrating that the S-state system is involved in $^{18}O_2$ production from H_2O_2. KCN also abolishes the weak light $^{18}O_2$ evolution and inhibits $^{18}O_2$ uptake in strong light. It appears that the low light intensity signal, also when measured as flash-induced signal, is inverted by KCN to an uptake phenomenon which, when analyzed under flash light, exhibits a periodicity of 2. It is suggested that part of the KCN effect observed with such preparations is due to an effect on cytochrome *b*-559. The effect of SHAM on oxygen uptake in strong light seems to indicate that *O. chalybea* owns in addition a CN^--insensitive respiratory pathway. Hence, in *O. chalybea* our method permits the distinction of at least three types of oxygen uptake: one is associated with Photosystem II and the S-state system, whereas the two other types apparently belong to the respiratory pathway.

Introduction

One of the old essentially unsolved questions in plant physiology is whether during photosynthesis respiration continues unchanged as in the dark, is enhanced or inhibited [1]. Early experiments by Brown and Webster [2] and Brown and Weiss [3] using the oxygen isotope $^{18}O_2$ have not been able to solve the question [4]. As a contribution towards the solution of the problem we have tempted to analyze oxygen gas exchange in the filamentous cyanobacterium *Oscillatoria chalybea* with mass spectrometric techniques recently described [5]. In cyanobacteria the photosynthetic electron transport chain and the respiratory chain are all located within the same membrane. Moreover, under conditions of nitrate assimilation nitrate reductase in contrast to the

Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; SHAM, salicylhydroxamic acid.

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eukaryotic condition is also located in the thylakoid membrane. As we had observed in earlier studies that a number of Photosystem II properties were changed in dependence on the nitrogen source on which the cyanobacteria had been grown [6,7] we decided to make by means of mass spectrometry a complete analysis of oxygen gas exchange in the light with thylakoid preparations from the filamentous cyanobacterium *O. chalybea*, grown on different nitrogen sources. In the present paper we analyze the properties of preparations of *O. chalybea* grown on either nitrate, ammonium sulfate or arginine as the sole nitrogen source. In addition, these preparations were compared to preparations which had been obtained from cultures grown in air on a medium without the addition of any of the above-described nitrogen components.

Materials and Methods

Cyanobacteria. Oscillatoria chalybea was obtained from the algal collection in Göttingen und cultured on different nitrogen sources in the medium as described earlier [6,7].

Thylakoid preparations of the filamentous cyanobacterium were prepared as described earlier [7] from 20-day-old cultures grown on either nitrate or ammonium sulfate or arginine as the sole nitrogen source. The mucoids on the filaments were digested with glucuronidase (Boehringer, Mannheim) and the cell walls with lysozyme (Sigma) and cellulase (Kinki Yakoult, Japan), as described in detail earlier [7].

The assay suspension usually contained an aliquot of *Oscillatoria* thylakoid preparation, equivalent to approx. 70 μg chlorophyll in 2 ml 0.06 M Tricine and 0.03 M KCl (pH 7.5).

Mass spectrometry. All assays were performed with a modified magnetic-sector field mass spectrometer 'type Delta' from Finnigan (Bremen, F.R.G.). The experimental set-up, including the valve system used, is described in detail in earlier publications [5,11]. Signals were recorded on an SE 130-03 BBC Metrawatt three-channel recorder. Light flashes of 8 μs duration were provided by a Stroboscope (1539 A of General Radio) and usually spaced 300 ms apart. Light source for the strong white light condition ($2 \cdot 10^4$ lx) was a projector lamp (Prado Universal Leitz, F.R.G.). For the low light conditions a normal incandescent bulb (Osram) was used.

Measuring procedures essentially corresponded to those described earlier [11]. 2 ml of the *Oscillatoria* preparation equivalent to approx. 70 μg chlorophyll in buffer containing only $H_2{}^{16}O$, hence no ^{18}O-label were given on the teflone membrane of the measuring cell and were allowed to sediment on the membrane. Injections of defined gas mixtures into the closed system established the desired experimental conditions with respect to the gas phase. Throughout this paper the gas phase in equilibrium with the liquid phase correspond to air (21% O_2, 79% N_2) in which half of the natural $^{16}O_2$ was substituted by $^{18}O_2$. For comparison purpose all assays were run under strictly identical conditions in particular with thylakoid preparations from the respective cultures corresponding to 70 μg of chlorophyll.

Results

In the dark or in weak light filamentous cyanobacteria show in comparison to other organisms such as *Chlorella* only very low O_2-uptake activity [20] or at the limit no oxygen uptake at all (Figs. 1 and 2). Hence, normal respiratory activity can a priori not be high under our conditions tested. Since the respiratory as well as the photosynthetic electron-transport chains are located within the same membrane an intimate mutual influenceability of the metabolic pathways is obvious. Thus, although dark respiration seems to be generally low in nitrate-, in ammonium sulfate- or arginine-grown cells, preparations from these cells react in a very subtle way already to very dim light conditions (less than 10 lx). Nitrate- and ammonium sulfate-grown preparations react upon such dim light with an immediate transient stimulation of oxygen uptake followed by a steady-state uptake rate greater than in the preceding dark period (Fig. 1). Switching off the light shows that, compared to the subsequent dark rate, the rate in the light was inhibited, since switching off the light leads again to a fast transient stimulation of O_2-uptake followed by a

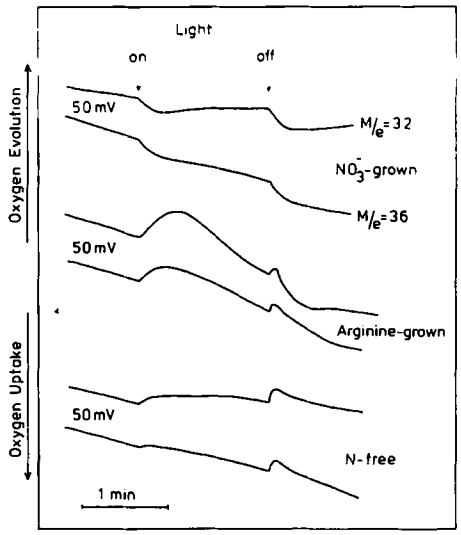


Fig. 1. Oxygen gas exchange measured at very low light intensity less than 10 lx (dim white light) by mass spectrometry in thylakoid preparations of *O. chalybea* cells, grown on nitrate or arginine as the sole nitrogen source in the medium or for comparison without any added nitrogen source to the medium (N-free). The gas atmosphere was air (21% O_2, 79% N_2) in which part of the natural oxygen ($^{16}O_2$) was substituted by $^{18}O_2$. The aqueous assay medium was made up of only $H_2{}^{16}O$-containing buffer, hence contained no ^{18}O-label.

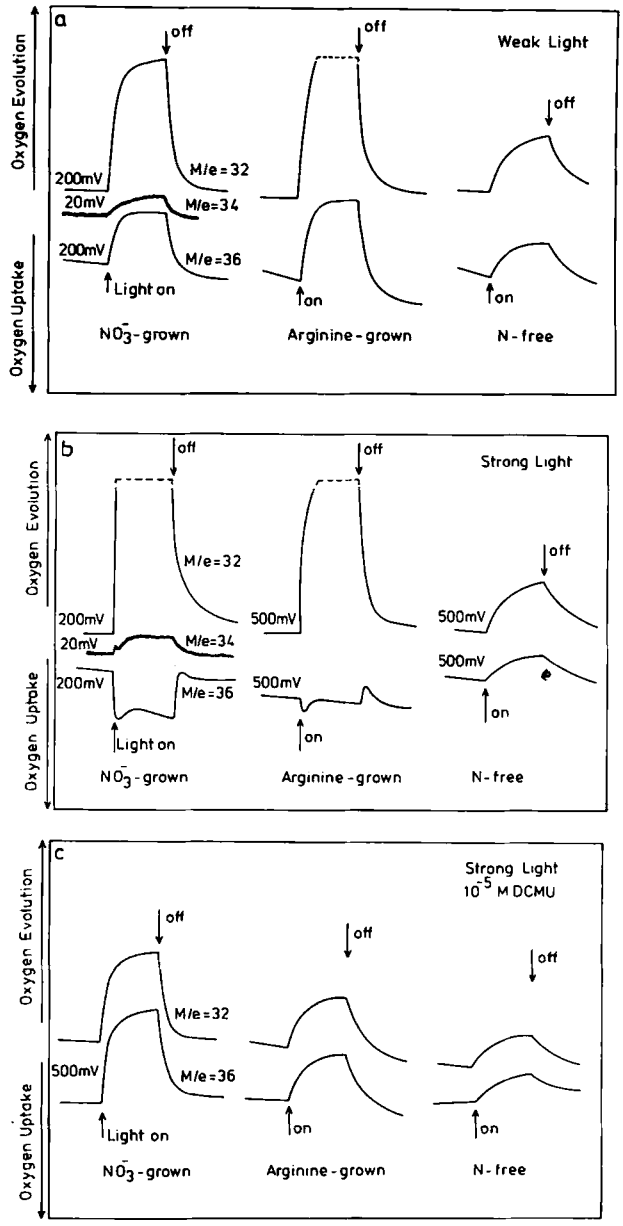


Fig. 2. Same experimental set-up as in Fig. 1 but measurement at white light (a) at 80 lx (weak light); (b) at $2 \cdot 10^4$ lx (strong light); and (c) at $2 \cdot 10^4$ lx in the presence of 10^{-5} M DCMU.

steady-state rate which is higher than that in the preceding illumination period. Arginine-grown cells or cells grown on nitrogen-free medium react in a comparable fashion upon dim light by a transient reversal of the dark oxygen-gas exchange, followed by a steady-state rate which is lower, hence inhibited, in comparison to the dark rate. This is clearly seen from the transient upon turning off the light (Fig. 1). Fig. 1 clearly demonstrates that any O_2-uptake phenomenon detectable in the dark or in dim light is low. We, therefore, proceeded in our analysis to light conditions in which photosynthesis occurs. In the following we analyzed the oxygen metabolism in weak light (80 lx) and in strong light ($2 \cdot 10^4$ lx). Fig. 2a demonstrates that in weak light (80 lx) none of the preparations of cells grown either on nitrate or ammonium sulfate, or on arginine or on N-free medium shows a net O_2-uptake phenomenon. In the experiment shown in Fig. 2 preparations of ammonium sulfate-grown cells behave like nitrate-grown ones and are therefore not contained in the figure. Addition of $^{18}O_2$ to the gas phase permits to decide whether oxygen is taken up in the light [8–10]. As the control experiments in Fig. 2a show, no net $^{18}O_2$-uptake phenomenon is observed in weak light. To the contrary $^{18}O_2$ added to the gas phase appears as $^{18}O_2$ evolution (Fig. 2a). It should be emphasized again that all experiments in the present paper (as described in Materials and Methods) contain no $H_2{}^{18}O$ label in the aqueous assay phase. Simply part of the $^{18}O_2$ of normal air in equilibrium with the liquid phase has been substituted by $^{18}O_2$. As seen from Fig. 2a no mixed label ($^{18}O^{16}O$, i.e., mass 34) is observed. The result clearly demonstrates that $^{18}O_2$-evolution observed in such an experiment (Fig. 2a) cannot come from water nor can it be due to a reaction mechanism (e.g., $H_2{}^{18}O_2 \rightarrow H_2{}^{18}O + {}^{18}O_2$) in which $H_2{}^{18}O$ is a reaction product. Furthermore the experiment shows that in weak light under such condition, as judged from the curve shape also part of the $^{16}O_2$-evolution observed contains this phenomenon. We have shown by means of mass spectrometry in a previous paper by flash light analysis that *Oscillatoria* exhibits in the region of Photosystem II a fast $^{18}O_2$-uptake phenomenon which leads to the production of H_2O_2 which is decomposed by the S-state system [11]. The observation clearly fits into and confirms observations by Åkerlund [12] and Schröder and Åkerlund [13] or Johansen [14] who show that Photosystem II of spinach not only produces but also uses H_2O_2 in its electron-transport system. The present observation clearly shows that in *Oscillatoria* the phenomenon is not only observed under flash light conditions but at least also under natural low light intensities. In strong light ($2 \cdot 10^4$ lx) the same assay permits the detection of a substantial $^{18}O_2$-uptake phenomenon which is fully comparable in preparations of nitrate- or ammonium sulfate-grown cells (Fig. 2b).

Arginine-grown cells show a fast, although small, transient $^{18}O_2$-uptake followed by a nearly full inhibition of $^{18}O_2$-uptake, whereas preparations of cells grown in N-free medium exhibit in comparison to the weak light condition of Fig. 2a an unchanged behaviour, namely the above described $^{18}O_2$-evolution (Fig. 2b). It should be borne in mind that mass 32 measures under these conditions essentially $^{16}O_2$-evolution originating from the water-splitting reaction. Addition of DCMU to the assay and measurement of the gas exchange under strong continuous light fully inhibits any $^{18}O_2$-uptake as evidenced by Fig. 2c. Instead, a positive mass 36 signal appears in preparations of nitrate-, ammonium sulfate- or arginine-grown cells, whereas in

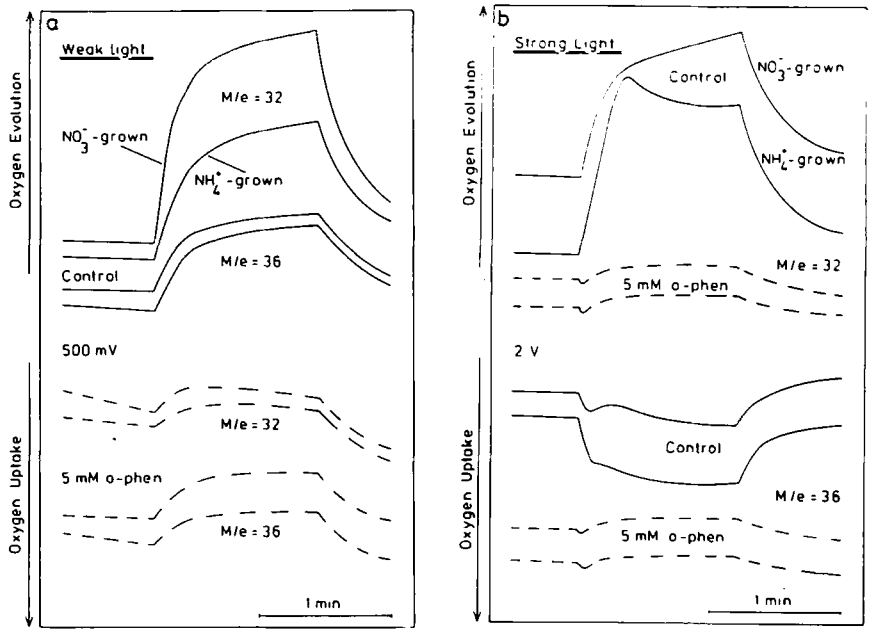


Fig. 3. Oxygen gas exchange measured by mass spectrometry in thylakoid preparations of nitrate- and ammonium sulfate-grown cells of *O. chalybea*. Solid lines: control experiments for masses 32 and 36 without addition. Dashed lines: assay in the presence of 5 mM *o*-phenanthroline. (a) Weak light; (b) strong light. The labeling conditions are identical to those in Fig. 1.

those of N-free medium not much change seems to be induced by DCMU addition. Fig. 2c does not permit to say that under the condition of strong continuous light the H_2O_2 producing and decomposing process is insensitive to DCMU, since the net phenomenon seems not to exist under natural continuous strong light condition, being the only condition in which a relatively good coupling of Photosystem II to Photosystem I is realized in *Oscillatoria chalybea*. It should be noted that the DCMU-sensitivity reported for the phenomenon under flash light illumination is only partial [11]. The light intensity of $2 \cdot 10^4$ lx used in Fig. 2b and c is saturating whereas the phenomenon shown in Fig. 2a is light limited. A general feature in many cyanobacteria seems to be an insufficient coupling of the two photosystems [20]. This has been verified for *Oscillatoria chalybea* by fluorescence induction kinetics [6]. A possible interpretation of Fig. 2 is that in *Oscillatoria* under conditions where Photosystem II is preponderantly working [6,7] and reducing equivalents are insufficiently transferred to Photosystem I, the H_2O_2 producing and decomposing cycle described by Åkerlund [12], Schröder and Åkerlund [13] and Johansen [14] is particularly well working. These conditions are produced by low light intensity or intermittent light together with high oxygen partial pressure [11] or as shown here by the addition of DCMU even under strong light condition (Fig. 2c). *o*-Phenanthroline is a typical inhibitor of Photosystem II [18] and inhibits the signals of masses 32 and 36 in weak as well as in strong light (Fig. 3a and b). In *Oscillatoria* only under strong light an appreciable respiratory O_2-uptake seems to occur, provided the cells have been grown on nitrate or ammonium sulfate (Fig. 2). Only under these conditions sufficient electrons seem to be available at the 'cytochrome *b/f*' site to be funneled towards the cytochrome *c*-type terminal oxidase. This electron transport is under our experimental conditions in the coupled state, hence involved in ATP production, as evidenced by the fact that the uncoupler CCCP stimulates this $^{18}O_2$-uptake rate (Fig. 4a). In arginine-grown cells, as said above, no appreciable apparent $^{18}O_2$-rate (hence also low respiration) is seen under strong light illumination. However, addition of CCCP reveals a particularly well-coupled respiratory electron-transport chain (Fig. 4b). It should be noted that CCCP also affects the mass 32 signal in strong light but interestingly also both signals, the mass 32 as well as 36 signal, in the weak light condition (Fig. 4c and d).

The effect of CCCP on Photosystem II has been clearly interpreted by Renger [15,16] who was able to show that ADRY-reagents such as CCCP affect Photosystem II by acceleration of the deactivation of S-states [14]. Fig. 4c can be fully interpreted in this sense. Thus, CCCP practically fully abolishes the mass 32 as well as that of mass 36 under weak light illumination in preparations of nitrate-, ammonium- and arginine-grown cells. The fact that CCCP affects the positive mass 32- and 36 signals observed in the weak light assay (Fig. 4d) adds one more evidence to the fact that the H_2O_2 producing and decomposing reaction sequence described by Åkerlund [12]. Johansen [14] and ourselves [11] is linked to the S-state system.

In the following we tested the effect of cyanide on the gas exchange under our experimental conditions.

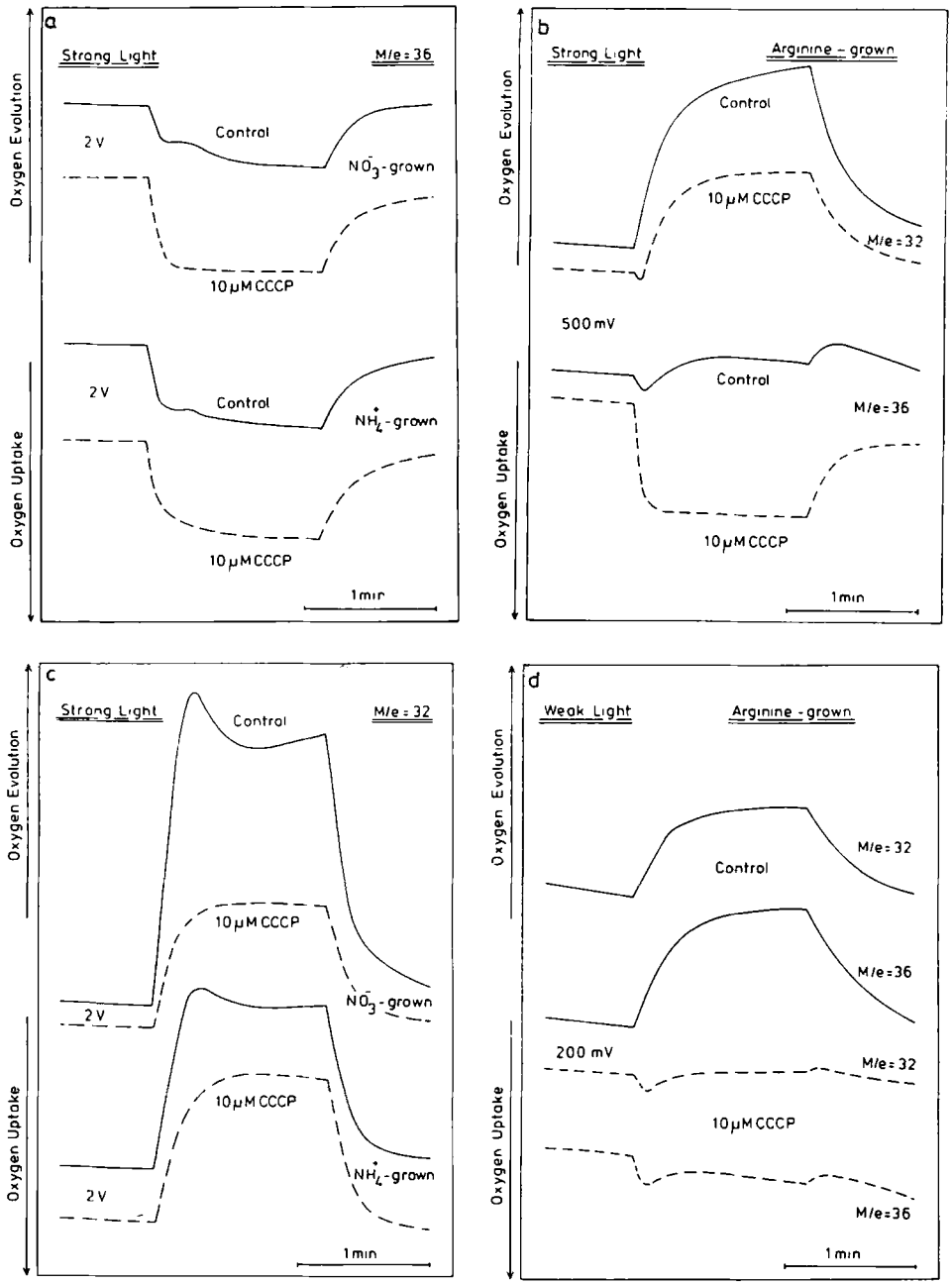


Fig. 4. Oxygen gas exchange measured by mass spectrometry as mass 32 and mass 36 in thylakoids of nitrate-, ammonium sulfate and arginine-grown cells of *O. chalybea*. Solid lines are control experiments without addition, dashed lines are experiments in the presence of 10 μM CCCP (carbonylcyanide *m*-chlorophenylhydrazone). (a) Nitrate- and ammonium sulfate-grown assays in strong light ($2 \cdot 10^4$ lx). Measurement of mass 36; (b) arginine-grown assays in strong light, measurement of masses 32 and 36; (c) nitrate- and ammonium sulfate-grown assays in strong light, measurement of mass 32; and (d) arginine-grown assays in weak light (80 lx), measurement of masses 32 and 36. The labeling conditions are identical to those in Fig. 1.

The observation is that cyanide has an effect on gas exchange in several respects. 1 mM KCN affects all signals summarized in Fig. 2a and b. In strong light the uptake phenomena measured as mass 36 are strongly inhibited and so is oxygen evolution measured as mass 32 (Table I). This has been tested for preparations of nitrate- and ammonium sulfate-grown cells, and demonstrates that the respiratory and photosynthetic electron-transport chains are both sensitive to KCN. In weak light the positive mass 36 signal and also the mass 32 signal are inverted by cyanide to an apparent O_2-uptake phenomenon. This is valid for preparations of nitrate-, ammonium sulfate- or arginine-grown cells. The phenomenon is not only seen under continuous light but can be easily verified also by flash light analysis. Fig. 5 shows a positive mass 36 signal, hence oxygen evolution produced by ten actinic flashes. Addition of KCN transforms the evolution signal to an

TABLE I

Effect of cyanide on the oxygen gas exchange measured by mass spectrometry in thylakoids of O. chalybea + denotes oxygen evolution; − denotes oxygen uptake

Nitrogen source	Relative units for strong light				Relative units for weak light			
	$m/e = 32$		$m/e = 36$		$m/e = 32$		$m/e = 36$	
	Control	1 mM KCN	Control	1 mM KCN	Control	1 mM KCN	Control	1 mM KCN
Nitrate	+4496	+1104	−1248	−512	+558	−38	+356	−63
Ammonium sulfate	+5852	+1012	−1860	−772	+542	+59	+386	−378

uptake signal. The phenomenon has a periodicity of 2 and might be due to the effect of KCN on cytochrome *b*-559 which causes that electrons are directly given back to oxygen by an enzyme of Photosystem II.

If the gas exchange is measured in the presence of 1 mM SHAM, it appears that *Oscillatoria* exhibits a SHAM-sensitive oxygen uptake. In strong light it is seen that in preparations of nitrate- and ammonium sulfate-grown cells the O_2-uptake phenomenon, measured as mass 36, clearly reacts to SHAM (Fig. 6). This might imply that *Oscillatoria* also owns a cyanide-insensitive respiratory pathway [17]. The fact that SHAM also exerts some effect on photosynthetic O_2-evolution also might indicate that a flavine enzyme is associated with Photosystem II as supported by work of Pistorius and Gau [19]. It should be noted that the amino acid oxidase activity of this enzyme is not SHAM sensitive [18]. In weak light SHAM does not appreciably influence the mass 32 signal and certainly not at all the mass 36 signal. Most interestingly the oxygen gas exchange thus analyzed seems not at all sensitive to SHAM in preparations of arginine grown-cells (experiments not shown).

Discussion

When analyzing the entire oxygen gas exchange in thylakoid preparations of *O. chalybea* by means of mass spectrometry two principal observations are made: first, the gas exchange pattern depends on the type of nitrogen source present in the growth medium of the cyanobacterium and second the gas exchange is typically different in weak light when compared to light-saturating conditions. In the dark, oxygen uptake and hence also respiration is very low in these cyanobacteria irrespective of growth conditions, an observation which is

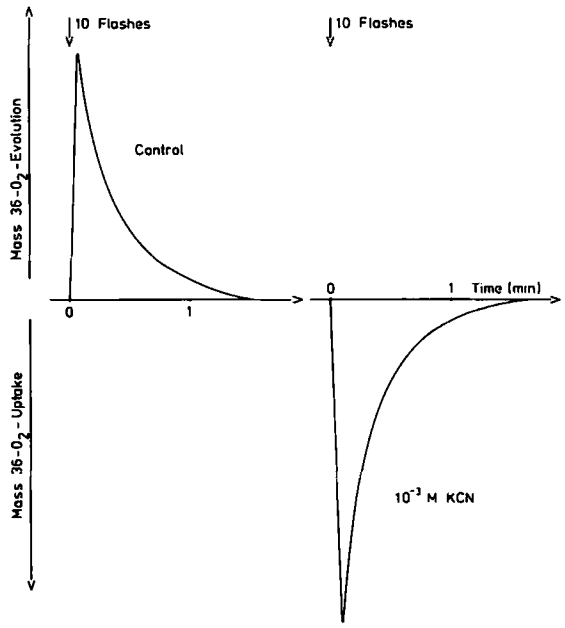


Fig. 5. Oxygen evolution measured by mass spectrometry as mass 36 in thylakoids of nitrate-grown *O. chalybea* as the consequence of 10 actinic flashes. Addition of KCN to the assay (1 mM in the medium) transforms the evolution to an uptake signal. Labeling conditions as in Fig. 1.

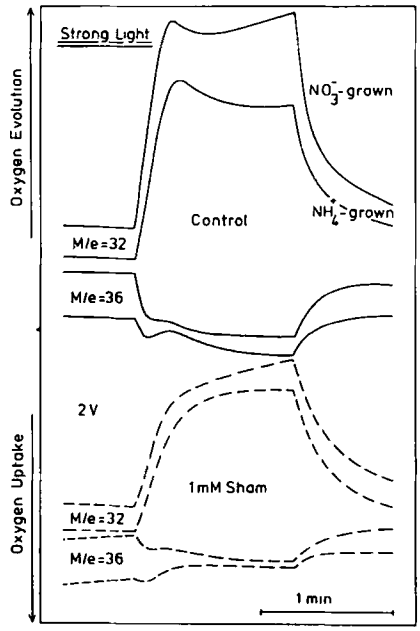
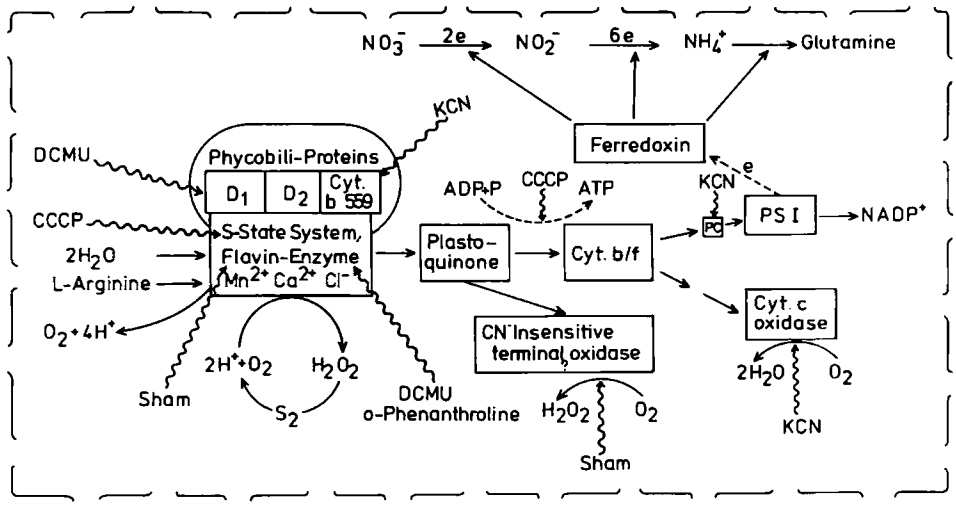


Fig. 6. Effect of 1 mM salicylhydroxamic acid in the assay on oxygen gas exchange in thylakoids of nitrate- and ammonium sulfate-grown *O. chalybea* measured by mass spectrometry as masses 32 and 36 in strong white light ($2 \cdot 10^4$ lx). Labeling conditions are identical to those in Fig 1.

already known from the literature [20]. In weak light, that is light in which photosynthesis is light limited, absolutely no net oxygen uptake and therefore practically no respiratory activity is observed (Figs. 2a, 3a and 4d) irrespective of the growth conditions of the cyanobacteria. Instead in weak light, by labeling the gas atmosphere with $^{18}O_2$, an $^{18}O_2$-evolving phenomenon is observed which, as described earlier [11], can only be due to an extremely fast $^{18}O_2$-uptake phenomenon in the region of the S-state system, leading to the formation of $H_2{}^{18}O_2$ which then is decomposed to $^{18}O_2$, yielding the evolution signal observed as mass 36. As demonstrated in this paper, but as indirectly already proven earlier [11] respiratory activity which would lead via oxygen reduction to $H_2{}^{18}O$ does strictly not account for the phenomenon. This conclusion is made, since $H_2{}^{18}O$ formation and subsequent photosynthetic water splitting of the pool of $H_2{}^{18}O$ and $H_2{}^{16}O$ would inevitably lead to mass 34 oxygen that is a preponderantly mixed label ($^{16}O^{18}O$) of oxygen evolution, which is not observed. As shown in this paper this possibility does anyway not apply, as respiratory activity is very low in the dark and is apparently inhibited in low light (Figs. 1, 2a and 3a). But the fact that no mixed label is observed also excludes that the $H_2{}^{18}O_2$ formed is decomposed via a catalase-like mechanism such as $2H_2{}^{18}O_2 \rightarrow 2H_2{}^{18}O + {}^{18}O_2$, since this mechanism also inevitably would lead to $H_2{}^{18}O$ formation which then via photosynthesis should lead to mixed labelled O_2 ($^{16}O^{18}O$). This is indeed the case, if $H_2{}^{18}O$ is added to the aqueous assay phase as shown by our earlier experiments [5]. We feel that our earlier experiments on the content of mass 34 as demonstrated in Figs. 3c and 4 in Ref. 11 or Figs. 2a and 2b in this paper do not permit the conclusion that half of the label would go into $H_2{}^{18}O$. An obvious explanation could be that $H_2{}^{18}O_2$ is decomposed by the water-splitting system (the S-state system) yielding just as in the water-splitting reaction itself only oxygen and protons $H_2O_2 \rightarrow 2H^+ + {}^{18}\overline{O_2}$. The possibilities of reaction mechanisms leading to O_2 production with H_2O_2 as source or intermediate is clearly discussed by Johansen [14]. This oxygen production seems to be managed by S_2, hence needs only the accumulation of two positive charges. The phenomenon occurs in *O. chalybea* only under light limiting conditions and responds to inhibitors of Photosystem II (Fig. 3a) and ADRY-reagents (Fig. 4d) [15]. One of the principal conditions of the photosynthetic apparatus in *O. chalybea* in low light seems to be an insufficient coupling of electron flow between the two photosystems [6,7]. Under these conditions apparently no or only few electrons arrive at or can be diverted from the cytochrome *b/f* site towards the terminal oxidase, thus leading to the formation of water. It should be borne in mind that work of Åkerlund and Schröder [12,13] has shown that inside-out vesicles from spinach thylakoids lacking two of the extrinsic peptides of Photosystem II exhibit this H_2O_2-producing and H_2O_2-utilizing reaction sequence. Cyanobacterial Photosystem II as that of *O. chalybea* lacks these 17- and 24 kDa-peptides, making the situation in *O. chalybea* comparable to that in salt-washed inside-out vesicles in which Schröder and Åkerlund observe an "increased accessibility for the hydrogen peroxide donation site" [13]. Therefore, under light-limiting conditions oxygen produced by Photosystem II or available in the immediate ambient atmosphere is recycled in Photosystem II similarly or equivalent to the O_2/H_2O_2 cycle described by Åkerlund [12] and Schröder and Åkerlund [13] for inside-out vesicles of spinach thylakoids. Only in high saturating light intensity ($2 \cdot 10^4$ lx) an appreciable $^{18}O_2$-uptake phenomenon which might be related to respiration is observed (Figs. 2b and 4a). This is valid for preparations from nitrate-, ammonium sulfate- and arginine-grown cells, whereas cells from cultures without exogenous N-source in the medium still exhibit the H_2O_2 decomposing reaction (Fig. 2b).

It should be noted that cyanobacterial cultures from different nitrogen sources differ considerably with respect to their requirement for ATP and reducing equivalents [20]. Nitrate-grown cells, in which the nitrate-reducing system with its considerable ATP-requirement is located in the thylakoid membrane, differ with respect to several Photosystem-II properties from ammonium-grown cells [6,7]. Most of the observed differences seem to be due to the difference in the molecular structure of the thylakoid membrane which in the nitrate-grown condition has to accommodate in comparison to ammonium cells nitrate and nitrite reductase in the membrane. However, no difference in the properties of the described Photosystem II phenomenon is observed between preparations of ammonium- and nitrate-grown cells. Beside interesting aspects of the arginine metabolism [18,19], preparations of arginine-grown cells used in the present paper were supposed to exploit in comparison to ammonium-grown cells a potential influence of the heterotrophic condition which (e.g., when bypassing the GOGAT-system) has a lesser requirement for reducing equivalents than nitrate- or ammonium-grown systems. Interestingly, arginine-grown cells exhibit an extremely well-coupled respiratory electron transport at high light intensities (Fig. 4b). It seems as if only in high light intensities sufficient coupling of electron flow between the two photosystems exists, permitting electron transport to the terminal oxidases of the cyanobacterial respiratory chains (scheme). Under these conditions the O_2/H_2O_2-cycle is not operative. However, if this coupling of electron transport is disconnected by herbicide action on the reducing side of photosystem II the O_2/H_2O_2-cycle seems to reappear (Fig. 2c). In this case even in strong light H_2O_2-production and its decomposition by Photosystem II takes



Scheme I. Interaction between the respiratory and photosynthetic electron transport in *O. chalybea*. The participation of a flavin enzyme in Photosystem II is taken from a hypothetical model in *A. nidulans* developed by Pistorius and Gau [19].

place, being apparently the only photochemical activity that is still possible. In contrast to the action on the reducing side of Photosystem II an inhibitor which exclusively acts on the reaction center itself as shown for *o*-phenanthroline, abolishes also the O_2-evolving phenomenon from H_2O_2.

Acknowledgement

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