# Interrelationship of Oxygen and Nitrogen Metabolism in the Filamentous Cyanobacterium *Oscillatoria chalybea*

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By means of mass spectrometric analysis we have been able to demonstrate $H_2O_2$-production and its decomposition by photosystem II in thylakoids of the filamentous cyanobacterium *Oscillatoria chalybea*. This $H_2O_2$-production and its quasi simultaneous decomposition by the S-state system can be readily demonstrated in flash light illumination (K. P. Bader and G. H. Schmid, Biochim. Biophys. Acta **936**, 179–186 (1988)) or as shown in the present paper in continuous light at low light intensities. These light conditions correspond essentially to the culturing condition of the organism on nitrate as the sole nitrogen source. Under these conditions, however, electron transport between the two photosystems seems to be mostly disconnected and respiratory activity practically non existent. Under these conditions, on the other hand, nitrate reductase is induced and nitrate reduced. The present paper addresses the question how this organism might solve the metabolic problems of nitrate reduction with such an electron transport system. Tested under high light intensities under which the organism would not grow at all, electron transport between the two photosystems is optimally linked and the system funnels part of its photosynthetically produced electrons into a conventional cyanide-sensitive respiratory electron transport chain and even into an alternative Sham-sensitive (cyanide-insensitive) respiratory chain. This is made possible by the overweight of photosystem II capacity in comparison to photosystem I activity as reported in this paper. Under the conditions described, the cyanobacterium grows also on arginine as the sole nitrogen source. Most interestingly under these conditions nitrate reductase induction is not shut off as is the case with other aminoacids like ornithine or alanine in the medium. Nitrite reductase is not induced in these bacteria, if grown on arginine as the sole nitrogen source. This observation is discussed in context with the fact that arginine is a major storage product (cyanophycin) in this organism and that the observed photosystem II mediated $H_2O_2$-production might be correlated with arginine metabolism.

## Introduction

In cyanobacteria photosynthetic and respiratory electron transport occur in the same membrane. Moreover, if nitrate is used as nitrogen source, the enzymes of the nitrate reducing system are in addition located in the thylakoid membrane [1]. This close local relationship necessarily implicates interference and interrelationships of the three electron transport systems. Electron carriers common in the three systems demonstrate this interrelationship. Thus, electrons transported in the photosynthetic electron transport chain and arriving in sufficient quantity at the cyt $b_6$/$cyt_f$ site are funnelled into the respiratory chain in direction of the terminal cyt oxidase towards oxygen [2]. Sufficient electron pressure might even permit funnelling of electrons at the plastoquinone site into an additional alternative respiratory system [2]. An interrelationship between photosynthetic oxygen evolution and nitrogen-metabolism is suggested by work of Pistorius and co-workers [3, 4], who show in *Anacystis nidulans* that a 36 kDa peptide belonging to the reaction center complex of photosystem II exhibits under defined experimental conditions the properties of an arginine oxidase [3]. It appears that under conditions under which this enzyme is active photosystem II is inhibited [4]. Recently, we have been able to demonstrate in the region of the water splitting side of photosystem II $H_2O_2$-formation and its immediate decomposition by the S-state system, practically at the formation site itself [5]. Theoretically, $H_2O_2$ could be the product of the arginine oxidase reaction described by Pistorius *et al.* [3]. It should be noted that arginine is a N-storage product stored in form of cyanophycin, occurring in high amount in the bacteria when grown on nitrate. In the present

*Abbreviations:* Sham, salicylhydroxamic acid; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; MOPS, (3-[N-Morpholino]-propanesulfonic acid).

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paper we make a first attempt to correlate certain aspects of the photosystem II activity with nitrogen metabolism, in particular with that of arginine.

## Materials and Methods

*Cyanobacteria: Oscillatoria chalybea* was obtained from the algal collection in Göttingen and cultured on different growth media as described in earlier papers [6, 7].

*Protoplast and thylakoid preparations* of the filamentous cyanobacterium were prepared according to earlier described procedures [7] from 10–14 day old cultures grown on either nitrate, ammonium sulfate or arginine as the sole nitrogen source. The mucoid layer of the cells was digested with glucuronidase (Boehringer, Mannheim) and the cell walls with lysozyme (Sigma) and cellulase (Kinki Yakoult, Japan) as described in earlier publications [6, 7]. For preparations of "French press particles" thylakoids were passed through the French press at 10,000 psi.

*Mass spectrometry* was performed exactly as described earlier [5, 8] 98% $^{18}O_2$ was purchased from CEA-Oris, Bureau des Isotopes Stables, Gif-sur-Yvette, France, and 99% atom % $H_2{}^{18}O$ came from Ventron. The assays were performed with a modified magnetical sector field mass spectrometer type "Delta" from Finnigan, Bremen F.R.G., equipped with a two directional focussing device "Nier type I". The measuring cell used is laboratory made and described earlier [8, 9].

*Light reactions* are described and referred to in an earlier publication [10].

*Flash spectroscopy* of $X_{320}$, $P_{700}$ and $P_{820}$ was carried out in a laboratory built set-up according to the principles described by Rüppel and Witt [11] and by Witt [12]. The assay conditions in these measurements corresponded to those described earlier [13].

*Nitrate-reductase* activity was measured according to Herrero *et al.* [14] in an assay containing protoplasts, thylakoids or thylakoid fragment preparations corresponding to 30–50 μg of chlorophyll. The assay volume of 1 ml contained beside the cyanobacterial material 100 μmol $NaHCO_3/Na_2CO_3$ buffer, pH 10.5, 10 μmol $KNO_3$, 4 μmol methylviologen and 10 μmol sodium dithionite in 0.1 ml 0.3 M $NaHCO_3$. The assay was incubated for 15–20 min at 30 °C and the formation of nitrite measured photometrically at 540 nm.

*Nitrite reductase* activity was measured according to Flores *et al.* [15]. The assay contained as described under nitrate reductase cyanobacterial protoplasts, thylakoids or thylakoid fragment preparations corresponding to 30–50 μg chlorophyll. 1 ml assay contained in addition to the cyanobacterial material 25 μmol MOPS-NaOH buffer pH 7.2, 100 μmol $NaNO_2$, 5 μmol methylviologen, 20 μmol sodium dithionite in 0.1 ml 0.3 M $NaHCO_3$. The assay was incubated for 30 min at 30 °C and the disappearance of nitrite measured at 540 nm.

*Electron microscopy* of *Oscillatoria chalybea* was carried out according to standard procedures described in our ultrastructural study of tobacco mutant chloroplasts [16].

## Results

If oxygen gas exchange is measured in the light in the filamentous cyanobacterium *Oscillatoria chalybea,* the type of gas exchange observed depends on the light intensity. A principal difference exists between assays in low and in strong light. In low light (to this type belong also flash light experiments) one observes $O_2$-evolution which is in part due to water splitting and in part due to $H_2O_2$-oxidation mediated by the S-state system [5]. It appears that under these light conditions the cells practically do not respire [2]. It should be noted that dark respiration is practically non-existent [2]. Fig. 1 shows the oxygen gas exchange in a thylakoid preparation of *Oscillatoria chalybea* grown on nitrate as the sole nitrogen source, measured at low light intensity of approx. 100 lux white light. The assay is carried out in a buffer system described in [2, 5] containing only normal water that is $H^{16}O$. The gas phase, however, con-

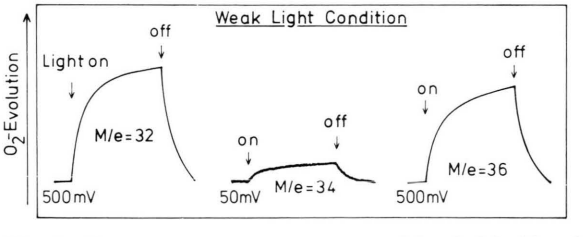



Fig. 1. Oxygen gas exchange measured in thylakoids of nitrate-grown *Oscillatoria chalybea* at low light intensity (80 lux) by means of mass spectrometry. The gas phase was air (21% $O_2$ and 79% $N_2$) in which part of the natural $^{16}O_2$ was substituted by $^{18}O_2$. The aqueous medium contained only $H_2{}^{16}O$-containing buffer, hence no $^{18}O$-label.

tained besides $^{16}O_2$ some $^{18}O_2$ in order to detect $O_2$-uptake phenomena. It is seen that no apparent $O_2$-uptake is observed. However, one observes $^{16}O_2$-evolution *and* $^{18}O_2$-evolution. The $^{16}O_2$-evolution could obviously come from water. The observed $^{18}O_2$-evolution, however, is not so readily explained. The conditions here use thylakoid preparations and continuous light and represent to a certain extent *in vivo* conditions. The experiment described under these natural conditions confirms the result obtained by flash light illumination in particle preparations [5]. We had shown there that $^{18}O_2$ is taken up in the flash. Due to the labeling density and the isotope distribution $^{18}O_2$-evolution could not come from any source that implicated water in its reaction mechanism. The compound formed by photosystem II was $H_2O_2$ that was decomposed by the S-state system producing only protons and oxygen [5]. Fig. 1 clearly shows that under *in vivo* conditions in low light intensity the same reaction occurs. If the observed $^{18}O_2$-evolution occurred *via* respiration and intermediate formation of $H_2{}^{18}O$ that was thereafter split by photosystem II, we should first have observed an $^{18}O_2$-uptake, a lower $^{18}O$-labeling density and above all $^{16}O^{18}O$ (Mass 34)-formation according to the formation rules of dioxygen : Ö : : Ö : from a mixture of $H_2{}^{16}O$ and $H_2{}^{18}O$ [5]. Under these conditions electron transport between photosystem II and photosystem I seems to be badly linked as evidenced by the activity of the methylviologen reduction with water as the electron donor (Table I). Moreover, Table I shows that in protoplast and thylakoid preparations from nitrate-grown cells photosystem II activity exceeds that of photosystem I.

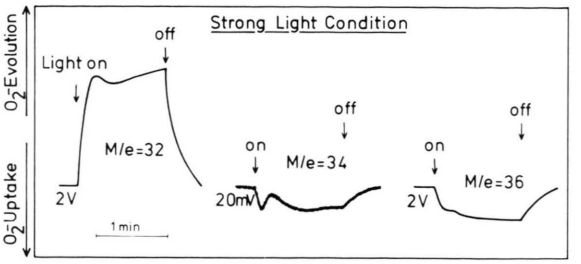



Fig. 2. Oxygen gas exchange measured in thylakoids of nitrate grown *Oscillatoria chalybea* at high light intensity ($2 \times 10^4$ lux). Experimental conditions as in Fig. 1.

In strong continuous light, however, in the same preparation and in the same assay the oxygen evolved comes exclusively from the water splitting reaction (no $^{18}O_2$-evolution is observed!). Fig. 2 shows that under this and only under this condition electron transport from photosystem II to photosystem I occurs and a respiratory $O_2$-uptake measured as $^{18}O_2$-uptake is observed. With our methods we can measure under this light condition not only a cyanide-sensitive but as well as cyanide-insensitive, that is a Sham-sensitive alternative respiratory oxygen uptake (Fig. 3). With sufficient generation of electrons orginating from the water splitting reaction in high light intensity the membrane condition of the cyanobacterium apparently permits electron transport over the two photosystems and electrons arriving at the plastoquinone and cyt $b_6$/cyt$_f$ site are funnelled for energy conservation into the respiratory chains. The observed excess of photosystem II activity seems to be advantageous to this situation (Table I). From our experiments it appears that under

Table I. Photosystem II and photosystem I activities in different preparations of the filamentous cyanobacterium *Oscillatoria chalybea* in dependence on the nitrogen source in the growth medium.

| Reaction | Activities in μmol $O_2 \cdot$ mg chlorophyll$^{-1}$ h$^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| | Protoplasts | | Thylakoids | | French press particles | |
| | $NO_3^-$-grown | $NH_4^+$-grown | $NO_3^-$-grown | $NH_4^+$-grown | $NO_3^-$-grown | $NH_4^+$-grown |
| $H_2O \rightarrow$ Fericyanide | 38 | 36 | 45 | 42 | 29 | 28 |
| + $CaCl_2$ | 58 | 45 | 52 | 50 | 27 | 32 |
| $H_2O \rightarrow$ Phenyl-*p*-benzoquinone | 187 | 87 | 152 | 96 | 25 | 26 |
| + $5 \times 10^{-5}$ M DCMU | 33 | 13 | 28 | 28 | 24 | 26 |
| DCPiP/ascorbate → methylviologen + DCMU | 192 | 233 | 219 | 194 | 317 | 875 |
| $H_2O \rightarrow$ methylviologen | 35 | 29 | 25 | 19 | 0 | 0 |

Thylakoid preparations and French press particles came from the same algal batch. Protoplast preparations have been prepared separately.

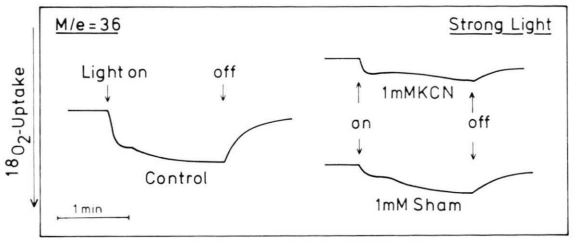



Fig. 3. Cyanide sensitive $^{18}O_2$-uptake and Sham-sensitive $^{18}O_2$-uptake in nitrate-grown *Oscillatoria chalybea* measured at high light intensity ($2 \times 10^{-4}$ lux). Labeling conditions are as described in Fig. 1. Sham concentration in the assay 1 mM, cyanide concentration in the assay, 1 mM.

low light intensities the cyanobacteria produce with photosystem II in an unknown reaction $H_2O_2$ which they decompose in the S-state system by 2 light quanta [5]. The overall effect is a recycling phenomenon of oxygen. The reaction is designated in the following discussion as $H_2O_2/O_2$-cycle.

As the cyanobacterium can only be cultivated under low light conditions, we have attempted to study the electron transport properties of *Oscillatoria chalybea* that has been cultivated in 800 lux white light in a light/dark cycle of 12/12 h. Table I shows that different types of preparations which range from protoplasts to thylakoids and thylakoid fragments exhibit, depending on the nature of the nitrogen source of the cells, which was in our assay cultures either nitrate or ammonium sulfate, different activities in the region of photosystem II and photosystem I. It is seen that preparations from nitrate cultures, with the exception of the French press fragments, generally exhibit better photosystem II activities than preparations from ammonium cultures. However, with all preparations, regardless on what nitrogen source growth had occurred, it is seen that electron transport between the photosystems is badly coupled (Reaction $H_2O \rightarrow$ methylviologen, Table I). If these electron transport properties are checked with the methods of flash light spectroscopy by measuring the absorption changes attributed to the compounds $P_{680}$, $P_{700}$ and $X_{320}$, this property is particularly well verified (Table II). The reaction of electron transport between $H_2O \rightarrow P_{700}$ shows that less than half of the electrons arrive at $P_{700}$ compared to the capacity of photosystem I activity itself. The $X_{320}$-signal measured in preparations of the three culture types shows that preparations of nitrate cultures exhibit signals practically twice as big as those of ammonium sulfate cultures. The fact that the properties and probably the structure of photosystem II depends on the nature of the nitrogen source in the growth medium is demonstrated beyond the observations of Table II with other methods. Thus, it can be shown that room temperature fluorescence emission in thylakoids from nitrate cultures is lowered by DCMU which is apparently not the case in ammonium cultures (Fig. 4 and 5). It appears that the condition of ammonium sulfate cultures corresponds to that described in the literature for *Chlorella* and higher plants (Fig. 5a and b).

The question how this cyanobacterium solves certain metabolic tasks with such a photosynthetic and

Table II. Electron transport properties in thylakoid preparations of the filamentous cyanobacterium *Oscillatoria chalybea* in dependence on the nitrogen source in the growth medium. Measurement by flash spectroscopy.

| Reaction | Nitrate-grown | $NH_4^+$-grown | N-free |
|---|---|---|---|
| [$\Delta I/Io \times 10^{-5}$] | | | |
| $H_2O \rightarrow P_{700}$ | 7.55 | 8.35 | 11.0 |
| $H_2O \rightarrow P_{700}$ + DCMU | 0.79 | 2.02 | 2.19 |
| DCPiP/ascorbate → methylviologen + DCMU | 18.4 | 15.6 | 16.4 |
| $P_{820}$ | 3.35 | 2.14 | 2.92 |
| $P_{820}$ + DCMU | 0.39 | 0.66 | 1.34 |
| $X_{320}$ | 10.50 | 5.97 | 9.27 |
| $X_{320}$ + DCMU | 0 | 0 | 0 |

Concentration of e-donors and acceptors in the assay: DCPiP $10^{-4}$ M, methylviologen $10^{-8}$ M, ascorbate $10^{-2}$ M, chlorophyll concentration 7.5 µg/ml for nitrate and ammonium-grown preparations, 5 µg/ml for N-free preparations, DCMU $10^{-4}$ M; age of cultures 14–27 days. Average of at least 20 determinations.

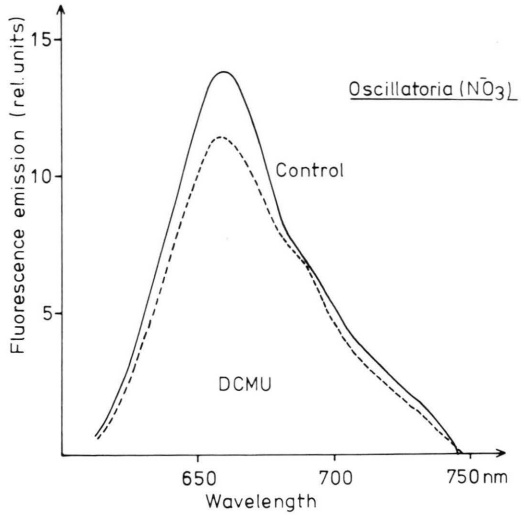



Fig. 4. Room temperature fluorescence emission spectrum of thylakoids from nitrate-grown *Oscillatoria chalybea* in the presence and absence of $10^{-4}$ M DCMU. Excitation by blue light (plexiglasfilter) 380 $<$ nm $\lambda <$ 500 nm.

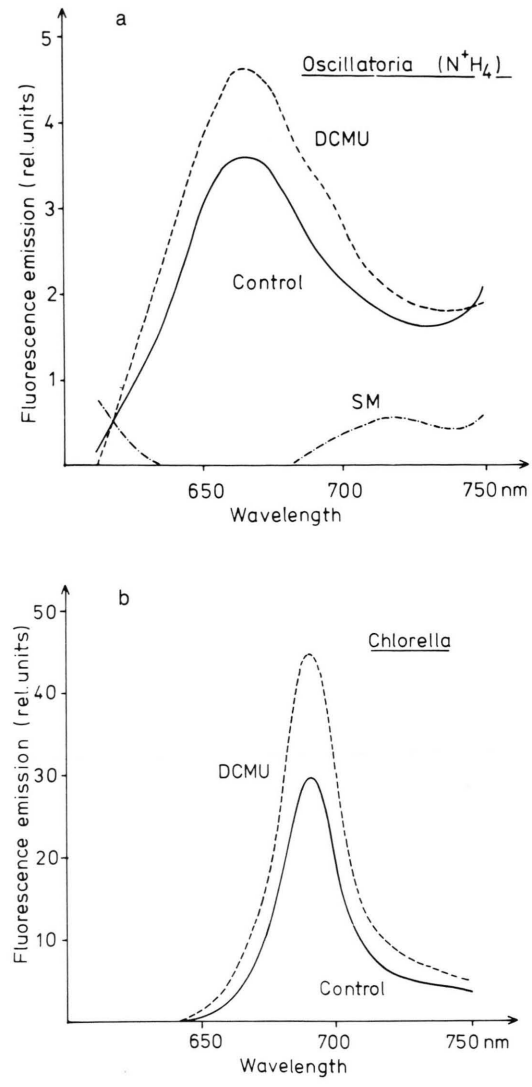



Fig. 5. a) Room temperature fluorescence emission spectrum of thylakoids from ammonium sulfate-grown *Oscillatoria chalybea* in the presence and absence of $10^{-4}$ M DCMU; b) Room temperature fluorescence emission spectrum of *Chlorella* cells (*Chlorella vulgaris* (211–11 h)) in the presence and absence of $10^{-4}$ M DCMU.

respiratory electron transport apparatus demonstrated in Fig. 1 and 2, in Table I and II and referred to under [2, 3], is certainly the question to ask. The first point concerns nitrate reduction. Preparations of *Oscillatoria chalybea* grown under low light conditions (700–1000 lux white light) exhibit nitrate reductase activity if cultivated on either nitrate, nitrite or arginine as the sole nitrogen source (Table III). If cultured on ammonium or without a nitrogen source in the medium this activity is repressed as manifold reported in the literature (for review see [1]). Disregarding the question why as seen in Table III this cyanobacterium permits itself the luxury to induce nitrate reductase, if grown on arginine, the obvious question concerns first the mechanism of nitrate reduction in the nitrate culture. According to the literature reducing equivalents and ATP originating from photosynthetic electron transport are used to reduce nitrate [1]. Usually ferredoxin is the supposed reductant. As the cyanobacterium grows under the described growth conditions of 800 lux and as nitrate reductase activity is observed (Table III) there is no doubt that nitrate is used as nitrogen source and is reduced. According to Tables I and II this reductant cannot be comprehensively formed in the region of photosystem I. If one does not wish to assume existence of a special ferredoxin specifically belonging to photosystem II which is reduced in a more or less unknown manner [17], one could assume ATP formation which in this case had to take place in the region of photosystem II (in our $O_2$/$H_2O_2$-cycle for example). Together with this, one could take into account the possibility of NADH or NADPH formation *via* a pathway of the kind of a "reverse electron transport" described by Böhme [18]. Depending on the specificity of the system,

Table III. Nitrate reductase activities in the filamentous cyanobacterium *Oscillatoria chalybea* in dependence on the nature of the nitrogen source in the growth medium.

| Nitrogen source | Nitrate reductase nmol $NO_2^-$ reduced × mg chlorophyll$^{-1}$ × h$^{-1}$ |
|---|---|
| $NO_3^-$ | 13,500 |
| $NO_2^-$ | 12,900 |
| $NH_4^+$ | 1,700 |
| arginine | 12,800 |
| citrulline | 10,300 |
| alanine | 3,000 |
| ornithine | 1,900 |
| agmatine | 1,000 |
| nitrogen-free | 2,700 |

Table IV. Nitrite reductase activity in the filamentous cyanobacterium *Oscillatoria chalybea* in dependence on the nature of the nitrogen source in the growth medium.

| Nitrogen source | Nitrite reductase activity nmol $NO_2^-$ formed × mg chlorophyll$^{-1}$ × h$^{-1}$ |
|---|---|
| $NO_3^-$ | 5,700 |
| $NO_2^-$ | 6,000 |
| $NH_4^+$ | 370 |
| arginine | 970 |
| ornithine | 970 |
| alanine | 510 |
| citrulline | 820 |
| agmatine | 920 |
| nitrogen-free | 0 |

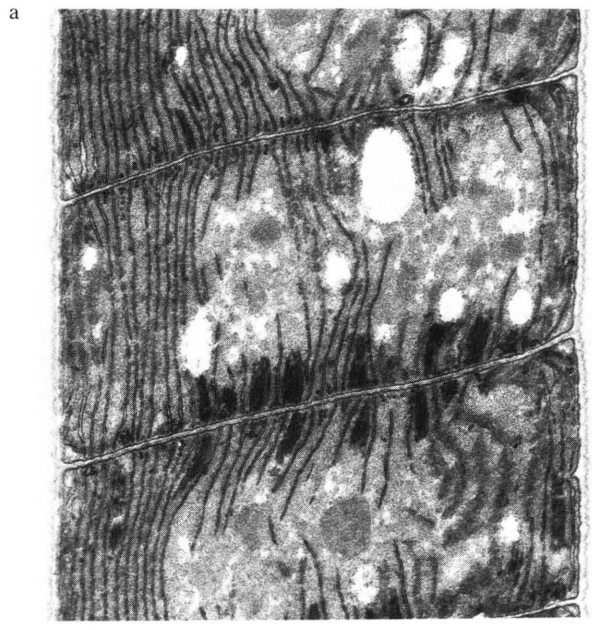
NADPH or NADH could reduce ferredoxin which then in the known fashion [1] would be used in nitrate reduction. This is actually under investigation but preliminary studies do not speak in favour of such a possibility.

The fact that arginine as the sole nitrogen source in the medium permits nitrate reductase activity to occur in such cells not only shows that the regulatory principle, which represses synthesis of nitrate reductase, if reduced nitrogen, for example $NH_4^+$ ccurs in the system, does not function properly. It shows also the peculiar role which arginine apparently plays in nitrogen metabolism of cyanobacteria [3, 4]. It is apparently the circumstance that arginine is a nitrogen storage product in these bacteria which matters. Growth on arginine might simulate arginine mobilization from cyanophycin which would correspond to a depletion of reduced nitrogen and therefore makes the bacteria synthesize nitrate reductase. Other aminoacids as alanine or ornithine do not show this phenomenon (Table III). Agmatine does not seem to play a role in arginine degradation whereas citrulline which lacks in comparison to arginine only one imino group is almost as effective as arginine and permits the induction of nitrate reductase. This regulation error which is obviously committed with respect to nitrate reductase, when cells are grown on arginine or citrulline, is not committed with respect to nitrite reductase (Table IV).

According to the literature cyanophycin (poly-L-arginyl-poly(L-aspartic acid) is a nitrogen storage compound and is used under conditions of nitrogen starvation [19] and seems to serve also as a carbon source under $CO_2$ starvation [20, 21]. On the other hand the literature reports that cyanophycin is accumulated, when cells are starved for light [21]. In context with the hypothetical interpretation advanced in the present paper, namely that at low light intensity the $H_2O_2$ production observed might have to do with arginine mobilization and its oxidation by photosystem II [3] we have looked by means of electron microscopy at the ultrastructure of *O. chalybea* in nitrate- and ammonium sulfate-grown cells. The overall observation is that cyanophycin bodies occur in larger number and are *per se* bigger in nitrate-grown *Oscillatoria* when compared to ammonium sulfate-grown cells (Fig. 5 and 6). Hence, under the culturing conditions used for *Oscillatoria chalybea* in the present paper cyanophycin is accumulated in nitrate-grown cells.

## Discussion

The oxidation of hydrogen peroxide by photosystem II has been repeatedly described in the literature. The conditions under which this compound can be oxidized or the causal connections in its oxidation process are still under investigation [22–24]. Thus, Schröder and Åkerlund found that NaCl washing of inside-out vesicles of spinach thylakoids, which removes the two extrinsic 16 and 23 kDa peptides, creates the condition which permits the accessibility of $H_2O_2$ to its electron donation site at the donor side of photosystem II [22]. $CaCl_2$-washing of detergent treated photosystem II particles removes all three extrinsic polypeptides *i.e.* the 33, 23, and 16 kDa peptides. In this condition $H_2O_2$ is electron donor for photosystem II-mediated photoreductions [23]. In high salt concentrations $H_2O_2$ seems to extract in this type of particles manganese which when added back



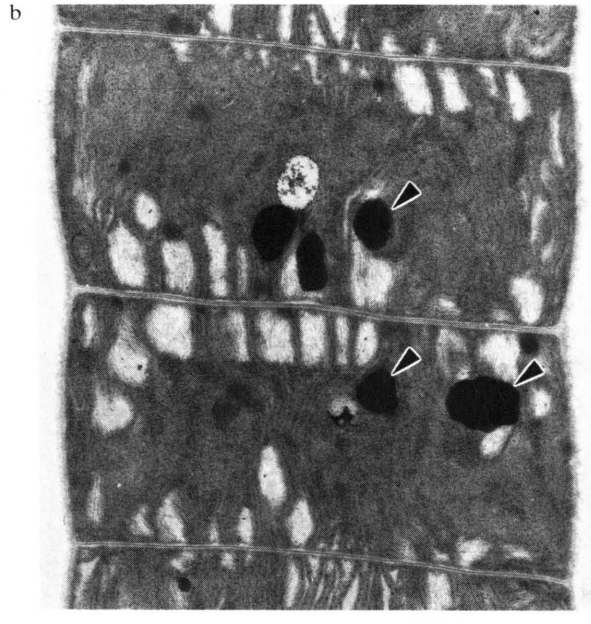
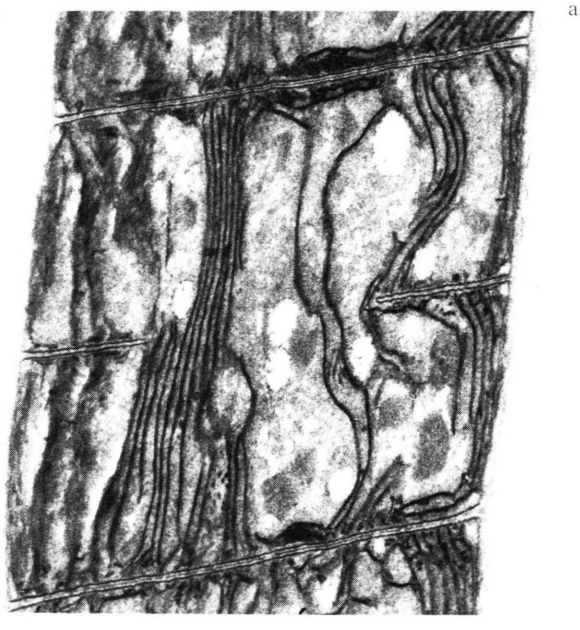




Fig. 6. Ultra structure of nitrate-grown *Oscillatoria chalybea*. a) Permanganate fixation. Magnification 24,000; b) glutaraldehyde fixation and postfixation with $OsO_4$. Stained with uranylacetate and lead citrate; arrows indicate cyanophycin bodies. Magnification 24,000.



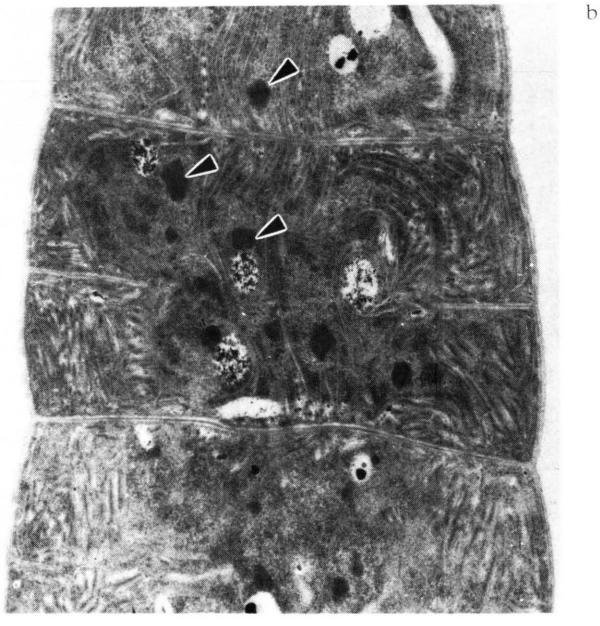




Fig. 7. Ultra structure of ammonium sulfate-grown *Oscillatoria chalybea*. a) Permanganate fixation magnification 24,000; b) glutaraldehyde fixation magnification 24,000; arrows indicate polyhedral bodies; experimental conditions as Fig. 6.

to the preparation in form of $Mn^{2+}$ can reactivate $H_2O_2$-oxidation [23]. Recent work by Sandunsky and Yocum shows that in chloride-depleted thylakoid preparations it is a pool of free or loosely bound manganese, released from the oxygen evolving complex in the thylakoid lumen which permits $H_2O_2$-oxidation [24]. Although these papers demonstrate that $H_2O_2$ can be oxidized by photosystem II they do not present evidence for its production in the region of photosystem II. Our previous papers [2, 5] together with the present one demonstrate $H_2O_2$-production in *Oscillatoria chalybea* by photosystem II. The origine of its production is unknown yet and is actually under investigation, but work by Pistorius and co-workers [3, 4] demonstrates that a 36 kDa polypeptide which belongs to the active oxygen evolving photosystem II complex in *Anacystis nidulans*, exhibits under defined conditions the properties of an amino acid oxidase in particular those on an arginine oxidase [3]. Such a reaction would produce $H_2O_2$. It should be noted, that we have not produced the direct evidence yet which would support the work by Pistorius [3, 4]. However, application of this hypothesis to our system could for example imply that under conditions in the life cycle of *O. chalybea* where the water splitting reaction is inoperative [3] arginine oxidation could take place. If one looks at the condition of photosystem II in a cyanobacterium in comparison to that of higher plants one point could be the principal absence of the two extrinsic 16 and 23 kDa peptides, a condition which since it implies lesser binding affinity for chloride could mean, kind of chronical lack of chloride in such cyanobacteria. At the same time, according to the observation by Schröder and Åkerlund [22] this could permit $H_2O_2$-oxidation by photosystem II, if it is produced. In line with this observation under conditions described earlier [5] and, as shown in Fig. 1, $H_2O_2$ produced by photosystem II is immediately decomposed to protons and $O_2$ [5]. From the labeling density of the $^{18}O_2$ evolved and the promptness of it evolution it follows that the site of production is locally very close to its side of decomposition which is the S-state system [5]. That both reactions occur in the lumen of the thylakoids is evidenced by the fact that the observed $O_2$-evolution can not be influenced by adding neither $H_2O_2$ nor catalase to the external reaction medium [25]. Our experiments in Fig. 1 clearly show that this $H_2O_2$-production and its decomposition is part of the $O_2$-metabolism in our cyanobacterium. From the presence of a large number cyanophycin bodies in nitrate-grown *Oscillatoria* (Fig. 6) it can be concluded that our growing conditions have permitted nitrate assimilation, despite the fact that at the low light intensities in the growth stage where the cyanobacterium has been tested (Table I) several properties do not permit to say where the conventional reductant and the necessary ATP for nitrate reduction would come from. An obvious possibility to be tested could be that nitrate reduction and the built-up of the arginine containing storage products shown in Fig. 5 has occurred in the growth period during the first 10 days. Thereafter, our cyanobacterium seems to make, in a second growth phase, use of the stored cyanophycin, using it as a nitrogen and a carbon source, an activity which would only require photosystem II [3, 5]. Alternating operation cycles of photosynthesis with production of reducing equivalents and ATP and the subsequent reduction of nitrogen in a condition where no or little $O_2$ is evolved have been reported for a heterocyst-free *Oscillatoria* species [26].

*Acknowledgements*

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