Investigations about various Possible Functions of the L-Amino Acid Oxidase in the Cyanobacterium *Anacystis nidulans*

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Z. Naturforsch. **44c**, 370–377 (1989); received December 23, 1988

Dedicated to Prof. Dr. Achim Trebst on the occasion of his 60th birthday

Cyanobacteria, *Anacystis nidulans*, L-Amino Acid Oxidase, Photosystem II, L-Arginine Catabolism

The cyanobacterium *Anacystis nidulans* was grown on nitrate or L-arginine as sole nitrogen source and in the presence of different divalent cation concentrations (1 mM $MgSO_4$ and 0.1 mM $CaCl_2$ or 0.1 mM $MgSO_4$ and 0.05 mM $CaCl_2$). The L-amino acid oxidase previously reported to be present in *Anacystis nidulans* (E. K. Pistorius and A. E. Gau, Biochim. Biophys. Acta **849**, 203, 1986) was shown to be involved in L-arginine catabolism in cells grown with the lower divalent cation concentration. Under these conditions L-arginine was partly degraded *via* 2-ketoarginine and 4-guanidinobutyrate. On the other hand, at higher cation concentrations the L-amino acid oxidase activity seemed to be not sufficient to provide enough NH_4^+ from L-arginine for cell growth. Under those conditions photosystem II activity was initially reduced, and growth on L-arginine could only start after photosystem II activity increased again and after arginase was induced. The arginase pathway was functional in *A. nidulans* grown on L-arginine independently of the divalent cation concentration in the medium. A tentative scheme of the various functional roles of the L-amino acid oxidase protein in *A. nidulans* is given. This model combines the here presented and the previous results and suggests that the L-amino acid oxidase is functional in photosynthetic and respiratory activities as well as in L-arginine degradation in *A. nidulans*. All these activities of the L-amino acid oxidase protein are greatly influenced by the divalent cation concentration in the growth medium.

Introduction

In microorganisms the L-arginine metabolism has been shown to be extremely complex. Evidence has been presented that a great variety of degradative pathways for L-arginine exists in microorganisms and that frequently one organism has more than one of those degradative pathways [1]. In addition to that, the metabolism of arginine in cyanobacteria is of particular interest since the urea cycle contributes to CO_2 fixation [2] and due to the fact that some cyanobacteria contain a polymer of L-aspartate and L-arginine called cyanophycin [3]. We have previously shown that the cyanobacterium *Anacystis nidulans* contains an L-amino acid oxidase with a high specificity for the basic L-amino acids (L-arginine being the best substrate). The L-amino acid oxidase activity of this enzyme is inhibited by cations as well as by anions [4, 5]. When the cells are broken by French press treatment, a substantial amount of the detectable activity of this enzyme is found in the soluble fraction. However, a certain part of this enzyme is tightly associated with the thylakoid membrane, and the protein can still be detected in purified PS II complexes of *A. nidulans* [6, 7]. Our initial reason to suspect that possibly a connection might exist between the L-amino acid oxidase activity in thylakoid membranes and photosynthetic water oxidation was based on the observation that $CaCl_2$ has an antagonistic effect on these two reactions: $CaCl_2$ stimulates photosynthetic water oxidation but inhibits the L-amino acid oxidase activity [8]. This observation together with a number of additional results has led to our hypothesis that the water-oxidizing enzyme might have evolved from an L-arginine dehydrogenase which originally (in an anaerobic atmosphere) donated electrons coming from L-arginine to the plastoquinone pool from where the electrons could be transported either to photosystem I or the cytochrome oxidase, since in cyanobacteria the respiratory and photosynthetic electron transport chain partly use the same electron carriers [9]. Later in evolution (in an aerobic atmosphere) this flavoprotein could also use O_2 as electron acceptor.

Abbreviations: Chl, chlorophyll; PS, photosystem.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/89/0500–0370 $ 01.30/0

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In this paper we were mainly interested to investigate whether this flavoprotein with L-amino acid oxidase activity could still function as a substrate dehydrogenase using plastoquinone instead of O_2 as electron acceptor and whether this enzyme might have a role in L-arginine catabolism when the cells are grown on L-arginine as sole nitrogen source. Moreover, since our hypothetical model implies a close interaction between L-arginine metabolism and photosynthetic O_2 evolution, we also examined whether growth on L-arginine has an effect on PS II.

Materials and Methods

Growth of cells

Anacystis nidulans (Synechococcus leopoliensis) B 1402-1 was obtained from the Sammlung von Algenkulturen, Universität Göttingen. Normally the cells were grown in 250 ml culture bottles at 38 °C in a culture medium according to Kratz and Myers [10] with some modifications: 1×10^{-3} M $MgSO_4$, 5.7×10^{3} M K_2HPO_4, 1×10^{-2} M KNO_3, 1.1×10^{-4} M $Ca(NO_3)_2$, 5.6×10^{-4} M Na_3-citrate, 1.6×10^{-5} M $FeCl_3$, 1.3×10^{-5} M Na_2-EDTA, 1.4×10^{-5} M $FeSO_4$ and in addition the following microelements: 5×10^{-7} M $CoSO_4$, 4.6×10^{-5} M H_3BO_3, 9.2×10^{-6} M $MnCl_2$, 7.7×10^{-7} M $ZnSO_4$, 1×10^{-7} M Na_2MoO_4, and 3.2×10^{-7} M $CuSO_4$. The medium was autoclaved and had approximately a pH of 8. 60 µl cells were inoculated into 250 ml medium and air enriched with CO_2 (2%) was sparged through the cultures. Illumination was performed from the top (40 cm distance) with lamps from Philips (120 W, PAR 38, cold beam – 7000 lux). After cultures had grown for two days and reached a cell density of about 2 µl cells per ml, the cells were harvested by centrifugation, washed twice with water and then inoculated into the various media used in the experiments: When L-amino acids were used as nitrogen source, KNO_3 was omitted, $Ca(NO_3)_2$ was replaced by $CaCl_2$ and L-arginine or L-alanine was added to the autoclaved medium to give a final concentration of 5×10^{-3} or 1.5×10^{-2} M, respectively. The amino acids were sterilized by filtration through a cellulose-nitrate filter of 0.45 µm pore size (Sartorius). In the experiments with the regular cation concentration the medium was used as above. The experiments with the "reduced cation concentration" were performed at $MgSO_4$ and $CaCl_2$ concentrations of 1×10^{-4} M and 0.5×10^{-4} M, respectively. The comparative experiments given in this paper were all done in a water bath with illumination from the side (15 cm distance with neon tubes: Radium NL, 40 W 25-1, white and Osram L, 40 W 30-1, warm white – 6000 lux).

Preparations of cell suspensions and French press extract

The cells were harvested by centrifugation after 1, 2 or 3 days, washed once with water and resuspended in water to give a cell density of 100 µl cells $\times$ ml^{-1} for the experiments with the cell suspensions. For the experiments with the French press extract, the cells were resuspended in 20 mM potassium phosphate buffer, pH 7 (100 µl cells $\times$ ml^{-1}) and passed twice through a French pressure cell at 137.8 MPa.

Measurements with cell suspensions

O_2 uptake or O_2 evolution was measured in a Gilson oxygraph (model IC-OXY) fitted with a Clark type electrode. The solutions were saturated with air, and the reaction temperature was 20 °C. For the O_2 evolution measurements the reaction chamber was illuminated with a Halogen lamp (24 V, 250 W) from Spindler and Hoyer, Göttingen. The light was filtered through a 2% $CuSO_4$ solution and a red glass filter RG 1(610) from Schott, Mainz. For the O_2 uptake measurements in the dark the reaction mixture contained in a total volume of 1.85 ml: 54 mM Hepes-NaOH, pH 7, and 5 to 20 µl *Anacystis* cells. When indicated in the figures or tables, 5 mM L-arginine, 50 mM $CaCl_2$, 1 mM KCN and 0.8 mM phenyl-*p*-benzoquinone were added. For the O_2 evolution in the light the reaction mixture contained in a total volume of 1.85 ml: 54 mM Hepes-NaOH, pH 6.5, 0.8 mM phenyl-*p*-benzoquinone and 1 to 10 µl *Anacystis* cells.

Enzymatic assays with French press extract

All enzymatic activities were measured as NH_4^+ production from the corresponding substrates: L-amino acid oxidase activity as NH_4^+ production from L-arginine in the presence of EDTA (optimizes the L-amino acid oxidase [5] but inhibits the arginase), arginase activity as NH_4^+ production from L-arginine in the presence of $MnCl_2$ (activates the arginase but inhibits the L-amino acid oxidase) and excess of urease to decompose the formed urea, 4-guanidinobutyrase as NH_4^+ production from 4-gua-

nidinobutyrate in the presence urease, and urease activity without further additions. The arginase in *A. nidulans* was completely inhibited by EDTA or by dialysis against phosphate buffer (not shown) and could be reactivated by the addition of $MnCl_2$. The reaction mixtures for the enzyme assays contained in a total volume of 1.5 ml: a) L-amino acid oxidase assay: 0.07 M Tricine-NaOH, pH 9, 0.01 M L-arginine, 0.01 M EDTA and 100 µl French press extract; b) arginase assay: 0.07 M Tricine-NaOH, pH 9, 0.01 M L-arginine, 0.01 M $MnCl_2$, 50 µg urease S (from *Canavalia ensiformis*, 77 units/mg, from Boehringer, Mannheim) and 100 µl French press extract; c) 4-guanidinobutyrase assay: 0.07 M Tricine-NaOH, pH 9, 0.01 M 4-guanidinobutyric acid, 50 µg urease and 100 µl French press extract, d) urease assay: 0.07 M Tricine-NaOH, pH 9, 0.01 M urea and 100 µl French press extract. Reaction mixtures were incubated for 1 h at room temperature, and the reaction was stopped by the addition of 100 µl 2 N H_2SO_4. After centrifugation and neutralization with 1 N NaOH, NH_4^+ was determined enzymatically as previously described [5].

Results and Discussion

Growth on nitrate or L-arginine as nitrogen source in the presence of different divalent cation concentrations

The cyanobacterium *A. nidulans* was grown on nitrate for 2 days, then harvested and transferred to media containing either nitrate or L-arginine as nitrogen source. In addition, the divalent cation concentration of the medium was altered, because the L-amino acid oxidase in *A. nidulans* is inhibited by ions [4, 5]. Although most of the ions in the medium are present in concentrations which are too low to expect a pronounced effect on the enzyme, the divalent cation concentration (especially the Mg^{2+} and Ca^{2+} concentration) is high enough to cause together approximately 50% inhibition, if we assume that the divalent cation concentration is the same inside the cell as present outside. However, the concentration of the ions could be higher or lower at the side of the L-amino acid oxidase action – especially in the light. As representative examples for the effects which we want to show here, we have used the regular divalent cation concentration (1 mM Mg^{2+} and 0.1 mM Ca^{2+}) and a reduced divalent cation concentration (0.1 mM Mg^{2+} and 0.05 mM Ca^{2+}) – referred to throughout the paper as regular and reduced cation concentration, respectively. Omitting Ca^{2+} and leaving the Mg^{2+} concentration unchanged proved not to be sufficient to obtain the results as presented.

The growth curves and the absorption spectra (after 24 h of growth) of the *Anacystis* cells grown on nitrate or L-arginine in the presence of the two cation concentrations are given in Fig. 1 and 2, respectively. As previously shown by Wallen and Allan [11] and demonstrated in Fig. 1, *Anacystis* cells can grow on L-arginine as sole nitrogen source. When the medium contained the reduced cation concentration, growth on L-arginine started without any delay and proceeded equally well on L-arginine as on nitrate. After three days growth ceased as a result of divalent cation deficiency. However, when the medium contained the regular cation concentration, growth on L-arginine showed a substantial lag phase of about 24 h. After the lag phase the cells grew equally well (or even better) on L-arginine as on nitrate. Since the doubling time for *A. nidulans* under our growth conditions is 12 to 14 h, this delay of about 24 h seems to be an extremely long lag phase. During this period of accomodation, cells reduced their pigment content and turned yellowish (Fig. 2 and Table I) but recov-

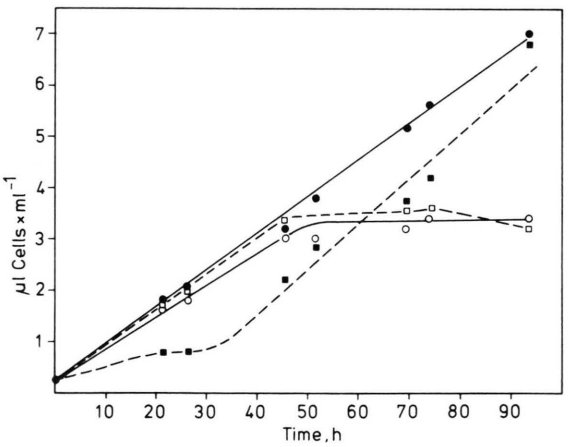

Fig. 1. Growth of *A. nidulans* on nitrate or L-arginine as N-source in the presence of different divalent cation concentrations. *A. nidulans* was grown in a medium containing: A: nitrate and the regular cation concentration (1 mM $MgSO_4$ and 0.1 mM $CaCl_2$), ●——●, B: L-arginine and the regular cation concentration ■– – –■, C: nitrate and the reduced cation concentration (0.1 mM $MgSO_4$ and 0.05 mM $CaCl_2$) ○——○, D: L-arginine and the reduced cation concentration □– – –□.

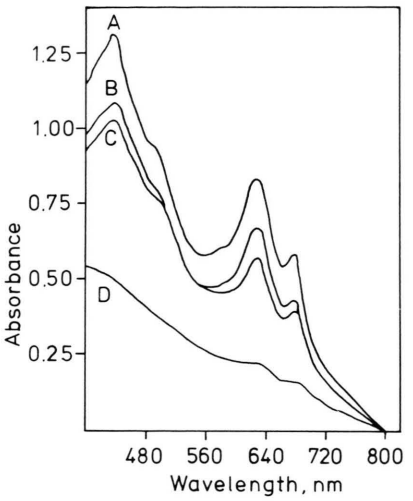

Fig. 2. Absorption spectra of *A. nidulans* cells. The cells were the same as in Fig. 1 and the spectra were recorded after 24 h of growth and at a cell concentration of 1 µl cells × ml^{-1}. A: Cells grown on nitrate and the regular cation concentration, B: cells grown on nitrate and the reduced cation concentration, C: cells grown on L-arginine and the reduced cation concentration and D: cells grown on L-arginine and the regular cation concentration.

ered to their normal appearance after that period (not shown). When the cells were grown on L-alanine as a control, this extended lag phase in the presence of the regular cation concentration was not observed (not shown).

Influence of various growth conditions on photosynthetic O_2 evolution and L-amino acid oxidase activity in cell suspensions

Chlorophyll content, rates of photosynthetic O_2 evolution with phenyl-*p*-benzoquinone as electron acceptor and L-amino acid oxidase activity with L-arginine as substrat in cells grown under the various conditions are given in Table I. Cells grown on nitrate and the regular cation concentration showed values for photosynthetic O_2 evolution of 100 to 250 µmol O_2 evolved × ml^{-1} cell suspension (100 µl cells) × h^{-1} on all three days, while the cells grown on L-arginine and the regular cation concentration had a substantially reduced rate of O_2 evolution on the first day but regained their O_2 evolving activity on the second day when growth also started to proceed normally. This drop in PS II activity on the first day was not seen when cells were grown on L-alanine. On the contrary, O_2 evolving activity had a tendency to increase (not shown). In cells grown on the reduced cation concentration, O_2 evolution was normal on the first and second day but decreased on the third day. However, this decrease was seen with cells grown on L-arginine as well as on nitrate, and therefore seemed to be due to a deficiency in Mg^{2+} and/or Ca^{2+}. Most likely, this decrease in O_2 evolution due to a deficiency in cations is related to the phenomena already described by Becker and Brand [12].

In all cell types L-arginine stimulated the O_2 uptake (Table I). Cells grown on nitrate with the regular cation concentration had practically no O_2 uptake without further addition of L-arginine to the reaction mixture, while all the other cell types showed an O_2 uptake due to endogenous substrates – especially the cells grown on L-arginine in the presence of the reduced cation concentration.

Table I: Influence of various growth conditions on chlorophyll content, photosynthetic O_2 evolution (and L-amino acid oxidase activity) in cell suspensions of *A. nidulans*. *A. nidulans* was grown on nitrate or L-arginine as nitrogen source and in a medium containing either the regular or the reduced divalent cation concentration. Photosynthetic O_2 evolution was measured with phenyl-*p*-benzoquinone as electron acceptor and the O_2 uptake in the dark was measured in the absence or presence of added L-arginine to the cell suspensions (100 µl cells × ml^{-1}) as described under Materials and Methods.

Growth time:	Divalent cation concentration in growth medium: Regular N-source of growth medium: Nitrate	Regular L-Arg	Reduced Nitrate	Reduced L-Arg
A. Chlorophyll content: mg Chl × ml^{-1} cell suspension				
1. Day	0.34	0.08	0.26	0.23
2. Day	0.64	0.43	0.44	0.34
3. Day	0.66	0.50	0.42	0.36
B. Photosynthetic O_2 evolution: µmol O_2 × ml^{-1} cell suspension × h^{-1}				
1. Day	104	4	135	97
2. Day	159	98	147	96
3. Day	206	118	54	11
C. O_2 uptake in the presence and in the absence (in parenthesis) of added L-arginine: µmol O_2 × ml^{-1} cell suspension × h^{-1}				
1. Day	9 (0)	6 (5)	14 (4)	11 (7)
2. Day	17 (0)	16 (7)	23 (7)	30 (14)
3. Day	14 (0)	6 (3)	25 (4)	26 (13)

Table II. The influence of $CaCl_2$, KCN and phenyl-*p*-benzoquinone on the L-arginine stimulated O_2 uptake in cell suspensions of *A. nidulans*. The O_2 uptake in the dark was measured with cell suspensions (100 µl cells × ml^{-1}) and the various additions given in the Table as described under Materials and Methods.

Additions	*Anacystis* grown for 2 days on nitrate and the regular cation concentration	*Anacystis* grown for 2 days on L-arginine and the reduced cation concentration
	µmol O_2 taken up × ml^{-1} cell suspension × h^{-1}	
− L-Arg	0	14.9
+ L-Arg	16.8	29.5
+ L-Arg + $CaCl_2$	0	7.9
+ L-Arg + KCN	26.1	14.9
+ L-Arg + phenyl-*p*-benzoquinone	10.0	7.3

Table II shows the effect of various compounds on O_2 uptake caused by the addition of L-arginine to the cell suspensions: $CaCl_2$ acts as an inhibitor of the L-amino acid oxidase, cyanide as an inhibitor of the catalase and of the terminal cytochrome oxidase and phenyl-*p*-benzoquinone as an artificial electron acceptor at the side of the plastoquinone pool. In these experiments we wanted to investigate to what extent this flavoprotein would transfer electrons either directly to O_2 to form H_2O_2 or to the electron transport chain to give an O_2 uptake by way of the cytochrome oxidase. Previous results have already indicated that addition of cyanide to *Anacystis* cell suspensions supplemented with L-arginine could cause either an apparent stimulation of the O_2 uptake by inhibiting the decomposition of the formed H_2O_2 by catalase or an inhibition of the O_2 uptake by inhibiting the cytochrome oxidase [13]. The L-amino acid oxidase is not inhibited by KCN. In Table II two examples for the effects of those substances are given. The results confirm that cyanide can cause a stimulation or inhibition of the O_2 uptake. Moreover, phenyl-*p*-benzoquinone always gave a partial inhibition of the O_2 uptake. Those results indicate that in all here examined cell types, L-arginine dependent O_2 uptake was due in part to a direct transfer of electrons to O_2 and in part to a transfer of electrons into the electron transport chain. Whether the transfer of electrons to O_2 or to the electron transport chain would prevail, was greatly variable (as expected with whole cells). However, in all examined cells both ways were possible.

There was another interesting observation related to the H_2O_2 formed by the L-amino acid oxidase reaction. Part of this H_2O_2 seemed to be compartmentalized – most likely in the lumen space of the thylakoids – thus making this H_2O_2 inaccessible to catalase (at least not in the dark). As shown in Fig. 3, the *Anacystis* cells supplemented with L-arginine switched from an O_2 uptake in the dark to an O_2 evolution in the light under conditions where there was no O_2 evolution from H_2O (only very low concentrations of CO_2 and no artificial electron acceptor present). This light-dependent O_2 evolution was cyanide sensitive. Since there have been several reports that PS II can catalyze a H_2O_2-dependent O_2 evolution [14–16], it seems likely that part of the H_2O_2 formed in the L-amino acid oxidase reaction

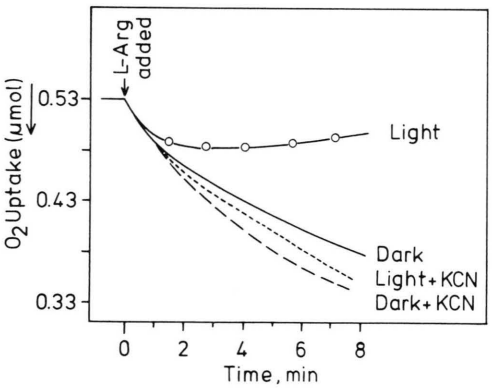

Fig. 3. Influence of light on the L-arginine dependent O_2 uptake. The O_2 uptake or O_2 evolution was measured as described under Materials and Methods. The reaction mixture contained in a total volumen of 1.85 ml: 54 mM Hepes-NaOH, pH 7, and 5 µl *Anacystis* cells (grown for 2 days on nitrate and the regular cation concentration). At the point indicated L-arginine (5 mM) was added and the O_2 uptake or evolution was measured in the dark and in the light, in the presence and absence of 1 mM KCN.

can only be decomposed by PS II in the light. The conclusion that this light-dependent O_2 evolution from H_2O_2 was catalyzed by PS II, was supported by the observation that this reaction worked better in the cells grown on the regular cation concentration than in the cells grown on the reduced cation concentration (not shown).

Influence of the various growth conditions on some L-arginine metabolizing enzymes

Ammonium production in the dark by the L-amino acid oxidase in *A. nidulans* cell suspensions was in the range of 2 to 8 nmol NH_4^+ produced $\times$ μl^{-1} cells $\times$ min^{-1} (in medium with regular or reduced cation concentration). Those values were somewhat lower but in the same range as those given by Flores *et al.* [17] for *A. nidulans*. In the same paper the rates for nitrate assimilation were reported to be about 1 to 4 nmol NO_3^- assimilated $\times$ μl^{-1} cells $\times$ min^{-1}. Therefore, the activity of the L-amino acid oxidase in *A. nidulans* should be high enough to provide the cells with sufficient NH_4^+ from L-arginine for growth – at least during the initial growth phase when the cells switch from nitrate to L-arginine as nitrogen source and before other L-arginine metabolizing enzymes have been induced.

Although amino acid oxidases are scarcely involved in the catabolism of basic L-amino acids, there are a few exceptions [1]. In *Pseudomonas putida e.g.* it has been shown that the cells can use L-arginine as sole carbon and nitrogen source and that L-arginine is oxidized by an L-amino acid oxidase to give 2-keto-arginine which is further degraded to 4-guanidino-butyraldehyde and 4-guanidinobutyrate. The latter is converted by a 4-guanidinobutyrase to urea and 4-aminobutyrate [1, 18]. In case this pathway is functional in *A. nidulans,* we should be able to detect the corresponding enzymes of this pathway, as *e.g.* 4-guanidinobutyrase activity. We have also investigated whether in addition other L-arginine degrading pathways might be induced when the cells are grown on L-arginine. We selected the arginase pathway, since arginase had been shown to be functional in several cyanobacteria [1, 19, 20]. The results are summarized in Table III. In all tested growth conditions, L-amino acid oxidase can be detected in French press extracts as already shown in Table I for cell suspensions. Moreover, urease also seems to be constitutive. Although the activity is substantially higher in cells grown on L-arginine than in cells grown on nitrate. On the other hand, the 4-guanidinobutyrase activity as a measure for the L-amino acid oxidase pathway [18] can only be detected in cells grown under reduced cation concentration on L-arginine and to a minor extent in the cells grown on nitrate with the reduced cation concentration. This enzyme can not be detected in cells grown with the regular cation concentration (values < 1 should be considered as zero). On the other hand, the arginase which is absent in nitrate grown cells, is induced in cells grown on L-arginine in the presence of the reduced as well as regular cation concentration.

In summary, the results clearly show that the arginase pathway is functional in *A. nidulans* when the cells are grown on L-arginine as nitrogen source. The induction of this enzyme being absent in cells grown on nitrate, is independent of the cation concentration

Table III: Influence of various growth conditions on some L-arginine metabolizing enzymes in French press extracts of *A. nidulans*. The various enzymatic activities were determined in French press extracts as described under Materials and Methods. The cell suspensions used for the preparation of the French press extracts contained 100 μl cells $\times$ ml^{-1}.

Growth time:	Divalent cation concentration in growth medium: Regular	Regular	Reduced	Reduced
	N-source of growth medium: Nitrate	L-Arg	Nitrate	L-Arg
A. Arginase activity: μmol $NH_4^+ \times ml^{-1}$ extract $\times h^{-1}$				
1. Day	0.6	14.9	1.6	4.6
2. Day	0.4	9.7	0.7	15.8
3. Day	0.5	15.4	0.9	22.0
B. Urease activity: μmol $NH_4^+ \times ml^{-1}$ extract $\times h^{-1}$				
1. Day	4.7	21.9	3.9	20.1
2. Day	1.9	20.0	3.0	17.8
3. Day	2.8	6.6	3.7	18.1
C. L-Amino acid oxidase activity: μmol $NH_4^+ \times ml^{-1}$ extract $\times h^{-1}$				
1. Day	5.2	10.0	9.9	16.3
2. Day	7.5	17.7	8.4	20.8
3. Day	6.2	13.8	13.3	21.1
D. 4-Guanidinobutyrase activity: μmol $NH_4^+ \times ml^{-1}$ extract $\times h^{-1}$				
1. Day	0.2	0.9	0	1.9
2. Day	0	0.4	1.4	4.5
3. Day	0.2	0.4	1.3	8.1

in the medium. However, the L-amino acid oxidase pathway *via* 4-guanidinobutyrate [18] only seems to play a role in arginine catabolism when the cells are grown on L-arginine in the presence of the reduced cation concentration and seems to be totally suppressed at the regular cation concentration. Under those conditions the cells behaved for about 24 h as if they were nitrogen deficient and reduced PS II activity (Table I). This reduction of PS II activity explains the long lag phase of 24 h, since the cells did only start to grow on L-arginine in the presence of the regular cation concentration after PS II activity has increased again and arginase (and possibly other not yet examined pathways) has been induced. Obviously, under these conditions, the NH_4^+ production by the L-amino acid oxidase is not sufficient (although not zero – not shown) for the cells to grow. Whether the reduction of the PS II activity is only due to a general stress situation associated with the degradation of D_1 [21, 22] or whether L-arginine may possibly have a direct effect on PS II under conditions of nitrogen starvation can not be decided at the present time and requires additional work.

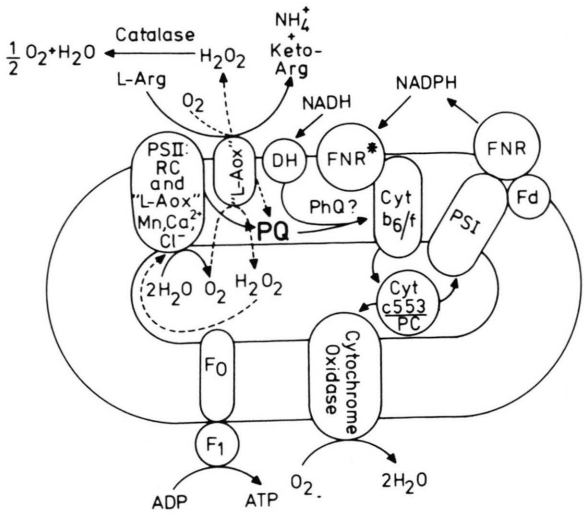

Fig. 4. Tentative scheme for the various possible functions of the L-amino acid oxidase in the photosynthetic and respiratory electron transport chain as well as in L-arginine degradation of the cyanobacterium *A. nidulans*. RC: reaction center of PS II consisting of D_1, D_2 and cytochrome b_{559} binding peptides; L-AOX: L-amino acid oxidase; DH: NADH dehydrogenase; PQ: plastoquinone; PhQ: phylloquinone; Cyt b_6/f: cytochrome b_6/f complex; PC: plastocyanin; Cyt c_{553}: soluble cytochrome *c*; FNR: ferredoxin-$NADP^+$ reductase active in the light; FNR*: FNR active in the dark as NADPH dehydrogenase; Fd: ferredoxin; F_0F_1: ATP synthetase.

Concluding Remarks

A tentative scheme for the various suggested functional roles of the L-amino acid oxidase protein in the cyanobacterium *A. nidulans* is given in Fig. 4. This scheme is adapted from a scheme which was recently published by Scherer *et al.* [23] and which we have modified to the extent that we added the suggested L-amino acid oxidase reactions but omitted those parts which were not relevant to here presented experiments. Our previous [6, 7] and here presented results can be best explained assuming the L-amino acid oxidase protein does exist in two forms: one part of the enzyme is only loosely associated with the outer side of the thylakoid membrane (as also shown to be the case for NAD(P)H dehydrogenases in some cyanobacteria [9, 24, 25]. The other part of the enzyme is transported to the inner side of the membrane and most likely processed to a lower molecular weight form (unpublished results). In our model this part (as previously suggested) [6, 7] becomes incorporated into the PS II complex and functions in photosynthetic water oxidation (after activation by Mn^{2+}, Ca^{2+} and Cl^-) in connection with the reaction center complex [26, 27] which in our model would only catalyze the photochemical charge separation. Some cyanobacteria have a rather loosely organized thylakoid membrane [28], and this might be the reason why we could still detect the various activities of this flavoprotein. Moreover, in *A. nidulans* this enzyme interacts well with O_2 in the gas phase which might be totally prevented in more tightly structured thylakoid membranes.

However, there might be a number of reasons why it could be an advantage to the *Anacystis* cells to have a certain amount of the L-amino acid oxidase only loosely associated with the thylakoid membrane, although we otherwise believe that the major function of this enzyme is in the photosynthetic water oxidation [6, 7]. Firstly, the L-amino acid oxidase could be used for immediate NH_4^+ production from L-arginine before any other L-arginine degrading systems which are not constitutive in *A. nidulans*, are induced.

Secondly, the enzyme could function as a L-arginine dehydrogenase to use L-arginine as electron donor for the electron transport chain in a comparable fashion as reported for *Escherichia coli* where a

D-amino acid dehydrogenase or a proline oxidase have been shown to be connected with the respiratory system [29]. Since cyanobacteria have an incomplete citric acid cycle [30], the cells might not produce enough NADH as a source of reducing equivalents for the respiratory chain. This is in agreement with the rather low activity of the NADH dehydrogenase in cyanobacteria [9]. Therefore, L-arginine which can be formed by the alternative CO_2 fixation pathway by way of citrulline in cyanobacteria [2], could be used in place of NADH as an immediate electron donor under some conditions.

Thirdly, the enzyme can also directly interact with O_2 to form H_2O_2 which partly is decomposed by catalase and partly in a light-dependent reaction (most likely by PS II). This ability of the enzyme might have been an advantage in the early stages of evolution when O_2 was rare in the atmosphere, since the enzyme could scavenge the O_2 evolved by PS II as H_2O_2. By using such an $O_2 \rightarrow H_2O_2$ cycle in the lumen (as suggested in Fig. 3) a microaerobic atmosphere could possibly be created in the cells even under conditions when photosynthetic O_2 evolution proceeded. This might be an interesting aspect to consider, since some *Synechococcus* species are able to fix N_2 [31, 32].

All those above-mentioned activities of the L-amino acid oxidase in *A. nidulans* are strongly influenced by the ions in the medium (especially by the divalent cations). The L-amino acid oxidase activity (O_2 or plastoquinone as electron acceptor) and the associated NH_4^+ production are inhibited by ions, while the suggested water-oxidizing activity of this enzyme in PS II requires Mn^{2+}, Ca^{2+} and Cl^- for activity.

Acknowledgement

The financial support of the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

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