RESEARCH PAPER

Detection of an L-amino acid dehydrogenase activity in Synechocystis sp. PCC 6803

Sarah Schriek, Uwe Kahmann, Dorothee Staiger, Elfriede K. Pistorius, and Klaus-Peter Michel*

Lehrstuhl für Molekulare Zellphysiologie, Universität Bielefeld, Universitätsstr. 25, D-33615 Bielefeld, Germany

Received 23 October 2008; Revised 8 December 2008; Accepted 9 December 2008

Abstract

The protein Slr0782 from Synechocystis sp. PCC 6803, which has similarity to L-amino acid oxidase from Synechococcus elongatus PCC 6301 and PCC 7942, has been characterized in part. Immunoblot blot analysis showed that Slr0782 is mainly thylakoid membrane-associated. Moreover, expression of slr0782 mRNA and Slr0782 protein were analyzed and an activity assay was developed. Utilizing toluene-permeabilized cells, an L-arginine-stimulated O₂ uptake became detectable in Synechocystis sp. PCC 6803. Besides oxidizing the basic L-amino acids L-arginine, L-lysine, L-ornithine, and L-histidine, a number of other L-amino acids were also substrates, while D-amino acids were not. The best substrate was L-cysteine, and the second best was L-arginine. The L-arginine-stimulated O₂ uptake was inhibited by cations. The inhibition by o-phenanthroline and salicylhydroxamic acid suggested the presence of a transition metal besides FAD in the enzyme. Moreover, it is shown that inhibitors of the respiratory electron transport chain, such as KCN and 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone, also inhibited the L-arginine-stimulated O₂ uptake, suggesting that Slr0782 functions as an L-arginine dehydrogenase, mediating electron transfer from L-arginine into the respiratory electron transport chain utilizing O₂ as electron acceptor via cytochrome oxidase. The results imply that Slr0782 is an additional substrate dehydrogenase being able to interact with the electron transport chain of the thylakoid membrane.

Key words: L-amino acid oxidase/dehydrogenase, L-arginine catabolism, Synechocystis sp. PCC 6803, electron transport chain.

Introduction

In general, L-amino acid oxidases (L-Aoxs) are homodimeric flavoproteins containing a non-covalently bound FAD as cofactor in each subunit (Bright and Porter, 1975; Curti et al., 1992; Meister, 1965). They catalyze the oxidative deamination of L-amino acids to produce the corresponding 2-keto acid, ammonium, and hydrogen peroxide by utilizing molecular oxygen as electron acceptor. L-Aoxs occur in insects (Ahn et al., 2000), snake venoms (Massey and Curti, 1967; Meister, 1965; Du and Clemetson, 2002; Torii et al., 1997), and are also found in bacteria (Coudert, 1975; Bredahl et al., 1994), fungi (Kusakabe et al., 1980; DeBusk and Ogilvie, 1984; Niedermann and Lerch, 1990), algae (see below), snails (Ehara et al., 2002), and mammals (Nakano et al., 1966).

The crystal structure of the snake venom L-Aox from Calloselasma rhodostoma (Pawelek et al., 2000) and Agkistrodon halys pallas (Zhang et al., 2004), and of the bacterial L-Aox from Rhodococcus opacus (Faust et al., 2007) have been published. A phylogenetic tree of the evolutionary distances of a number of L-Aoxs has recently been given (Gau et al., 2007; Machéroux et al., 2001; Nishizawa et al., 2005).

Besides L-Aoxs, which utilize molecular O₂ as electron acceptor leading to hydrogen peroxide formation, a few
amino acid dehydrogenases have been described in the literature, which are associated with a respiratory electron transport chain and which donate electrons to the quinone pool. Subsequently, these electrons are transferred to a terminal cytochrome oxidase leading to the reduction of molecular oxygen to water. This reaction is inhibited by KCN. Such a membrane-bound amino acid oxidase is, for example, a D-amino acid dehydrogenase being associated with the respiratory electron transport chain in E. coli (Franklin and Venables, 1976; Olsiewski et al., 1980; Jones and Venables, 1983; Anraku and Gennis, 1987). D-alanine was shown to be the most active substrate, and the enzyme is suggested to contain FAD and a non-heme (heme AE) iron.

Another Aox, which is associated with the respiratory electron transport chain, is a L-cysteine oxidase in the cytoplasmic membrane of Neisseria meningitides (Yu and DeVoe, 1981). This enzyme is inhibited by o-phenanthroline and salicylhydroxamic acid (SHAM) implying the presence of a metal cofactor besides the presence of FAD. The function of this enzyme in the metabolism is yet unclear. A membrane-associated L-Aox is also present in Proteus rettgeri (Duerre and Chakrabarty, 1975) and in Proteus mirabilis (Pelmont et al., 1972). The Proteus rettgeri enzyme has a high specificity for basic L-amino acids. Both enzymes are suggested to interact with the respiratory electron transport chain.

Information on L-Aoxs in photosynthetic organisms is still rather scarce. Three eukaryotic marine phytoplankton species (Pleurochrysis, Prymnesium, and Amphidinium) have been shown to contain cell-surface located L-Aoxs for the utilization of L-amino acids as a nitrogen source (Palenik and Morel, 1990a, b). These L-Aoxs have broad substrate specificity and low \( K_m \) values in the \( \mu \text{M} \) range. In the unicellular green alga Chlamydomonas reinhardtii, either two L-Aoxs or two forms of the same L-Aox are present, which become induced when no primary nitrogen source is available to produce ammonia (Munoz-Blanco et al., 1990; Piedras et al., 1992; Vallon et al., 1993). The marine red alga Gymnogongrus flabelliformis contains an L-Aox catalyzing the oxidative deamination of basic L-amino acids, L-citrulline and L-methionine, and of the non-proteinogenic amino acid L-gigantnine (Fujisawa et al., 1982). An L-Aox with a somewhat broader substrate specificity is also present in the calcareous marine red alga Amphiorea crassissima Yendo (Ito et al., 1987). Moreover, it has been shown that the red sea weed Chondrus crispus contains an L-Aox located in the apoplast, which has a function in pathogen defence (Weinberger et al., 2005).

Previously, it has been shown that the two closely related mesophilic cyanobacteria Synechococcus elongatus PCC 6301 and Synechococcus elongatus PCC 7942 (subsequently named S. elongatus PCC 6301/PCC 7942) possess an L-Aox, which catalyzes the oxidative deamination of the basic L-amino acids L-Arg>L-Lys>L-Orn>L-His utilizing \( \text{O}_2 \) as electron acceptor resulting in the formation of the corresponding 2-keto acid, ammonium, and hydrogen peroxide, an activity, which is strongly inhibited by cations (Pistorius et al., 1979; Pistorius and Voss, 1980; Engels et al., 1992; Gau et al., 2007). The L-Aoxs of S. elongatus PCC 6301/PCC 7942 are in part located in the soluble protein fraction of the periplasm and in part in the spheroplast fraction, mainly precipitating with the membrane fraction (Bockholt et al., 1996; S Schriek, unpublished results). The L-Aoxs of S. elongatus PCC 6301/PCC 7942 contain FAD as cofactor and are encoded by the aoxA genes (synpcc7942_0369, synpcc7942_0946) (Gau et al., 2007). The two L-Aoxs are 100% identical and encode proteins of 54 kDa with a calculated isoelectric point of 7.9. When the aoxA gene in S. elongatus PCC 7942 was insertionally inactivated, cells no longer grew with L-arginine as the sole N-source, suggesting that this enzyme is the only one that enables the cells to utilize extracellularly added L-arginine as a N-source (Bockholt et al., 1996).

Both cyanobacteria contain an additional gene with similarity to the aoxA gene, which has been called aoxB, being synl144_c for S. elongatus PCC 6301 and being synpcc7942_0369 for S. elongatus PCC 7942 (Gau et al., 2007). The product of the aoxB gene has not yet been characterized biochemically. Whether AoxB is the thylakoid membrane-bound L-Aox, catalyzing the conversion of phenylalanine to phenylpyruvic acid (Loeffelhardt, 1977), can not yet be answered.

A similar L-Aox activity with high specificity for basic L-amino acids, with L-arginine being the best substrate, has also been detected in Synechococcus cedrorum PCC 6908 (Gau et al., 2007). Moreover, a bioinformatic analysis of 24 cyanobacterial genomes revealed the presence of one or two gene(s) with similarity to AoxA in 10 other cyanobacterial species (Gau et al., 2007; Schriek, 2008; Schriek et al., 2007).

In the present paper, our interest was focused on the AoxA-similar protein Slr0782 in Synechocystis sp. PCC 6803 for two main reasons. Although L-Aox activity could easily be detected in S. elongatus PCC 6301/PCC 7942 by measuring L-arginine-stimulated \( \text{O}_2 \) uptake, which is inhibited by cations such as CaCl\(_2\), in cell suspensions as well as in cell-free extracts of Synechocystis sp. PCC 6803, we had not been able to detect such activity. The second reason is related to the observation that a complex interrelationship seems to exist between L-arginine catabolism and photosynthesis/respiration in Synechocystis sp. PCC 6803, especially when the light intensity during growth was set to 200 \( \mu \text{mol} \text{ photons m}^{-2} \text{ s}^{-1} \) (Schriek et al. 2008; Schriek 2008; Stephan et al., 2000). Previous results have shown that a PsbO-free Synechocystis sp. PCC 6803 mutant was much better able to utilize L-arginine as the sole N-source than the wild type (WT), suggesting that a change on the donor side of photosystem II (PSII) has a substantial influence on L-arginine catabolism. Therefore, we were interested in finding conditions under which an L-Aox activity was detectable in Synechocystis sp. PCC 6803 and to see whether such results might help to explain why a PsbO-free mutant is better able than WT to utilize L-arginine as the sole N-source when the light intensity during growth corresponds to approximately 200 \( \mu \text{mol} \text{ photons m}^{-2} \text{ s}^{-1} \).
Materials and methods

Cyanobacterial strains, growth conditions, and cell harvest

The cyanobacterial strain *Synechocystis* sp. PCC 6803 WT was obtained from the Pasteur Culture Collection of Cyanobacterial Strains, Paris, France. The PsbO-free mutant was the same as described earlier (Engels et al., 1994). Cyanobacteria were cultivated in 250 ml gas wash bottles (16 cm in height and 4.5 cm in diameter), which were placed in a water bath (size 40×70×25 cm) set at 30 °C. Illumination was with six beams (Philips Cool Spot, angle 12°, 150 W) placed 36 cm above the culture bottles. The ambient illumination at the outside of the culture bottles corresponded to 200 μmol photons m⁻² s⁻¹. The light intensity was determined with a LI-250 light meter with a LI-190SZ quantum sensor (Li-Cor, Lincoln, Nebraska, USA) measuring photons between 400–700 nm. Since the cultures revealed a higher growth rate at 200 μmol photons m⁻² s⁻¹ than at an illumination of 50 μmol photons m⁻² s⁻¹ (not shown), this light regime obviously did not cause substantial photoinhibitory damage. The BG11 medium was continuously bubbled via two manometers (Porter Instruments, Hatfield, USA) with 20 l h⁻¹ 2% CO₂-enriched air. Growth was performed with a slightly modified BG11 medium (Stephan et al., 2000) with nitrate as the N-source (17.7 mM sodium nitrate) or with nitrate-free BG11 medium containing 5 mM L-arginine-HCl to which 50 mM EPPS-NaOH pH 7.5 was added to prevent acidification. After 48 h of growth with nitrate or L-arginine the pH of the CO₂-aerated BG11 medium was between 7.0 and 7.5 (Nodop et al., 2006). Growth of the PsbO-free mutant was performed as described above, except that the growth medium contained kanamycin sulphate (7.5 mg l⁻¹). The standard culture inoculum corresponded to an absorbance of 0.3 at 750 nm (OD₇₅₀ nm). Growth rates were determined by measuring the OD₇₅₀ nm of cell suspensions. The chlorophyll content was determined according to previously published protocols (Grimme and Boardman, 1972).

RNA isolation and slot-blot RNA–DNA hybridization analysis

Isolation of total RNA was performed as described previously (Michel et al., 2003). The isolation protocol was improved by an on-column DNase digestion step with the RNase-free DNase set from Qiagen (Qiagen, Hilden, Germany) according to the manufacturer’s recommendation. The *rnpB* probe was used to ensure equal loading.

Preparation of cell-free extracts, SDS PAGE, and immunoblotting

Preparation of cell-free extracts, SDS PAGE, and immunoblotting were performed as previously described (Tölle et al., 2002). Cells were harvested by centrifugation, resuspended in 50 mM TRIS-HCl, pH 7.4, containing 5 mM EDTA-NaOH, pH 7.4, 1 mM benzamidine, 0.1 mM PMSF, and broken in a Hybrid Ribolyser (FastPrep Instrument, Q-biogene, Heidelberg, Germany). The extract was centrifuged for 1 min at 16,200 g at 4 °C, and the supernatant was used. Samples corresponding to 15 μg proteins were denatured for 20 min at 60 °C using a 20 mM dithiothreitol- and 4% SDS-containing buffer and subjected to TRIS-glycine SDS PAGE. Subsequently, the proteins were transferred to BA-85S nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany) as described previously (Michel et al., 1996). The antisera used were the anti-PsbA (dilution 1:2,000) (Engels et al., 1992) and anti-PetA (dilution 1:1,500) (Michel et al., 2003; Pietsch et al., 2007). The anti-Slr0782 antiserum was raised in rabbits against heterologously expressed and affinity-purified Slr0782 (Pineda Antikörper-Service, Berlin, Germany) (Schriek, 2008). The second antibody was a peroxidase-coupled swine anti-rabbit IgG (dilution 1 to 250–500) or an alkaline phosphatase-conjugated goat anti-rabbit IgG (dilution 1:500, DAKO A/S, Glostrup, Denmark). Immunoblot staining was performed either with the ECL Detection Kit (GE Healthcare, Munich, Germany) or with nitrotetrazolium blue/X-phosphate as substrate for the alkaline phosphatase.

Immunocytochemistry and sucrose density centrifugation

*Synechocystis* sp. PCC 6803 cells were grown and harvested as described above. Cell pellets were washed three times with EM buffer (50 mM KH₂PO₄-Na₂HPO₄, pH 7).

### Table 1. Oligonucleotides used for slot-blot RNA-DNA hybridization

| Primer | Name        | Amplified product | DNA sequence 5’→3’ direction |
|--------|-------------|-------------------|-----------------------------|
| sll0782 | slr0782-F   | 1325 bps          | CACATCTCGTGCTCTGATGG        |
|        | slr0782-R   |                   | CCGAGTCAAGGTTGCGACATC       |
| sll1077 | speB2-F     | 1054 bps          | CACGAGAGAGGTTGCACAGG        |
|        | speB2-R     |                   | CAAAACGGTGTATAGGCCGCGT      |
| sll1022 | argD-F      | 1224 bps          | GTTGTGATATCCGTTGAGG         |
|        | argD-R      |                   | TTTCGTGTCCGTCACCACTA        |
| sll0370 | gabb-F      | 895 bps           | GCCCGAGAATACCTTGCGG         |
|        | gabb-R      |                   | GTTCAATGCTGCGACTGCA         |
|        | slr1469-F   | 599 bps           | GCCGGCCATGCTGCTTATCA        |
| mPB    | slr1469-R   |                   | TTTGACAGATGCGCCTGAC        |

**Detection of an L-amino acid dehydrogenase activity in *Synechocystis***
Embedding and cutting of ultra-thin sections was performed according to previously described protocols (Engels et al., 1997). Immunocytochemistry with the anti-Slr0782 antiserum at a dilution of 1:100 in TBS buffer was carried out according to Stephan et al. (2000). Sucrose density centrifugation was carried out as previously described (Omata and Murata, 1983, 1984).

**Toluene treatment of cells and measurements of L-arginine-stimulated oxygen uptake**

Permeabilization of *Synechocystis* sp. PCC 6803 cells was carried out as described before (Quintero et al., 2001). After organic solvent treatment, cells were kept on ice for 30 min in the dark. Afterwards, cells corresponding to 5 μg Chl were tested for their L-arginine-stimulated O₂ uptake activity in a Clark-type electrode. The assay consisted of a total volume of 3 ml containing 5 mM Tricine-NaOH pH 8.5, 0.5 mM MgCl₂, and 5 mM L-arginine. Inhibitors or other amino acids were added as indicated.

**Results**

The cyanobacterium *Synechocystis* sp. PCC 6803 contains the gene slr0782 encoding a protein with similarity to the *aoxA* gene encoding an L-Aox in *S. elongatus* PCC 7942 (Gau et al., 2007; Schriek et al., 2008). In the present paper, a partial characterization of the Slr0782 enzyme in *Synechocystis* sp. PCC 6803 is presented.

**Bioinformatic analysis of the slr0782 gene and the Slr0782 protein in Synechocystis sp. PCC 6803**

The gene slr0782 of *Synechocystis* sp. PCC 6803 is located on the chromosome between slr0750 encoding the histidine kinase. Genes *sasA* and *slr0751* both encode yet uncharacterized proteins. The gene slr0782 starts with a GTG start codon and consists of 1415 bps. It encodes a protein of 471 amino acid residues with a deduced molecular weight of 51, 404 kDa. Slr0782 contains 52 negatively and 38 positively charged amino acid residues resulting in a calculated isoelectric point of 5.19 (Expasy, ProtParam, http://www.exasy.ch/cgi-bin/protparam). Evaluation of the primary amino acid sequence with different software packages (DAS membrane prediction tool: http://www.sbc.su.se/~miklos/DAS/) revealed that Slr0782 contains one putative transmembrane helix at the N-terminus and another one at the C-terminus of the protein. Slr0782 contains a typical GXGXXG dinucleotide-binding motif at its N-terminal end (Wierenga et al., 1986; Macherox et al., 2001) (Fig. 1). The protein shares high similarity to the *aoxA* encoded gene product of *S. elongatus* PCC 6301/PCC 7942 (syc1144_c and synpcc7942_0369: 31% identical, 22% strongly similar, and 13% weakly similar amino acid residues), which has not yet been characterized. Slr0782 also shares similarity to FAD-containing monoamine oxidases (InterProScan, http://www.ebi.ac.uk/Tools/InterProScan/) (Gau et al., 2007). Although the similarity of Slr0782 to AoxA and AoxB is relatively high, especially in the FAD-binding domain, there also exists a major difference between the protein sequences. Slr0782 in *Synechocystis* sp. PCC 6803 contains the N-terminal twin-arginine motif of AoxA and AoxB in *S. elongatus* PCC 6301/PCC 7942 (Gau et al., 2007). A twin-arginine motif is part of a translocation pathway signal (Barks, 1996).

**Expression of Slr0782 on mRNA and protein level in *Synechocystis* sp. PCC 6803**

The steady-state transcript pool of the slr0782 mRNA and the concentration of the Slr0782 protein were investigated in *Synechocystis* sp. PCC 6893 WT and a PsbO-free mutant, when grown either with nitrate or L-arginine as the sole N-source and with an illumination of 200 μmol photons m⁻² s⁻¹ for 24 h, 48 h, and 72 h. The PsbO-free mutant was included in the investigation, since previous results provided evidence that the PsbO-free mutant was better able than WT to grow with L-arginine as the sole N-source, when the light intensity corresponded to 200 μmol photons m⁻² s⁻¹ (Stephan et al., 2000; Schriek et al., 2007, Schriek 2008). This suggests a complex interrelationship between L-arginine catabolism and photosynthesis/respiration, since lack of the Mn/Cu²⁺-stabilizing PsbO protein of PSI1 has a substantial effect on the ability of *Synechocystis* sp. PCC 6803 to utilize L-arginine.

**Slot-blot RNA–DNA hybridization experiments showed that the slr0782 mRNA level increased, when cells were grown with L-arginine as compared to the growth of cells with nitrate (Fig. 2). The increase was substantially higher in the PsbO-free mutant than in WT.** The same trend was
observed for the Slr0782 protein in immunoblot experiments utilizing an antiserum raised against the recombinant Slr0782 protein. As documented in Fig. 3, the Slr0782 concentration was higher in the mutant than in WT. It was also higher in L-arginine-grown mutant cells after 48 h of growth compared to nitrate-grown mutant cells. Thus, Slr0782 expression was substantially higher in the mutant than in WT. In the L-arginine oxidase/dehydrogenase pathway, as being characterized in *Pseudomonas putida* (Miller and Rodwell, 1971; Vanderbilt et al., 1975; Cunin et al., 1986; Lu, 2006), L-arginine is degraded to succinate by the enzymes L-arginine oxidase/dehydrogenase, 4-guanidine butyrase, 4-aminobutyrate transaminase, and succinate semialdehyde dehydrogenase. The transcripts encoding these enzymes were also up-regulated in the PsbO-free mutant of *Synechocystis* sp. PCC 6803, when grown with L-arginine (Fig. 2). This result suggests that in the PsbO-free mutant L-arginine can effectively be metabolized to succinate.

Localization of Slr0782 in *Synechocystis* sp. PCC 6803

Immunocytochemical investigation with the anti-Slr0782 antiserum and a gold-conjugated anti-rabbit-IgG as second antibody provided evidence that Slr0782 is almost exclusively located on the thylakoid membranes in the PsbO-free mutant of *Synechocystis* sp. PCC 6803 (Fig. 4A). Sucrose density centrifugation to obtain subcellular fractions confirmed the presence of a substantial amount of the Slr0782 protein in the thylakoid membrane fraction (Fig. 4B). Moreover, differential detergent membrane solubilization showed that part of the Slr0782 protein was found in a PSI1-enriched fraction, which had been solubilized by simultaneous treatment of membranes with octylglucoside and β-D-dodecyl maltoside according to the procedure given in Burnap et al. (1989). This suggests that either the Slr0782 protein is located close to the PSII complex in the thylakoid membrane or PSII and Slr0782 are co-solubilized by the detergent concentration used.

Partial characterization of the Slr0782 activity in *Synechocystis* sp. PCC 6803

In *S. elongatus* PCC 6301/PCC 7942 an L-arginine-stimulated O2 uptake, which is cation-inhibited, as for example, by CaCl2, could easily be detected in cell suspensions as well as in cell-free extracts (Pistorius and Voss, 1980; Engels et al., 1992; Gau et al., 2007). This activity has been shown to be due to the aoxA gene product (Gau et al., 2007).

---

**Fig. 2.** Transcript analysis of genes encoding enzymes of the L-amino acid dehydrogenase pathway in *Synechocystis* sp. PCC 6803. Slot-blot RNA–DNA hybridization was performed with total RNA extracted from *Synechocystis* sp. PCC 6803 WT and the PsbO-free mutant, and with Dig dUTP-labelled gene-specific probes. Cells were grown for 24, 48, or 72 h with nitrate or L-arginine as the sole N-source at a light intensity of 200 μmol photons m−2 s−1. After cell harvest, total RNA was extracted and 2 μg of RNA each were hybridized with gene-specific probes against the L-amino acid dehydrogenase slr0782 transcript, the 4-guanidinobutyrase slr1077 transcript, the 4-aminobutyrate transaminase slr1022 transcript, and the succinate semialdehyde dehydrogenase slr0370 transcript. The mpB probe was used to assure equal loading of RNA.

**Fig. 3.** Expression of the L-amino acid dehydrogenase Slr0782 in *Synechocystis* sp. PCC 6803 WT and the PsbO-free mutant under selected growth conditions. Cells were grown for 24, 48, or 72 h with nitrate or L-arginine as the sole N-source at a light intensity of 200 μmol photons m−2 s−1. Cell-free extracts corresponding to 50 μg of protein were resolved on SDS PAGE, transferred on to nitrocellulose membranes, and probed with the anti-Slr0782 antiserum (dilution 5,000). The anti-Slr0782 antiserum recognizes a 51 kDa band corresponding to the calculated molecular mass of Slr0782. A slower moving band of approximately 70 kDa is most likely due to an association of part of Slr0782 with lipids and/or cations altering the electrophoretic mobility during SDS PAGE. Immunoblot detection of AtaA (CF1 of ATP synthase) is given as an additional reference for equal protein loading.
Slr0782 has substantial similarity to AoxA, it was rather puzzling that so far we had not been able to detect such an L-arginine-stimulated O₂ uptake inSynechocystis sp. PCC 6803. Therefore, substantial efforts were made to find conditions under which such an L-arginine-stimulated O₂ uptake becomes detectable in Synechocystis sp. PCC 6803. This was not possible either with cell suspensions or with cell-free extracts. However, when toluene-permeabilized cells were used, an L-arginine stimulated O₂ uptake finally became detectable. For these experiments, Synechocystis sp. PCC 6803 WT and the PsbO-free mutant were cultivated with L-arginine and an illumination of 200 μmol photons m⁻² s⁻¹ for 48 h. The cells were harvested and permeabilized with toluene according to the protocol given by Quintero et al. (2001). The permeabilized cells were kept on ice for at least 30 min prior to use.

Toluene-treated Synechocystis sp. PCC 6803 cells have a low O₂ uptake based on the presence of endogenous substrate or substrates (Fig. 5). When L-arginine was added to toluene-treated Synechocystis cells, a substantial increase of O₂ uptake was observed in the PsbO-free mutant cells, while WT showed no or only a minor increase in oxygen consumption. When a low concentration of MgCl₂ (0.5 mM) was added to the reaction mixture, the O₂ uptake rate upon addition of L-arginine was stimulated 1.7-fold in the PsbO-free mutant cells and corresponded to 22.5 μmol O₂ taken up mg⁻¹ Chl h⁻¹ (Fig. 5). Under such conditions WT also showed a low L-arginine-stimulated O₂ uptake of 5.6 μmol O₂ taken up mg⁻¹ Chl h⁻¹. The slight enhancement of the O₂ uptake activity was seen with MgCl₂ but not with other alkaline earth metal ions such as Ca²⁺ or Sr²⁺. The higher activity in the PsbO-free mutant as compared to WT is in good agreement with the higher Slr0782 protein concentration in the mutant as compared to WT (Fig. 3; 48 h of growth).

Since the L-arginine-stimulated O₂ uptake was substantially higher in the PsbO-free mutant than in WT, all subsequent experiments were performed with toluene-permeabilized PsbO-free mutant cells grown with L-arginine as the sole N-source and with an illumination of 200 μmol photons m⁻² s⁻¹ for 48 h. Moreover, in all assay mixtures 0.5 mM MgCl₂ was added to optimize the O₂ uptake.
activity. As shown in Table 2, the enzyme acts stereospecifically on L-arginine as substrate. No activity was measurable with D-arginine as substrate. The basic L-amino acids, L-arginine, L-lysine, L-ornithine, and L-histidine, are substrates for the enzyme with L-arginine being the best substrate (Table 2), and the $K_m$ value for L-arginine is 3.2 mM. In addition to the tested basic L-amino acids, several other exogenously added L-amino acids were able to stimulate $O_2$ uptake in toluene-permeabilized cells. Among these, L-cysteine was very active and caused an $O_2$ uptake that was about 50% higher than that with L-arginine. Boiled toluenated cells did not cause an $O_2$ uptake, which proved that the measured $O_2$ uptake with L-cysteine is of an enzymatic nature and is not due to an autoxidation phenomenon. This is also supported by the fact that D-cysteine is not a substrate. Thus, the Slr0782 enzyme of *Synechocystis* sp. PCC 6803 oxidizes basic L-amino acids as does the AoxA enzyme of *Synechococcus elongatus* PCC 6301/PCC 7942, but Slr0782 has a broader substrate specificity than AoxA.

Although the L-arginine-stimulated $O_2$ uptake of *Synechocystis* sp. PCC 6803 is stimulated by low MgCl$_2$ concentrations of 0.5 mM, increased MgCl$_2$ concentrations had an inhibitory effect. 2 mM MgCl$_2$ gave 50% inhibition and 10 mM MgCl$_2$ completely inhibited $O_2$ uptake (Table 3). Besides Mg$^{2+}$, other divalent as well as monovalent cations inhibited the activity of Slr0782. The concentration necessary for a 50% inhibition of L-arginine-stimulated oxygen uptake for a selection of cations is given in Table 3. Moreover, the $O_2$ uptake was inhibited by o-phenanthroline, SHAM (salicylhydroxamic acid), KCN, and DBMIB (2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone). Since the AoxAs of *Synechococcus elongatus* PCC 6301/PCC 7942 are inhibited by cations as well as by o-phenanthroline, it is highly likely that these substances also inhibit Slr0782 in *Synechocystis* sp. PCC 6803. Most likely, the inhibition by SHAM is also due to a direct inhibition of Slr0782, since a membrane-associated L-cysteine oxidase in *Neisseria meningitides* has been shown to be inhibited by SHAM (Yu and DeVoe, 1981). By contrast, the inhibition of the L-arginine-stimulated $O_2$ uptake by DBMIB points to a participation of the cytochrome $b_{597}$ complex, and the inhibition by KCN points to a participation of a cytochrome oxidase in the L-arginine-stimulated $O_2$ uptake. Thus, the results suggest that the Slr0782 enzyme feeds electrons from L-arginine oxidation and also from the oxidation of other L-amino acids into the respiratory electron transport chain and does not directly interact with molecular oxygen. Therefore, it is concluded that the Slr0782 enzyme in *Synechocystis* sp. PCC 6803 is an L-arginine dehydrogenase and not an L-arginine oxidase. This would explain the great difficulties, which we had to detect this activity in the past. An activity of Slr0782 can obviously only be detected, when the enzyme is still connected to an intact respiratory electron transport chain, which does not seem to be the case in cell-free

---

**Table 2.** Oxygen uptake of toluene-treated PsbO-free *Synechocystis* mutant cells after addition of L- or D-amino acids

| Substrate     | Relative $O_2$-consumption (%) |
|---------------|--------------------------------|
| L-Arginine    | 100%                           |
| L-Lysine      | 65%                            |
| L-Methionine  | 44%                            |
| L-Histidine   | 44%                            |
| L-Cysteine    | 150%                           |
| L-Leucine     | 52%                            |
| L-Phenylalanine| 51%                           |
| L-Tyrosine    | 51%                            |
| L-Valine      | 40%                            |
| L-Threonine   | 40%                            |
| L-Alanine     | 37%                            |
| L-Glutamate   | 29%                            |
| L-Methionine  | 29%                            |
| L-Tryptophane | 28%                            |
| L-Proline     | 27%                            |
| L-Glycine     | 13%                            |
| L-Isoleucine  | 12%                            |
| L-Glutamine   | 9%                             |
| L-Aspartate   | nd$^a$                         |
| L-Asparagine  | nd$^a$                         |
| L-Arginine    | nd$^a$                         |
| L-Serine      | nd$^a$                         |
| L-Cysteine    | nd$^a$                         |

$^a$ nd, not detectable.

**Table 3.** Inhibition of L-arginine-stimulated oxygen uptake in toluene-treated PsbO-free *Synechocystis* mutant cells

| Inhibitors        | 50% inhibition |
|-------------------|----------------|
| NaCl              | 8.00 mM        |
| MgCl$_2$          | 1.66 mM        |
| SrCl$_2$          | 0.13 mM        |
| CaCl$_2$          | 0.09 mM        |
| ZnCl$_2$          | 0.20 mM        |
| CoCl$_2$          | 0.11 mM        |
| NiCl$_2$          | 0.09 mM        |
| o-Phenanthroline  | 0.05 mM        |
| Dipyridyl         | 0.01 mM        |
| SHAM              | 0.02 mM        |
| DBMIB             | 0.03 mM        |
| KCN               | 0.10 mM        |
| NaN$_3$           | 0.10 mM        |

---
Are L-arginine oxidation via the Slr0782 enzyme and photosynthetic water oxidation via PSII alternative reactions feeding electrons into the thylakoid membrane embedded electron transport chain?

As shown in Figs 2 and 3, when the PsbO-free mutant was grown with L-arginine as the sole N-source, the steady-state level of slr0782 mRNA and also the Slr0782 protein were up-regulated. If L-arginine oxidation and water oxidation represent alternative reactions feeding electrons into the plastoquinone pool, water oxidation should be down-regulated. As shown in Fig. 6, especially in the early phase of growth with L-arginine, the protein level for PsbA (PSII reaction AE center protein) was much lower in L-arginine-grown cells than in nitrate-grown cells. No large difference was seen for the cytochrome b$_6$f complex, which is required for photosynthetic as well as respiratory electron transport activity. Measurements of the fluorescence yield by pulse-amplified modulation (PAM) (Table 4) provided evidence that the PSII activity was highly reduced in cells grown with L-arginine as the sole N-source as compared to cells grown with nitrate, which is in agreement with the lower amounts of PSII reaction AE centre protein PsbA.

Discussion

The results presented here suggest that Slr0782 in *Synechocystis* sp. PCC 6803 is an L-amino acid dehydrogenase (L-amino acid:plastoquinone oxidoreductase) feeding electrons from L-amino acid oxidation into the respiratory electron transport chain. This implies that the measured L-arginine-stimulated oxygen consumption is mediated by a cytochrome oxidase and not directly by Slr0782. Thus, Slr0782 is not an L-amino acid oxidase, which donates electrons directly to O$_2$ to give hydrogen peroxide. To our knowledge, Slr0782 of *Synechocystis* sp. PCC 6803 is the first L-amino acid dehydrogenase in a cyanobacterium, for which an association with the thylakoid membrane-associated respiratory electron transport chain is shown.

Although the enzyme has a broad substrate specificity, it is likely that it functions mainly as an L-arginine dehydrogenase. The enzyme has a $K_m$ value in the mM range, and such a substrate concentration will, most likely, only be reached with L-arginine. This amino acid is taken up effectively by *Synechocystis* sp. PCC 6803 (Montesinos et al., 1997), is the end-product of an alternative CO$_2$ fixation pathway (Linko et al., 1957; Tabita, 1987, 1994), and might also accumulate at such a concentration intracellularly, when cyanophycin (multi-L-arginy-l-poly-L-aspartate) (Simon, 1971, 1987; Allen, 1984; Kolodny et al., 2006; Maheswaran et al., 2006) becomes degraded. The presence of an L-arginine dehydrogenase as an alternative substrate dehydrogenase sheds new light on the special role of L-arginine in the cyanobacterial metabolism and might contribute to a better understanding of the complex dynamic metabolism of cyanophycin as a N- and also C-reservoir (Simon, 1971, 1987; Allen, 1984, 1988; Mackerras et al., 1990a, b; Berg et al., 2000; Maheswaran et al., 2006) and of the complex interrelationship of L-arginine catabolism with photosynthesis/respiration (Schriek 2008, Schriek et al., 2007, Schriek et al., 2008, Stephan et al., 2000).

Our model (Fig. 7) implies that under conditions, under which the L-arginine concentration reaches a threshold level, L-arginine via Slr0782 and water via PSII are alternative electron donors to the electron transport chain. L-arginine oxidation only proceeds well, when a reduction of photosynthetic water oxidation exists as being the case in the PsbO-free mutant. This model could explain why such a big phenotypical difference between *Synechocystis* sp. PCC 6803 WT and the PsbO-free mutant. Cells were grown for 24, 48, or 72 h with nitrate or L-arginine as the sole N-source at a light intensity of 200 μmol photons m$^{-2}$ s$^{-1}$. Cell-free extracts corresponding to 50 μg protein were resolved on SDS PAGE, transferred on to nitrocellulose membranes, and probed with the antisera raised against PsbA (reaction centre protein D1 of PSII; dilution 1:2,000) and PetA (Cytb subunit of the Cyt b$_6$f complex; dilution 1:1,1500).

**Fig. 6.** Immunoblot analysis of selected electron transport-associated proteins in *Synechocystis* sp. PCC 6803 WT and the PsbO-free mutant. Cells were grown for 24, 48, or 72 h with nitrate or L-arginine as the sole N-source at a light intensity of 200 μmol photons m$^{-2}$ s$^{-1}$. Cell-free extracts corresponding to 50 μg protein were resolved on SDS PAGE, transferred on to nitrocellulose membranes, and probed with the antisera raised against PsbA (reaction center protein D1 of PSII; dilution 1:2,000) and PetA (Cytb subunit of the Cyt b$_6$f complex; dilution 1:1,1500).
Table 4. PAM measurements of photosynthetic yield of Synechocystis sp. PCC 6803 WT and the PsbO-free Synechocystis mutant.

Cells were grown for 24 h, 48 h or 72 h under constant illumination with 200 μmol photons m⁻² s⁻¹ either with nitrate or L-arginine as the sole N-source. Prior to PAM measurements, cells were washed once and concentrated to give a Chl concentration of 1 mg ml⁻¹ (see Materials and methods). The given activity values are representative of three independent experiments.

| Synechocystis sp. PCC 6803 | WT | PsbO-free mutant |
|----------------------------|----|-----------------|
| N-source                   | NO₃ | L-Arg | NO₃ | L-Arg |
|                           |     |       |     |       |
| Growth for 24 h            | 0.36| 0.08  | 0.16| 0.03  |
| Growth for 48 h            | 0.49| 0.01  | 0.30| 0.04  |
| Growth for 72 h            | 0.49| 0.11  | 0.32| 0.01  |

Fig. 7. Model of the function of the Slr0782 protein in Synechocystis sp. PCC 6803 as an alternative substrate dehydrogenase of the thylakoid membrane-associated electron transport chain. In this model, water via PSII (water:plastoquinone oxidoreductase) and L-arginine via Slr0782 (L-arginine:plastoquinone oxidoreductase) are alternative electron donors for the thylakoid membrane-associated electron transport system of Synechocystis sp. PCC 6803. Under high light conditions, as for example, growth with 200 μmol photons m⁻² s⁻¹, L-arginine oxidation via Slr0782 seems to proceed effectively only when a limitation in PSII exists, as being the case when PsbO is lacking. Moreover, CaCl₂ has an antagonistic effect on water oxidation (Ca²⁺ and Cl⁻ are stimulatory) and on L-arginine oxidation (Ca²⁺ and also other cations are inhibitory).

6803 WT and the PsbO-free mutant had previously been observed when grown with L-arginine under continuous light of about 200 μmol photons m⁻² s⁻¹ (Stephan et al., 2000; Schriek et al., 2008). In contrast to the PsbO-free mutant, L-arginine oxidation via the respiratory electron transport chain can not proceed effectively in WT, because water oxidation is optimized in the presence of PsbO. PsbO has been suggested to have a regulatory function for photosynthetic water oxidation (Spetea et al., 1994; Sherman et al., 1998; Tucker et al., 2001; De Las Rivas and Barber, 2004; Heide et al., 2004; Lundin et al., 2007). The signals that determine the ratio of the photosynthetic to the respiratory electron transport in the light is still mainly unknown (Vermaas, 2001).

Although our results provide evidence that genes encoding the enzymes of the entire L-arginine dehydrogenase pathway (L-arginine to succinate) for the utilization of L-arginine as a N- as well as the C-source are present in Synechocystis sp. PCC 6803 (Fig. 2), WT as well as the PsbO-free mutant were not able to grow with L-arginine in the presence of DCMU (not shown). DCMU is known to bind to the PsbA protein of PSII (Ort and Yocum, 1996; Barber and Kuhlbrandt, 1999).

Since the L-arginine-stimulated O₂ uptake in Synechocystis sp. PCC 6803 was not inhibited by DCMU, this observation suggests that a small amount of a functional PSII reaction centre has to be present for effective growth with L-arginine. If this is not the case, an imbalance of C- to N-metabolites or of ATP to NADPH might occur. No growth in the presence of DCMU was also reported for Aphanocapsa sp. PCC 6308 when cultivated with L-arginine (Weathers et al., 1978).

To elucidate further the complex interrelationship of this enzyme with the electron transport chain and with the overall L-arginine metabolism, the L-arginine dehydrogenase-containing multiprotein complex has to be isolated from the thylakoid membrane to allow for a more detailed characterization of this complex and the Slr0782 enzyme.

Acknowledgements

The fellowship of the NRW Graduate School of Bioinformatics and Genome Research for Sarah Schriek and the financial support of the DFG are gratefully acknowledged.
Detection of an L-amino acid dehydrogenase activity in Synechocystis

Loeffelhardt W. 1977. The biosynthesis of phenylacetic acids in the blue-green alga Anacystis nidulans: Evidence for the involvement of a thylakoid-bound L-amino acid oxidase. Bioscience C 31, 345–350.

Lu CD. 2006. Pathways and regulation of bacterial arginine metabolism and perspectives for obtaining arginine overproducing strains. Applied Microbiology and Biotechnology 70, 261–272.

Lundin B, Thuswaldner S, Shutova T, Eshaghi S, Samuelsson G, Barber J, Andersson B, Speetia C. 2007. Subsequent events to GTP binding by the plant PsbO protein: structural changes, GTP hydrolysis and dissociation from the photosystem II complex. Biochimica et Biophysica Acta 1767, 500–508.

Macherox P, Seth O, Bollschweiler C, Schwarz M, Kurfurst M, Au LC, Ghisla S. 2001. L-amino-acid oxidase from the Malayan pit viper Calloselasma rhodostoma. Comparative sequence analysis and characterization of active and inactive forms of the enzyme. European Journal of Biochemistry 268, 1679–1686.

Mackerras AH, de Chazal NM, Smith GD. 1990a. Transient accumulations of cyanoophycin in Anabaena cylindrica and Synechocystis 6803. Journal of General Microbiology 136, 2057–2065.

Mackerras AH, Youens BN, Weir RC, Smith GD. 1990b. Is cyanoophycin involved in the integration of nitrogen and carbon metabolism in the cyanobacteria Anabaena cylindrica and Gloeothecae grown on light/dark cycles? Journal of General Microbiology 136, 2049–2056.

Maheswaran M, Ziegler K, Lockau W, Hagemann M, Forchhammer K. 2006. PII-regulated arginine synthesis controls accumulation of cyanoophycin in Synechocystis sp. strain PCC 6803. Journal of Bacteriology 188, 2730–2734.

Massey V, Curti B. 1967. On the reaction mechanism of Crotalus adamanteus L-amino acid oxidase. Journal of Biological Chemistry 242, 1259–1264.

Meister A. 1965. Oxidative deamination. In: Bright HJ, Porter DJT, eds. Biochemistry of amino acids, Vol. 1. New York: Academic Press, 294–319.

Michel KP, Berry S, Hifney A, Pistorius EK. 2003. Adaptation to iron deficiency: A comparison between the cyanobacterium Synechococcus elongatus PCC 7942 wild-type and a DpsA-free mutant. Photosynthesis Research 75, 71–84.

Michel KP, Thole HH, Pistorius EK. 1996. IdA, a 34 kDa protein in the cyanobacteria Synechococcus sp. strains PCC 6301 and PCC 7942, is required for growth under iron and manganese limitations. Microbiology 142, 2635–2645.

Miller DL, Rodwell VW. 1971. Metabolism of basic amino acids in Pseudomonas putida. Intermediates in L-arginine catabolism. Journal of Biological Chemistry 246, 5053–5058.

Montesinos ML, Herrero A, Flores E. 1997. Amino acid transport in taxonomically diverse cyanobacteria and identification of two genes encoding elements of a neutral amino acid permease putatively involved in recapture of leaked hydrophobic amino acids. Journal of Bacteriology 179, 853–862.

Munoz-Blanco J, Hidalgo-Martinez J, Cardenas J. 1990. Extracellular deamination of L-amino acids by Chlamydomonas reinhardtii cells. Planta 182, 194–198.

Nakano M, Danoswki TS, Weitzel DR. 1966. Crystalline mammalian L-amino acid oxidase from rat kidney mitochondria. Journal of Biological Chemistry 241, 2075–2083.

Niedermann DM, Lerch K. 1990. Molecular cloning of the L-amino-acid oxidase gene from Neurospora crassa. Journal of Biological Chemistry 265, 17246–17251.

Nishizawa T, Aldrich CC, Sherman DH. 2005. Molecular analysis of the rebeccamycin L-amino acid oxidase from Lechevalieria aerocolonigenes ATCC 39243. Journal of Bacteriology 187, 2084–2092.

Nodop A, Suzuki I, Barsch A, Schroder AK, Niehaus K, Staiger D, Pistorius EK, Michel KP. 2006. Physiological and molecular characterization of a Synechocystis sp. PCC 6803 mutant lacking histidine kinase Slr1759 and response regulator Slr1760. Bioscience C 61, 865–878.

Olsiewski PJ, Kaczorowski GJ, Walsh C. 1980. Purification and properties of L-amino acid dehydrogenase, an inducible membrane-bound iron-sulfur flavoenzyme from Escherichia coli B. Journal of Biological Chemistry 255, 4487–4494.

Omata T, Murata N. 1983. Isolation and characterization of the cytoplasmic membranes from the blue-green alga Anacystis nidulans. Plant and Cell Physiology 24, 1101–1112.

Omata T, Murata N. 1984. Isolation and characterization of three types of membranes from the cyanobacterium Synechocystis PCC 6714. Archives of Microbiology 139, 113–116.

Ort DR, Yocum CF. 1996. Oxidative photosynthesis: The light reactions. Dordrecht: Kluwer Academic Publishers.

Palenik B, Morel FMM. 1990a. Amino acid utilization by marine phytoplankton: A novel mechanism. American Society of Limnology and Oceanography 35, 260–269.

Palenik B, Morel FMM. 1990b. Comparison of cell-surface L-amino acid oxidases from several marine phytoplankton. Marine Ecology Progress Series 59, 195–201.

Pawelek PD, Cheah J, Coulombe R, Macherox P, Ghisla S, Vrielink A. 2000. The structure of L-amino acid oxidase reveals the substrate trajectory into an energetically conserved active site. EMBO Journal 19, 4204–4215.

Pelmont J, Arlaud G, Rossat A-M. 1972. L-amino acid oxidases des envelopes de Proteus mirabilis: propriétés générales. Biochimie 54, 1359–1374.

Piedras P, Pineda M, Munoz J, Cardenas J. 1992. Purification and characterization of anl-amino-acid oxidase from Chlamydomonas reinhardtii. Planta 188, 13–18.

Pistorius EK, Jetschmann K, Voss H, Vennesland B. 1979. The dark respiration of Anacystis nidulans. Production of HCN from histidine and oxidation of basic amino acids. Biochimica et Biophysica Acta 585, 630–642.

Pistorius EK, Voss H. 1980. Some properties of a basic L-amino acid oxidase from Anacystis nidulans. Biochimica et Biophysica Acta 611, 227–240.

Quintero MJ, Montesinos ML, Herrero A, Flores E. 2001. Identification of genes encoding amino acid permeases by inactivation of selected ORFs from the Synechocystis genomic sequence. Genome Research 11, 2034–2040.
Schriek S. 2008. Interrelationship of photosynthesis, respiration, and L-arginine metabolism in the cyanobacterium Synechocystis sp. PCC 6803. PhD thesis, Bielefeld University, Bielefeld.

Schriek S, Rueckert C, Staiger D, Pistorius EK, Michel KP. 2007. Bioinformatic evaluation of L-arginine catabolic pathways in 24 cyanobacteria and transcriptional analysis of genes encoding enzymes of L-arginine catabolism in the cyanobacterium Synechocystis sp. PCC 6803. BMC Genomics 8, 437.

Schriek S, Aguirre-von-Wobeser E, Nodop A, Becker A, Ibelings BW, Bok J, Staiger D, Matthijs HC, Pistorius EK, Michel KP. 2009. Transcript profiling indicates that the absence of PsbO affects the coordination of C and N metabolism in Synechocystis sp. PCC 6803. Physiologia Plantarum 2008, July 133(3), 525–543.

Sherman LA, Meunier PC, Colon-Lopez MS. 1998. Diurnal rhythms in metabolism. A day in the life of a unicellular, diazotrophic cyanobacterium. Photosynthesis Research 58, 25–42.

Simon RD. 1971. Cyanophycin granules from the blue-green alga Anabaena cylindrica: a reserve material consisting of copolymers of aspartic acid and arginine. Proceedings of the National Academy of Sciences, USA 68, 265–267.

Simon RD. 1987. Inclusion bodies in the cyanobacteria: cyanophycin, polyphosphate, polyhedral bodies. In: Fay P, van Baalen C, eds. The cyanobacteria, Vol. 1. Amsterdam, New York, Oxford: Elsevier, 190–225.

Spetea C, Hundal T, Lundin B, Heddam M, Adamska I, Andersson B. 1994. Multiple evidence for nucleotide metabolism in the chloroplast thylakoid lumen. Proceedings of the National Academy of Sciences, USA 101, 1409–1414.

Stephan DP, Ruppel HG, Pistorius EK. 2000. Interrelation between cyanophycin synthesis, L-arginine catabolism and photosynthesis in the cyanobacterium Synechocystis sp. strain PCC 6803. Bioscience C 55, 927–942.

Tabita FR. 1994. The biochemistry and molecular regulation of carbon dioxide metabolism in cyanobacteria. In: Bryant DA, ed. The molecular biology of cyanobacteria, Vol. 4. Dordrecht, Boston, London: Kluwer Academic Publishers, 437–467.

Tabita FR. 1987. Carbon dioxide fixation and its regulation in cyanobacteria. In: Fay P, van Baalen C, eds. The cyanobacteria. Amsterdam, New York, Oxford: Elsevier, 95–118.

Tölle J, Michel KP, Krup J, Kahmann U, Preisfeld A, Pistorius EK. 2002. Localization and function of the IdiA homologue Sir1295 in the cyanobacterium Synechocystis sp. strain PCC 6803. Microbiology 148, 3293–3305.

Tori S, Naito M, Tsuruo T. 1997. Apoxin I, a novel apoptosis-inducing factor with L-amino acid oxidase activity purified from Western diamondback rattlesnake venom. Journal of Biological Chemistry 272, 9539–9542.

Tucker DL, Hirsh K, Li H, Boardman B, Sherman LA. 2001. The manganese-stabilizing protein (MSP) and the control of O2 evolution in the unicellular, diazotrophic cyanobacterium, Cyanothecae sp. ATCC 51142. Biochimica et Biophysica Acta 1504, 409–422.

Vallon O, Bulte L, Kuras R, Olive J, Wollman FA. 1993. Extensive accumulation of an extracellular L-amino-acid oxidase during gametogenesis of Chlamydomonas reinhardtii. European Journal of Biochemistry 215, 351–360.

Vanderbilt AS, Gaby NS, Rodwell VW. 1975. Intermediates and enzymes between alpha-ketoarginine and gamma-guanidinobutyrate in the L-arginine catabolic pathway of Pseudomonas putida. Journal of Biological Chemistry 250, 5322–5329.

Vermaas WFJ. 2001. Photosynthesis and respiration in cyanobacteria. Encyclopedia of life sciences, Vol. 1. London: Nature Publishing Group, 1–7.

Weathers PJ, Chee HL, Allen MM. 1978. Arginine catabolism in Aphanocapsa 6308. Archives of Microbiology 118, 1–6.

Weinberger F, Pohner G, Berndt ML, Bouarab K, Kloareg B, Potin P. 2005. Apoplastic oxidation of L-asparagine is involved in the control of the green algal endophyte Acrochaete operculata Correa & Nielsen by the red seaweed Chondrus crispus Stackhouse. Journal of Experimental Botany 56, 1317–1326.

Wierenga RK, Terpstra P, Hol WG. 1986. Prediction of the occurrence of the ADP-binding beta alpha beta-fold in proteins, using an amino acid sequence fingerprint. Journal of Molecular Biology 187, 101–107.

Yu EK, DeVoe IW. 1981. L-cysteine oxidase activity in the membrane of Neisseria meningitidis. Journal of Bacteriology 145, 280–287.

Zhang H, Teng M, Niu L, Wang Y, Wang Y, Liu Q, Huang Q, Hao Q, Dong Y, Liu P. 2004. Purification, partial characterization, crystallization and structural determination of AHP-LAAO, a novel L-amino-acid oxidase with cell apoptosis-inducing activity from Agkistrodon halys pallas venom. Acta Crystallographica D Biological Crystallography 60, 974–977.