A Role of the Q_B Binding Protein in the Mechanism of Cyanobacterial Adaptation to Light Intensity?*

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Growth of the unicellular blue-green alga *Anacystis nidulans* in media containing sublethal concentrations of DCMU-type inhibitors of photosynthetic electron transport in strong white light gave rise to shade type appearance in this organism, as characterized by an increased ratio of phycocyanin to chlorophyll and reduced ratios, both, of carotenoids to chlorophyll and of total chlorophyll to P700. Shade type in *Anacystis* was caused neither by phenolic inhibitors tested nor by those known to bind to the cytochrome b_6/f-complex. Surprisingly enough, the molar ratio of phycocyanin to chlorophyll in artificially shade adapted *Anacystis,* grown in strong white light in the presence of 10^{-6} M atrazine, was found to increase with temperature for a given light intensity and with light intensity for a given temperature.

Mutants of *Anacystis* with a reduced binding capacity for DCMU-type herbicides due to an amino acid exchange in the 32 kDa Q_B-binding polypeptide, also called D-1 protein, were observed to show shade type appearance in strong light, to respond very little to changes in light intensity and to show a reduced capability to further change their appearance to shade type by binding of competitors of Q_B to the 32 kDa polypeptide.

In *Anacystis* a concentration of atrazine (10^{-7} M), ten times lower than the one causing the highest rate of shade adaptation (10^{-6} M), was shown to induce an optimum in cell density, which in turn resulted in an optimum in light-dependent O_2 evolution. Both factors together might be responsible for the so-called greening effect observed in higher plants treated with sublethal concentrations of DCMU-type inhibitors of photosynthetic electron transport.

Introduction

While shade-type in higher plant chloroplasts is characterized by a low chlorophyll *a/b* ratio and a high degree of stacking compared to chloroplasts from sun plants, adaptation of the cyanobacterial photosynthetic apparatus upon transfer from high to low light intensity is known to be characterized by an increase in the ratio of phycocyanin to chlorophyll and decreased ratios, both, of carotenoids to chlorophyll as well as of total chlorophyll to P700 [1, 2]. A similar shift in the pigmentation is observed in strong light, when the unicellular blue-green alga *Anacystis nidulans* is exposed to sublethal concentrations of inhibitors of electron transport which are known to bind to a 32 kDa polypeptide which is also called the herbicide-binding or D-1 protein [3], a protein which is known to have a fast light-dependent turnover in a higher plant, the angiosperm *Spirodela* [4, 5] and also in the green alga *Chlamydomonas* [5, 6], and which is stable in the dark. The light-dependent degradation of the D-1 protein has been shown to be slowed down by DCMU-type inhibitors of photosynthetic electron transport [4, 5]. Moreover, this protein is thought to be involved in reversibly binding the secondary plastoquinone Q_B [4, 7] and bicarbonate [8].

With the perspective to localize the component which might give the signal for the adaptive reorganization of the cyanobacterial photosynthetic ap-

Abbreviations: Atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine; bromonitrothymol, 2-bromo-4-nitrothymol; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; dinoseb, 2,4-dinitro-6-isobutylphenol; DNOC, dinitro-*o*-cresol; DNP–INT, 2,4-dinitrophenylether of 2-iodo-4-nitrothymol; fenuron, N,N-dimethyl-N-phenylurea; iodonitrothymol, 2-iodo-4-nitrothymol; ioxynil, 4-hydroxy-3,5-diiodobenzonitrile; metribuzin, 4-amino-6-(1,1-isopropyl)-3-methyl-thio-1,2,4-triazin-5-one; PCC, Pasteur Culture Collection; phenisopham, isopropyl-N-[3-(N-ethyl-N-phenyl-carbamoyloxy)-phenyl]-carbamate; Q_B, secondary quinone electron acceptor; SAUG, Sammlung von Algenkulturen am Pflanzenphysiologischen Institut der Universität Göttingen; simazine, 4,6-bis(ethylamino)-2-chloro-*s*-triazine; terbutryn, 2-(*tert*-butylamino)-4-(ethylamino)-6-methyl-thio-*s*-triazine.

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paratus, inhibitors and conditions were screened with respect to a possible influence on the ratio of phycocyanin to chlorophyll in *Anacystis*. For comparison reasons, herbicide-resistant mutants were included in the investigations, mutants which had been obtained *via* exchange of the herbicide susceptible *psbA* gene (copy I, ref. 11) *versus* the resistant one.

A possible explanation for the so-called "greening effect" described in higher plants under the influence of sublethal inhibitor concentrations [9, 10] is presented.

Materials and Methods

Cyanobacteria, *Anacystis nidulans* (*Synechococcus leopoliensis* SAUG 1402–1, *Synechococcus* spec. PCC 6301), *Aphanocapsa* spec. (*Synechocystis* spec. SAUG 9279, PCC 6714) and mutant D5 of the *Anacystis nidulans* R2 (PCC 7942) with a reduced binding capacity for DCMU-type herbicides [11] were grown in standard mineral media. Batch cultures were harvested after 4–5 days growth in continuous white light. White light was obtained as described by Feierabend [12]. Electron transport inhibitors were added to the cultures in methanol, where necessary, up to a final methanol concentration of 0.1 per cent. Light-dependent O_2 evolution was measured with a Clark-type electrode (Hansatech) in saturating white light at the growth temperature of the cultures. Molar ratios of phycocyanin to chlorophyll were calculated from the absorption spectra of whole cells using the equations and coefficients given by Myers *et al.* [13]. Chlorophyll concentrations were measured using the procedure published by Klein and Vernon [14]. P700 was determined from the ascorbate-reduced minus ferricyanide-oxidized difference spectrum using an extinction coefficient of 64 $mM^{-1} \cdot cm^{-1}$ [15]. Routine absorption spectra were recorded with a Kontron Uvikon 810, reduced minus oxidized difference spectra with a Cary 118.

Results and Discussion

While an atrazine concentration of 10^{-5} M is lethal to *Anacystis* and one of 10^{-10} M allows for adaptation of the cells to the respective light intensity, 10^{-6} M atrazine induces shade type appearance in this cyanobacterium even in strong light; cultures grown in the presence of 10^{-10} M atrazine look exactly like the controls grown without any inhibitor (Fig. 1). Atrazine is not the only inhibitor of photosynthetic electron transport, though, sublethal concentrations

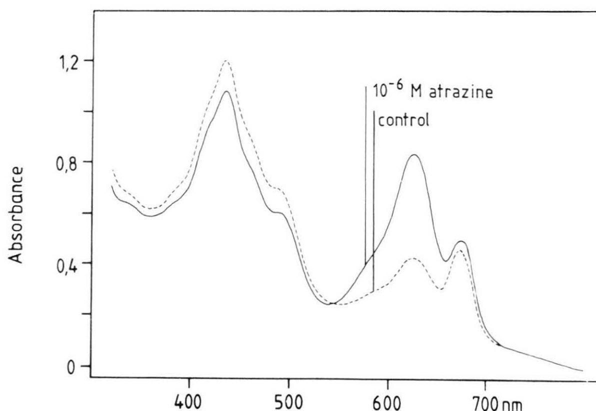

Fig. 1. Comparison of absorption spectra of *Anacystis* wild-type grown in the absence of any inhibitor (dashed line) and in the presence of 10^{-6} M atrazine (solid line) at 22 °C in white light (4500 lux).

Table I. Electron transport inhibitor-induced "shade adaptation" in *Anacystis*.

Inhibitor	Lethal concentration	Shade adaptation	Optimal concentration for shade adaptation
DCMU (diuron)	10^{-6} M	+++++	10^{-7} M
Fenuron	10^{-4} M	+	10^{-5} M, 10^{-6} M
Atrazine	10^{-5} M	+++++	10^{-6} M
Simazine	10^{-5} M	+++	10^{-6} M
Terbutryn	10^{-6} M	+++++	10^{-7} M, 10^{-8} M
Metribuzin	10^{-5} M	+++++	10^{-6} M
Phenisopham	10^{-4} M	+++++	10^{-5} M, 10^{-6} M

of which have been found to cause a reorganization of the cyanobacterial photosynthetic apparatus; so far, all DCMU-type inhibitors tested showed the same result except for lenacil and bromacil. There are, however, different concentrations which are optimal for the induction of shade type in *Anacystis* according to differences in the permeability of the cell envelope for certain inhibitors and according to different binding constants of the inhibitors to the thylakoid membrane (Table I). The result of the reorganization of the cyanobacterial photosynthetic apparatus upon 50 per cent inhibition of non-cyclic electron transport by binding of DCMU-type inhibitors to the D-1 protein is characterized by a very high ratio of phycocyanin to chlorophyll and a reduced ratio of carotenoids to chlorophyll compared to the situation in cells which were grown in the absence of any inhibitor. In this respect, there is no difference between shade type cells which are induced in high light intensity by DCMU-type herbicides and those naturally adapted to low light conditions, except that many times the ratio of phycocyanin to chlorophyll in artificially shade adapted cells is even higher than in those grown in shade. Moreover, the ratio of chlorophyll to P700 in cells grown in the presence of 10^{-6} M atrazine in strong light was found to be 110 compared to a ratio of 205 in control cultures without inhibitor. These data are in good agreement with those obtained by Öquist [2] for *Anacystis* grown in high and low light intensity, respectively. Also, concerning photosystem I activity the behaviour of artificially shade adapted cells parallels that of cells grown in shade [3].

With the aim, to localize the sensor for the adaptation of the cyanobacterial photosynthetic apparatus to light intensity, and with the idea that this sensor might be shared by the naturally occurring shade adaptation and by the one induced by p.e. sublethal concentrations of atrazine in strong white light, a wide variety of inhibitors of photosynthetic inhibitors was tested with respect to the capability to artificially induce shade adaptation in strong light. In contrast to DCMU-type inhibitors, neither the so-called phenolic inhibitors nor those thought to bind to the cytochrome b_6/f complex were ever observed to cause the induction of shade type in *Anacystis* in strong light; lethal concentrations were found for many of them, though. Occasionally, cells turned pale blueish green before finally dying in the presence of 10^{-5} M iodonitrothymol, a concentration which is ten times higher than the one found to completely block photosynthetic electron transport, 10^{-6} M which is lethal (Fig. 2). This phenomenon might be due to nonspecific binding of iodonitrothymol to the binding site of the DCMU-type inhibitors [16, 17]. Phenolic inhibitors tested besides iodonitrothymol were bromonitrothymol, dinitro-*o*-cresol (DNOC), dinoseb and ioxynil. Inhibitors known to bind to the cytochrome b_6/f complex used were DNP–INT and DBMIB. Unfortunately, the inhibition of electron transport by DBMIB is partially relieved during incubation of the cultures in the light. However, cultures stay inhibited with 10^{-5} M DBMIB in the media. Still, his inhibitor does not cause a change in pigmentation of *Anacystis*. Moreover, *o*-phenanthroline was used as an inhibitor; it proved to be

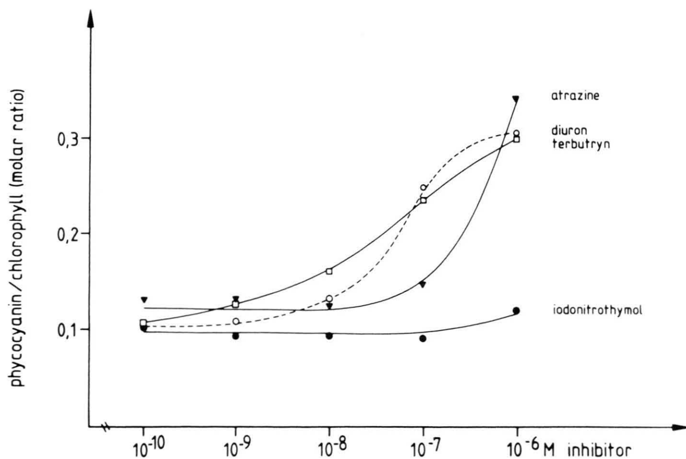

Fig. 2. Molar ratio of phycocyanin to chlorophyll in *Anacystis* wild-type grown in the presence of different inhibitors of photosynthetic electron transport. ▼——▼ Atrazine, ○– – –○ diuron (DCMU), □——□ terbutryn, ●——● iodonitrothymol. Batch cultures were grown in continuous white light (4500 lux) at 32 °C.

unable to evoke shade adaptation in strong light under the experimental conditions but, in addition, was found to inhibit shade adaptation in weak light.

From the fact, that only DCMU-type inhibitors induced shade type appearance in *Anacystis,* it was tentatively concluded that the protein they are known to bind to, the so-called herbicide binding protein, a polypeptide with a molecular weight of 32 kDa also called D-1 protein or, due to its apparent participation in the reversible binding of the secondary plastoquinone molecule Q_B [5, 7], Q_B binding protein, might be involved in – directly or indirectly – giving the signal for the reorganization of the photosynthetic apparatus. This protein is famous for its fast light-dependent turnover in higher plants and green algae [4, 5], its proteolytic degradation being slowed down by binding of DCMU-type inhibitors in strong light on one hand and by low light intensities on the other hand. The protein has been shown to be stable in darkness [5, 7].

Consequently, *Anacystis* mutants with reduced binding capacities for certain DCMU-type inhibitors [11, 18] due to conformational changes of the D-1 protein were tested for comparison reasons concerning their behaviour towards changes in light intensity and towards herbicide binding. These mutants had been obtained *via* exchange of the herbicide susceptible *psbA* gene (copy I, ref. [11]) *versus* the resistant one. In the mutant called D5 (Fig. 3) the two amino acids phe_{255} and ser_{264} of the wild-type had been replaced by tyrosine and alanine respectively [11, 18]. It is not known yet whether in this mutant in addition to the affinity for certain DCMU-type inhibitors also the affinity for the secondary plastoquinone Q_B is reduced compared to the wild-type, in other words whether, for the latter reason, there might be an impairment in photosynthetic electron transport.

Mutant D5 was observed to show shade type appearance in strong white light in the absence of any inhibitor (Fig. 3), suggesting, that due to the conformational change of the 32 kDa polypeptide, the speed of its degradation might be reduced, what in turn could be the signal for the adaptive reorganization of the photosynthetic apparatus to shade type in which a slow turnover is natural. Of course, one could also imagine an impairment in the reversible binding of Q_B resulting in a slow-down of non-cyclic electron transport and thus, finally, in an imbalance of the ratio of ATP to NADPH, to be the signal for the induction of shade type in strong light [19]; but in this case, any partial block of photosynthetic electron transport should evoke shade adaptation. Up to now, this could not be demonstrated.

Besides showing shade type character in strong light, mutant D5 responded very little to changes in light intensity and showed a reduced capacity to further change its appearance to shade type by binding of competitors of Q_B [5, 7] to the 32 kDa polypeptide (Fig. 3). Obviously, *Anacystis* upon mutation to herbicide resistance, upon herbicide binding in strong light as well as upon transfer from high to low light intensity uses the same tool for adaptation, the antenna pigment system; the questions now are, whether this tool is being used in response to the same signal and what the nature of the signal may be. It appears, that the 32 kDa polypep-

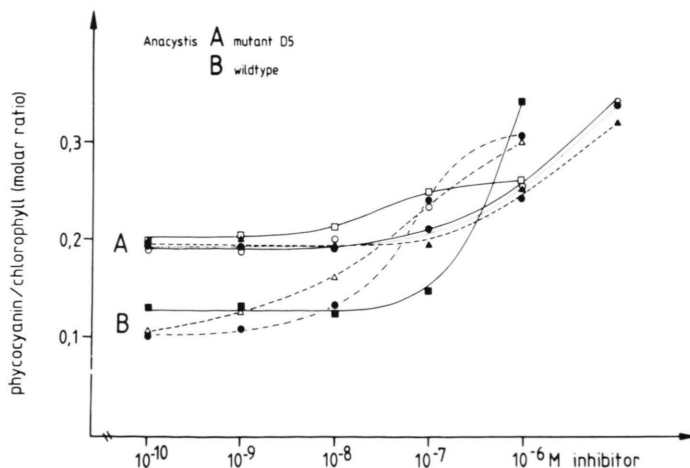

Fig. 3. Comparison of the atrazine resistant mutant D5 [11] of the *Anacystis* R2 (A) and the *Anacystis* wild-type (B) concerning their reaction to different inhibitors of photosynthetic electron transport. Intensity of white light was 4500 lux, growth temperature was 34 °C. Dependence of the molar ratio of phycocyanin to chlorophyll on inhibitor concentration.
A mutant D5 ● .... ● atrazine; □———□ diuron (DCMU); ○———○ terbutryn; ▲– – – – –▲ phenisopham.
B wild-type ■———■ atrazine; ●–·–·–·–● diuron (DCMU).

tide might play a role in this context as is also suggested by Mattoo and Edelman [4].

To further substantiate its role, the effects of light intensity and temperature on the inhibitor-induced shade adaptation were investigated. As can be seen in Table II, the molar ratio of phycocyanin to chlorophyll in *Anacystis*, growing in the presence of 10^{-6} M atrazine, increases with temperature for given light intensity and with light intensity for a given temperature. This light-dependent increase may be interpreted as a consequence of an enhanced synthesis of the 32 kDa polypeptide together with a degradation which is impaired by inhibitor-binding. At first sight, these results have nothing to do anymore with shade adaptation. The connecting link could be a similarity in the behaviour of the D-1 protein in both cases.

Table II. Molar ratio of phycocyanin to chlorophyll in *Anacystis* wild-type grown in the presence of 10^{-6} M atrazine in dependence on light intensity and temperature during growth.

Light intensity [lux]	22 °C	32 °C
190	0.181	0.275
400	0.193	0.281
650	0.220	0.285
1000	0.230	0.303
1700	0.270	0.315
2300	0.285	not det.
3000	0.307	not det.
4500	0.323	dot det.

Up to now, it has not been possible to prevent or relieve the herbicide-induced shade adaptation in strong light by simply feeding thiosulphate as a donor to photosystem I in *Anacystis*, thiosulphate utilization being independent of DCMU but inhibited by DBMIB [20] or by adding glucose as a carbon source to cultures of *Aphanocapsa*.

Obviously, the induction of shade type by a mutation to herbicide resistance on one hand and by binding of sublethal concentrations of DCMU-type inhibitors on the other hand is not restricted to cyanobacteria. Also triazine-resistant higher plants were demonstrated to have shade type character in strong light as shown by a low chlorophyll *a*/*b* ratio and a high degree of stacking [21]. Moreover, the term "greening effect" describes a phenomenon in higher plants upon treatment of the respective plants with sublethal concentrations of certain inhibitors of photosystem II [9, 10]. It appears, that this phenomenon might be caused by two factors, one of them the induction of shade type, the other one possibly an enhancement in cell division, as can be tentatively inferred from the data for cyanobacteria. Obviously (Fig. 4), 10^{-7} M atrazine, a concentration which is ten times lower than the one which leads to artificial shade adaptation (10^{-6} M) in *Anacystis*, caused an optimum in O_2 evolution per ml culture, a

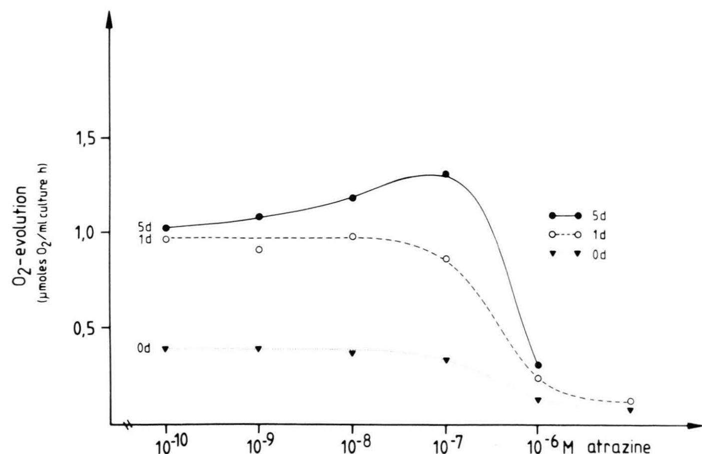

Fig. 4. O_2 evolution of *Anacystis* wild-type grown for a different number of days in the presence of atrazine. *Anacystis* from batch cultures in continuous white light (4500 lux) was tested right after addition of the herbicide (Od), and after 1 (1d) and 5 (5d) days growth at 32 °C, respectively.

result the reason for which is a higher cell density in these cultures than in those with more as well as in the cultures with less inhibitor. It is tempting to suggest that the "greening effect" observed in higher plants upon treatment with sublethal inhibitor concentrations might be the consequence of the combination of these two factors.

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