Partial Molecular Analysis of the psbA Gene in *Euglena gracilis* Mutants Exhibiting Resistance to DCMU and Atrazine

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Mutations conferring herbicide resistance have been detected in two new strains (ZR250 and ZR480) of *Euglena gracilis* Z by partial gene cloning and sequencing. These mutants were originally derived from Z cells grown in medium containing progressively increasing concentrations of DCMU. Each of these strains have been characterized by measuring their growth kinetics, O_2 evolution, and resistance to DCMU and atrazine.

Partial sequences of the psbA gene of these strains were compared to those published for strains Z and ZR25. The ZR250 and ZR480 strains were found to be double mutants. Besides the expected mutation S265A, they showed an additional point mutation at codon 219 (equivalent to codon 218 of other organisms). This mutation results in leucine being substituted by phenylalanine. For each of the ZR strains, two growth conditions (with or without DCMU in the medium) have been compared. The presence of the second mutation (at codon 219) leads to notable increase (20-fold) in resistance to DCMU, whereas the resistance to atrazine is only 2-fold. The presence of DCMU, as the selective agent, was responsible for an enhanced herbicide resistance, irrespective of the concentrations used. Substantial modifications in the rate of cell growth and O_2 yields were observed when the maximal concentration (480 μM) of DCMU was used. These modifications were reversible on withdrawal of the DCMU. Thus the reversible adaptive modifications also adds to the mutational effect observed.

Introduction

After several subcultures, *Euglena gracilis* Z, grown in photoorganotrophic conditions (33 mM lactate) at 25°C (Calvayrac, 1970) and in the presence of increasing concentrations of DCMU (25, 250 or 480 μM) exhibit a permanent adaptation to this herbicide (Calvayrac *et al.*, 1979 a, b and c; Troton *et al.*, 1986). This method enabled us to obtain three strains, ZR25, ZR250 and ZR480. In the strain ZR25, a single point mutation at codon 265 resulting in serine to alanine replacement in the chloroplast psbA gene encoding the D1 protein (32 kD) has been previously reported (Johanning-

meier and Hallick, 1987). More recently, a new strain (MSI) has been obtained in which serine 265 is replaced by threonine (Aiach *et al.*, 1992). In this work we present partial molecular analysis of the psbA gene: resistance to DCMU and atrazine and the adaptative mechanisms associated with it in two new strains of *Euglena gracilis* (strains ZR250 and ZR480) adapted to supersaturating concentrations of DCMU.

DCMU inhibits photosynthesis at the PSII level by blocking electron transfer between the primary electron acceptor Q_A and the secondary acceptor Q_B (for review see Hirschberg *et al.*, 1987). Fluorescence induction experiments (Ohad and Hirschberg, 1990) and trypsin treatments (Pfister *et al.*, 1981) have indicated that the herbicide is bound to the Q_B site at the D1 protein. The existence of a competition between herbicide molecules and the secondary electron acceptor Q_B in the "Q_B pocket" of the D1 protein has also been demonstrated (Vermaas and Arntzen, 1983; Trebst, 1987).

More recently, it has been shown that herbicide resistance can result from mutations of one or two

amino acids sites which weaken the herbicide binding in the Q_B pocket region (Ajlani *et al.*, 1989; Ohad and Hirschberg, 1992; Astier *et al.*, 1993 in *Synechocystis*, Gingrich *et al.*, 1988 in *Synechococcus*, and Wildner *et al.*, 1989 in *Chlamydomonas*). The primary structure of D1 protein may therefore influence O_2 evolution (Etienne *et al.*, 1990; Gowindjee *et al.*, 1992; Etienne and Kirilovsky, 1993).

In the present study, we investigated the relationship between mutations in a part of the psbA gene coding for the Q_B pocket in supersaturating DCMU-adapted *Euglena* strains and their resistance to DCMU and atrazine. We have also attempted to characterize the phenotype of each of the new strains. The resistance properties of different strains are compared either with DCMU or without DCMU in the culture medium. The putative adaptative mechanisms are discussed.

Materials and Methods

Strains and growth conditions

Wild-type (Z) *Euglena gracilis* Klebs and three DCMU-adapted strains (ZR25, ZR250 and ZR480) were grown under photoorganotrophic conditions (ZR25 = ZR, previously published by Johanningmeier and Hallick, 1987). Fresh cultures were inoculated at 5×10^4 cells ml^{-1} in a liquid medium at pH 3.5 (Calvayrac, 1970) containing 33 mM DL-lactate as sole carbon source, without DCMU for Z, ZR25$^-$, ZR250$^-$ and ZR480$^-$ or with DCMU (25, 250 or 480 μM) for ZR25$^+$, ZR250$^+$ and ZR480$^+$ respectively. The illumination conditions were constant at 20 μE m^{-2} sec^{-1} at 26 °C.

Photosynthetic oxygen evolution

The photosynthetic oxygen evolved by the cells (Z, ZR25, ZR250 and ZR480 strains) exposed to saturating light (600 μE m^{-2} sec^{-1}), was followed under conditions of maximal photosynthesis using a modified Clark-type electrode (Laval-Martin *et al.*, 1977) and at a controlled temperature of 25 °C. The experiments were carried out on cells resuspended (final concentration 10^6 cells ml^{-1}) in a buffer (50 mM Tris-HCl, pH 7.6 ; 1 mM MgCl$_2$) containing 20 mM NaHCO$_3$. The cell concentration used was verified and found not to provoke a screen effect. The photosynthetic efficiency (ratio

of maximal photosynthesis to chlorophyll concentration) was expressed as μmol O_2 h^{-1} nmol Chl^{-1}.

Photosynthesis inhibition kinetics

The inhibition kinetics on whole cells, under maximal photosynthesis conditions, were determined with successive dark/light transitions of 72 seconds each. Known amounts of herbicide (DCMU or atrazine), dissolved in isopropanol, were injected during the dark period. A final concentration of isopropanol, below 1.6%, was found not to alter the photosynthetic properties of the cells.

Characterization of Euglena gracilis resistance to herbicides

I_{50} (50% inhibition) values for whole cells were determined in wild-type as well as in adapted strains by assessing the concentrations of herbicide needed to block half of the maximal photosynthesis.

Resistance was expressed by the ratio: I_{50} adapted-strain/I_{50} wild-type.

Chlorophyll determination

Pigments were extracted with acetone-water (9:1 v/v) and measured with a Cary 219 spectrophotometer, using a kinetic method of controlled pheophytinization (Laval-Martin, 1985).

DNA isolation

Total DNA was isolated from 5 days old cultures (cell density = 2.7×10^6 cells ml^{-1}) of the four *Euglena* strains by the CTAB procedure (Roy *et al.*, 1992). DNA extraction was performed on 5×10^6 cells obtained after centrifugation (1000 x *g*, 10 min), followed by two distilled water washes. The pellet was resuspended and homogenized in 600 μl of extraction buffer. The RNase (DNase-free) treatment (20 μg ml^{-1}),(Eurogentec ME0230.10), performed at 37 °C for 30 min, was followed by a chloroform-isoamylalcohol (24:1) extraction. The DNA pellet was resuspended in 50 μl of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). DNA concentration was measured at 260 nm wavelength using a Hitachi U-1100 spectrophotometer.

Plasmid DNA was extracted from overnight cultures of *Escherichia coli* (DH5α strain) grown un-

der agitation in Luria-Bertani medium (LB). The bacteria were lysed according to the alkali extraction method (Sambrook *et al.*, 1989).

PCR

Two oligonucleotides (Eurogentec, Belgium) deduced from the sequence of the psbA gene (Karabin *et al.*, 1984) were used as primers : one corresponded to nucleotides 3112 to 3131 with an additional upstream Sma I site, and the other to nucleotides 3423 to 3440 (pEZC 514.3 clone of Hallick) with an additional downstream BamH I site. The reaction mixture was made up with Euro-taq DNA pol.(ME 0060.01) as per the conditions suggested by the suppliers (Eurogentec). Amplification reaction conditions were : 35 cycles at 94°C, 30 sec; 55°C, 45 sec; 75°C, 1 min and termination 8 min at 72°C. PCR reactions were performed on a PTC-100 programmable Thermal Controller.

Cloning experiments, competence and transformation

pUC 18 plasmid and PCR products from strains Z, ZR25, ZR250 and ZR480 were individually double-digested with Sma I and BamH I enzymes (Appligene) and the fragments were purified by centrifugal filtration (Zhu *et al.*, 1985). The PCR fragments obtained were then inserted into pUC 18 vector using the T4 DNA ligase (Eurogentec ME 0040.10), and used for transformation of *E. coli* strain DH5α (Chung *et al.*,1989). Transformants obtained were denominated pEC, pEC25, pEC250 and pEC480 respectively. The se-

lected clones were stored at -80°C, and used for direct sequencing after plasmid DNA extraction.

DNA sequencing

DNA sequences were determined directly on double-stranded templates from pEC, pEC25, pEC250 and pEC480 using the chain termination method (Sanger *et al.*, 1977). The sequenase T7 DNA polymerase kit from USB (United States Biochemical) and oligonucleotide primers from Eurogentec were used for the sequencing.

Results

Growth kinetics and photosynthetic O_2 evolution

In this study on ZR25, ZR250 and ZR480 adapted to 25, 250 or 480 μM of DCMU respectively *Euglena gracilis* Z strain served as reference. Each strain was grown either without herbicide (ZR25⁻, ZR250⁻ and ZR480⁻), or with herbicide in subsaturating concentration (ZR25⁺) or supersaturating concentrations (ZR250⁺ and ZR480⁺). The solubility of DCMU in water is 180 μM at 25°C.

Growth kinetics

Figure 1 A and B shows that the three resistant cultures, ZR25⁻, ZR250⁻ and ZR480⁻ display the same growth characteristics compared to the Z strain: generation times (GT=9hr) and final cell concentrations (2.7×10^6 cells ml⁻¹) being similar in both cases. However, the growth kinetics of ZR25⁺, ZR250⁺ and ZR480⁺ cultures are dependent on DCMU concentrations. A subsaturating
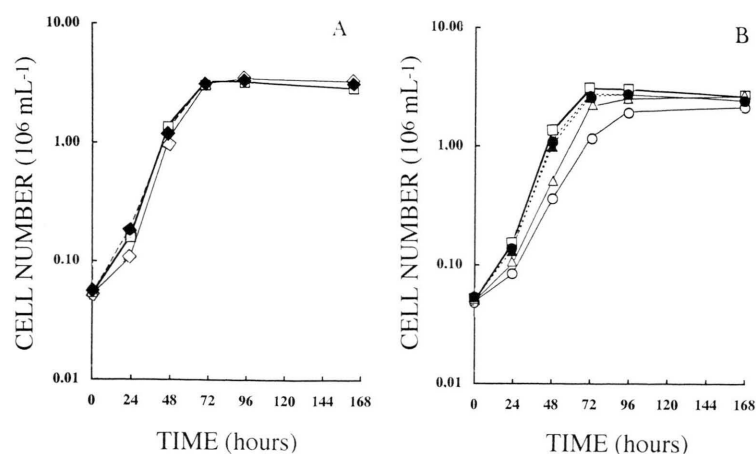

Fig. 1. DCMU effect on cell growth of *Euglena gracilis*, strain Z and DCMU-resistant strains, ZR25, ZR250 and ZR480. ZR⁺ = with DCMU in the culture medium (open symbol), ZR⁻ = whithout DCMU in the culture medium (broken lines and dark symbol). Panel **A**: Z, □; ZR25⁺, ◇; ZR25⁻, ◆. Panel **B**: Z, □; ZR250⁺, △; ZR480⁺, ○; ZR250⁻, ▲; ZR480⁻, ●.

dose (25 μM) does not affect the generation time as opposed to a supersaturating dose. Thus, a two-fold increase in the molar concentration of DCMU (480 μM) increased the generation time from 11 to 14 hours (Fig.1B). In all cases, the final cell concentrations were similar (2.7×10^6 cells ml^{-1}) except for the ZR480$^+$ culture which was slightly lower (2.2×10^6 cells ml^{-1}).

Photosynthetic efficiency

Irrespective of the growth conditions, photosynthetic efficiencies (see Materials and Methods) exhibit a peak on the second day of culture (Fig. 2). It is to be noted that these efficiencies (Fig. 2) were always lower when the herbicide was present in the culture medium. This may be due to the presence of a higher amount of chlorophyll in ZR250$^+$ and ZR480$^+$.

Q_B pocket analysis

The mutant sequences has been partially analysed in ZR250 and ZR480 strains grown on lactate medium supplemented with 250 or 480 μM of DCMU respectively, and compared to both Z and ZR25 strains.

Amplification and cloning of the PCR products

We have chosen to amplify and sequence the region that codes for the "Q_B pocket" of the D1

Upstream oligo: 5' TCCCCCGGGATTTTAATGCATCCATTTCA 3' 29 Mer

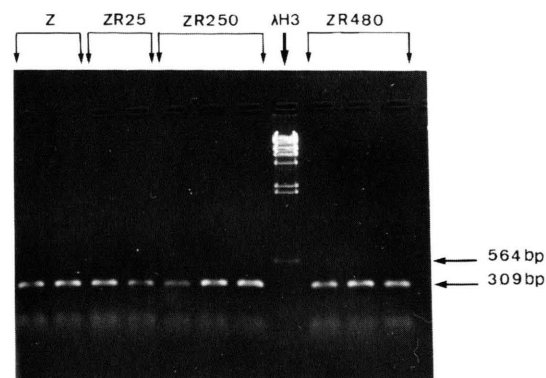Downstream oligo: 5' CCCGGATCCAAATGCCATAGTTGAAAC 3' 27 Mer

Fig. 3. Amplification by PCR of the chloroplast psbA sequence (309 bp) coding for 103 amino acids residues of the "Q_B pocket".

protein which corresponds to the extra-membrane 5 domain and to both the transmembrane IV and V domains (103 amino acid residues).

A unique band (309 bp) is obtained (Fig.3) in several independant PCR reactions for Z, ZR25, ZR250 and ZR480 strains. Two PCR products from each strain were cloned into pUC 18 (pEC, pEC25, pEC250 and pEC480). Two white clones of each pEC plasmid were saved for sequencing.
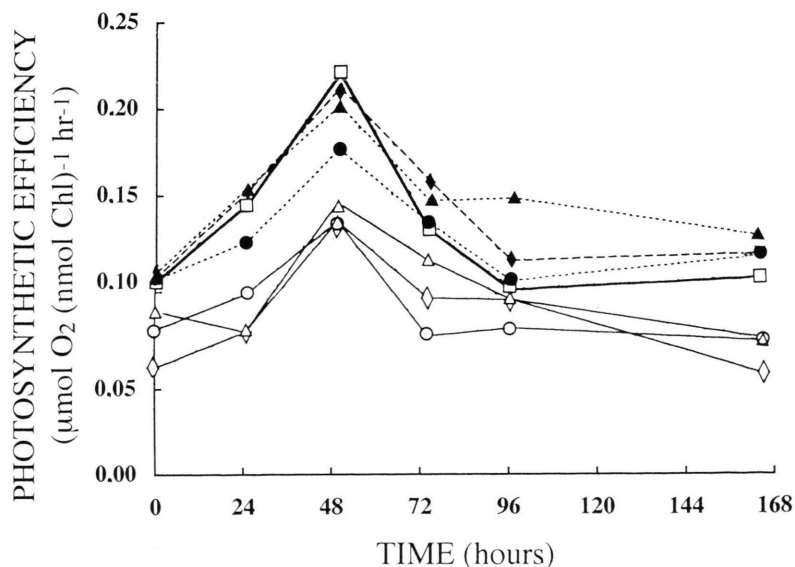

Fig. 2. Maximal photosynthetic efficiency in Tris-bicarbonate (see Materials and Methods) expressed as μmol O$_2$ h^{-1} (nmol Chl)$^{-1}$. ZR$^+$ = with DCMU in the culture medium (open symbol), ZR$^-$ = whithout DCMU in the culture medium (broken lines and dark symbol): Z, □; ZR25$^+$, ◇; ZR250$^+$, △; ZR480$^+$, ○; ZR25$^-$, ◆; ZR250$^-$, ▲; ZR480$^-$, ●.

Sequencing

Nucleotide sequences inserted into the pEC250 and pEC480 plasmids were compared to both Z (pEC) and ZR25 (pEC25) sequences.

Table I presents the nucleotide changes in the partial psbA gene and the corresponding amino-acid changes in D1 for each of the two resistant-strains compared to wild-type and ZR25. The point mutation at codon 265, serine to alanine change, (Johanningmeier and Hallick, 1987) is acquired by the two DCMU-adapted strains. Moreover, an yet unknown new point mutation is present at codon 219 in both the strains (leucine of Z strain is replaced by phenylalanine).

Fig. 4 presents the autoradiograms of DNA-sequencing gels pointing out the transversions for the two mutations. The absence of any signal in the T lane at codon 265 and in the G lane at codon 219 indicates that most, if not all, mutant psbA copies carry this modification.

Resistance assays with DCMU and atrazine

Inhibition kinetics and I_{50} determination

The inhibition kinetics were investigated each day on whole cells of Z and resistant-strains (ZR) grown with DCMU (ZR25$^+$, ZR250$^+$ and ZR480$^+$) or without DCMU (ZR25$^-$, ZR250$^-$ and ZR480$^-$).

Figure 5 shows a small increase in I_{50} as a function of the age of culture. The herbicide concentra-

tion required to block half the maximal photosynthesis is always higher for cells grown with DCMU. There are no significant differences between I_{50} of ZR250$^+$ and ZR480$^+$ on the one hand and I_{50} of ZR250$^-$ and ZR480$^-$ on the other hand. The use of distribution-free U test of Mann and Whitney gives significant differences at 5% for I_{50} obtained on the three strains ZR$^+$, compared to those obtained on the ZR$^-$ strains.

Table II presents the psbA and D1 mutations and the relative resistances of the herbicide-resistant strains of *Euglena*. Compared to Z, the D1 S265A single mutant (ZR25) is resistant to both the herbicides. However it is more resistant to DCMU (x 100) than to atrazine (x 30). The acquisition of a second D1 mutation L219F in ZR250 and ZR480, induces a strong increase of DCMU resistance (x 2000) and only a weak increase for atrazine resistance (x 2).

The presence of DCMU in the culture medium (sub or supersaturating doses) enhances the herbicide resistances: the ZR$^+$ (single and double mutants) are two-fold more resistant for DCMU than the ZR$^-$ and five-fold more for atrazine resistance (single mutant).

Discussion

In DCMU-adapted strains of *Euglena gracilis*, only two mutations conferring resistance are described. A serine to alanine change has been de-

Table I. Identification of two point mutations in a part of chloroplast psbA gene of ZR250 and ZR480 *Euglena* strains; these are transversions, in which a purine is replaced by a pyrimidine (codon 219) or vice versa (codon 265), so that a G∝C pair becomes a T∝A pair and vice versa. Corresponding mutated amino acids and domains IV and 5 are indicated (in italics).

Codon		219			265		
pEC (Z strain)	5'........TCT	TTG *L*	GTA	...............GCG	TCG *S*	TTC	..........3'
pEC25 (ZR25 strain)	5'........TCT	TTG *L*	GTA	...............GCG	**G**CG ***A***	TTC	..........3'*
pEC250 (ZR250 strain)	5'........TCT	TT**T** ***F***	GTA	...............GCG	**G**CG ***A***	TTC	..........3'
pEC480 (ZR480 strain)	5'........TCT	TT**T** ***F***	GTA	...............GCG	**G**CG ***A***	TTC	..........3'

NH$_2$........transmembrane domain IV...................⋊.............domain 5.............COOH

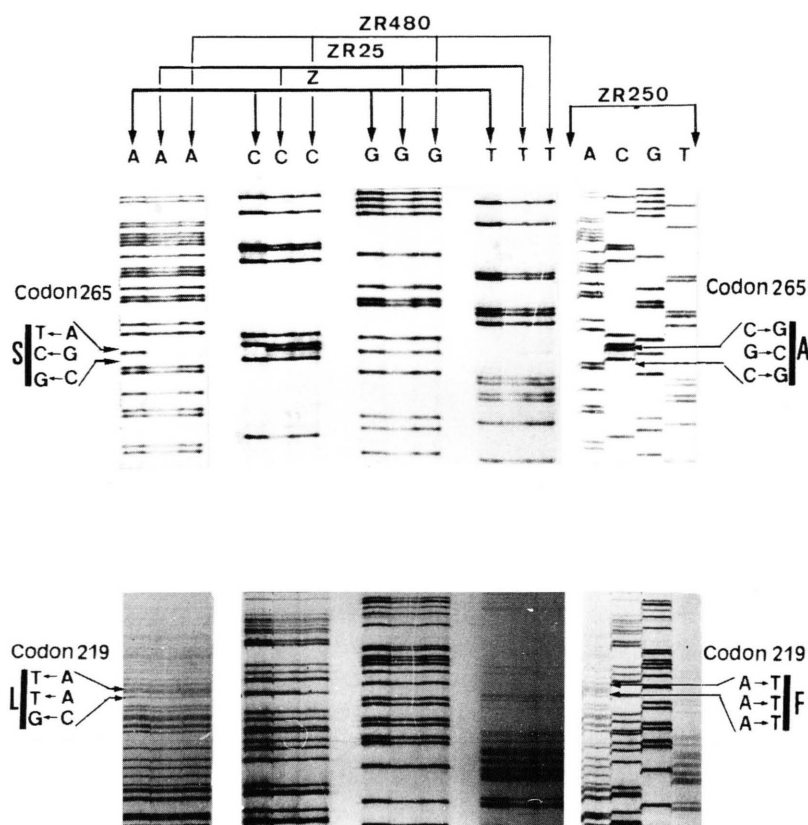* From results both Johanningmeier and Hallick (1987), and present.

Fig. 4. Autoradiograms of DNA-sequencing gels with downstream primer, identifying the transversions from a C (codon 219) and an A (codon 265) in Z strain to an A (in both ZR250 and ZR480 mutants) and a C in three *Euglena* mutants.
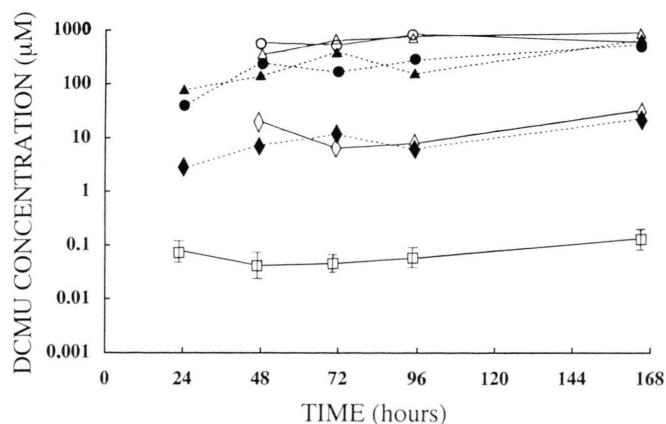
Fig. 5. Evolution of I_{50} as a function of time: I_{50} DCMU is the concentration in μM needed to block half of the maximal photosynthesis. ZR^+ = with DCMU in the culture medium (open symbol), ZR^- = whithout DCMU in the culture medium (broken lines and dark symbol): Z, □; $ZR25^+$, ◇; $ZR250^+$, △; $ZR480^+$, ○; $ZR25^-$, ◆; $ZR250^-$, ▲; $ZR480^-$, ●.
🜨 Standard errors are given for Z strain only. Standards errors though the same for other strains are not indicated on the figures for the sake of clarity.

Table II. Relative resistances of the herbicide-resistant strains of *Euglena gracilis* and corresponding D1 mutations.
*I_{50} (Z) are the means of I_{50} of the Z strain for each herbicide (μM).
a, b and c = p > 0.05 (Mann and Whitney's U test). All other data have a significant difference (p < 0.05).

Mutations	Strains	Relative resistances	
		DCMU *I_{50} (Z) = 0.09 μM$^+$ 0.02	Atrazine *I_{50} (Z) = 2.3 μM$^+$ 0.6
S265A Single mutant	ZR25$^+$	188	146
	ZR25$^-$	94	29
S265A L219F Double mutant	ZR250$^+$	4226 *(a)*	–
	ZR250$^-$	1922 *(b)*	63 *(c)*
S265A L219F Double mutant	ZR480$^+$	4045 *(a)*	–
	ZR480$^-$	1735 *(b)*	71 *(c)*

tected in the ZR strain at position 265. This position corresponds to codon 264 in other eukaryotic organisms. The difference is due to one additional amino acid at the NH$_2$-terminus, (Johanningmeier and Hallick, 1987). More recently, a serine to threonine change at the same position has been detected in MSI mutants (Aiach *et al.*, 1992). The former substitution weakens considerably the binding of DCMU whereas the latter restores this interaction to some extent because the resistance in ZR cells is 16-fold higher than resistance in MSI mutants (Aiach *et al.*, 1992). These data have been obtained on cells adapted to subsaturating DCMU concentrations (less than 180 μM) in photoorganotrophic conditions. In our study, supersaturating concentrations (250 or 480 μM of DCMU) have been used for the adaptation of cells (Calvayrac *et al.*, 1979 a and b).

ZR mutations and herbicide resistance

No alteration has been found in the region of the psbA gene coding for the "Q$_B$ pocket" of ZR25 even ten years after the work of Johanningmeier and Hallick (1987).

Mutation S265A found in the ZR25 strain is also present (Fig.4) in our two mutant strains (ZR250 and ZR480). An additional new point mutation is present at codon 219 replacing leucine by phenylalanine in the presumed transmembrane IV domain (Table I). Compared to the single mutant (ZR25), double mutants (ZR250 and ZR480) have acquired a greater resistance (20-fold) to DCMU (Table II). These results suggest, that in *Euglena* the amino acid residue at position 219 participates in the binding of DCMU with D1 protein.

Q$_B$ pocket mutations conferring herbicide resistance in various organisms are widely discussed in literature (for reviews, see Hirschberg *et al.* (1987) and Oettmeier (1992)). It appears that two amino acids are involved in DCMU binding: S264 and V219 in plants and in *Chlamydomonas*, S264 and F255 in cyanobacteria. Thus, in *Synechococcus* as well as in *Synechocystis*, the same double mutation S264A and F255L confers a variable degree of resistance, which can be explained by a difference in the amino acid environment (Ajlani *et al.*, 1989).

Two possible binding sites based on calculation of the intermolecular energy between amino acid residues of the D1 protein and functional groups on DCMU have been deduced (Mackay and O'Malley, 1993). One involves a hydrogen bond to H215 residue and the other to S264. In both the cases, L218 is the site of interaction with the DMA (dimethylamino) group of DCMU. In ZR double mutants, substitution of leucine by a more bulky molecule (phenylalanine) seems to provoke a steric hindrance which lowers the stability of the bond between DCMU and the Q$_B$-binding site. This may account for the strong resistance to DCMU noted in ZR250 and ZR480 strains and underlines the putative role of leucine in DCMU binding.

With regard to the electron transfer between Q_A and Q_B, it appears that the primary structure of D1 near the QB pocket influences oxygen evolution in cyanobacteria (Etienne and Kirilovsky, 1993). These authors have proposed three categories of mutants; of which, mutant S265A belongs to the second category. For this mutant, the equilibrium constant on the acceptor side is affected. The thermoluminescence band technique showed an accumulation of $Q_A^-Q_B$ due to a decrease in the equilibrium constant for the reaction $Q_A^-Q_B \Leftrightarrow Q_AQ_B^-$ accompanying DCMU resistance. In *Euglena* this decrease becomes more pronounced when the degree of DCMU-resistance increases (Farineau and Laval-Martin, 1995). Eventhough at present we do not have the experimental results for ZR480, we nevertheless presume it to be the same as for ZR250, since the two strains carry identical mutations.

Atrazine, like DCMU, is an urea/triazine-type inhibitor (Trebst, 1987). Studies of mutations conferring atrazine resistance pointed out that atrazine binding site is formed by the combination of three amino acids : A251, F255 and S264. When a serine is substituted by an alanine, the bond with the herbicide is broken and the alga develops resistance (Trebst *et al.*, 1985). Table II shows that cells of double mutants appear to be two-fold more resistant than those containing the single mutation. Thus the additional mutation, L219F, lowers slightly the affinity for atrazine in the Q_B-binding site. These results therefore differ from the ones reported earlier in literature (Aiach *et al.*, 1992; Farineau and Laval-Martin, 1995). This may perhaps be due to differences in our culture conditions and the differences in the parameters measured.

ZR strains in selective and non-selective conditions

The presence to supersaturating doses of DCMU in the culture medium results in considerable modifications in growth: generation times increase when DCMU concentrations increase (Fig.1), but cell productivity decreases only in case of DCMU concentrations exceeding 250 μM (Fig. 1). Moreover, the selective agent, whatever the dose used, affects the O_2 evolution. Photosynthetic efficiency, measured in Tris-bicarbonate buffer (Fig. 2), is always lower for ZR$^+$ cells than ZR$^-$ cells. However, no significant differences were noticed when they were measured in the culture medium. It has been previously shown that a modulation of the lipid environment in DCMU-resistant strains, in case of ZR25 and ZR250, contributed to the conformational stabilization of the protein-chlorophyll complexes, thereby maintaining the photosynthetic function (Troton *et al.*, 1986).

Selective conditions enhance DCMU resistance by a factor of 2. Ratios of resistance were maintained between ZR$^+$ double mutants and the ZR$^+$ single mutant on the one hand, and ZR$^-$ double mutants and the ZR$^-$ single mutant on the other hand (Table II). The occurance of the constant ratio cannot be explained easily: perhaps it may be correlated to the degree of perturbation in the Q_B^- stability and the rate of acceleration in the turnover of D1 protein (Ohad *et al.*, 1990). It is worth noting that inhibitors of the urea/triazine family prevent the trypsin cleavage of the D1 polypeptide at arginine 238 (Trebst, 1991). The presence of DCMU in the medium may slow down the D1 turnover even if its binding with D1 is considerably diminished in resistant cells.

Therefore an alternative explanation needs to be sought. As a matter of fact, it has been previously observed that the mutations of the D1 protein were accompanied by lipid changes in the thylakoid membranes. When DCMU resistant strains are grown without DCMU significant differences appear in fatty acids distribution. The unsaturation index of glycolipids decreases, which has a bearing on the membrane fluidity. For ZR$^+$ cells, DCMU resistance can be characterized by a more efficient electron transfer due to the difference in lipid composition of thylakoid membranes. The lipid modifications observed vary as a function of the logarithm of the herbicide concentration (Troton *et al.*, 1986).

In the same way, selective conditions enhance atrazine resistance (x 5 for the single mutant). The same adaptative mechanism exists most probably, in the two resistance-types. We infer that this mechanism operates by modifying the expression of the mutants. A weaker level of unsaturation of fatty acids was also found in atrazine-resistant cells of *Chenopodium* (Blein, 1980).

In this study ZR250 and ZR480 cells grown in non-selective conditions present a similar partial

genotype and an identical phenotype (growth kinetics, photosynthetic efficiency, chlorophyll amounts, respiration and herbicide resistance). However, significant differences are found in the catalatic activities of ZR250⁻ and ZR480⁻ cells (unpublished results). Earlier works (Calvayrac *et al.*, 1985) leads us to suppose that these differences reflect functional modifications in the oxidative metabolism. Moreover, high DCMU concentrations are known to affect the respiratory chain in *Saccharomyces* (Colson *et al.*, 1977). From these data, it appears that the main differences between these two strains are not observed in the chloroplastic compartment, but could probably be located in the mitochondrial or in the peroxisomal compartment. Unless otherwise shown we consider these two strains as different from each other.

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