GABACULINE-RESISTANT MUTANTS OF CHLAMYDOMONAS REINHARDTII WITH ELEVATED GLUTAMATE 1-SEMIALDEHYDE AMINOTRANSFERASE ACTIVITY

by

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Gabaculine-resistant mutants were obtained from UV-irradiated *Chlamydomonas reinhardtii* by selection on agar plates containing 2 or 4 mM-gabaculine. Genetic analysis of two of the mutants showed that the mutations conferring gabaculine resistance lie in non-allelic nuclear genes. These genes, *gab 1* and *gab 2* are linked to their respective centromeres and not to each other. Glutamate 1-semialdehyde aminotransferase activity in the soluble protein fraction of these mutants and a third one was 1.5 to 4 fold higher than in the wild type. Gabaculine inhibited the aminotransferase activity in the extracts from wild type and all three mutant strains of algae.

1. INTRODUCTION

Chlorophyll biosynthesis is regulated primarily at the enzymic steps catalysing the synthesis of δ -aminolevulinate (3). In *Chlamydomonas* (10) and in greening barley (5) the enzyme glutamate 1-semialdehyde aminotransferase catalyses the last step in δ -aminolevulinate synthesis. DL-gabaculine (3-amino-2,3-dihydrobenzoic acid) is a potent inhibitor of this enzyme (6). Gabaculine inhibits δ -aminolevulinate synthesis in *Chlorella* extracts (11) and chlorophyll synthesis in algae and higher plants (2). During gabaculine inhibition of chlorophyll synthesis in barley leaves, glutamate 1-semialdehyde accumulated (6). Therefore it was concluded that glutamate

1-semialdehyde aminotransferase participates in the chlorophyll biosynthetic pathway (6). It thus became apparent that gabaculine-resistant mutants could carry mutations affecting the enzyme glutamate 1-semialdehyde aminotransferase. In this paper we report on three gabaculine resistant mutants in *Chlamydomonas* which overproduce glutamate 1-semialdehyde aminotransferase activity.

2. MATERIALS AND METHODS

2.1. *Chlamydomonas* strains

Wild type *Chlamydomonas reinhardtii* DAN-GEARD (strain 137c *mt*⁺) and *arg 7-5 mt*⁻ (strain

110) were generously supplied by ROLAND LOPPE and RENÉ MATAGNE, University of Liège. ELIZABETH HARRIS at the Chlamydomonas Genetics Center, Duke University, kindly sent the following strains: CC125, (wild type *mt*⁺); CC255, (*mt*⁺ *nic* 13 *msr* 1 *act* 2 *pyr* 1 *Can*⁺) and CC631, *mt*⁺ *nic* 17. The nicotinamide auxotrophy of CC631 did not recombine with *mt*⁺, and *nic* 17 therefore is allelic with or close to *nic* 7. Using the strains CC125, CC255 and *arg* 7-5 *mt*⁺, the strain X11-17A (*mt*⁺ *nic* 13 *msr* 1 *act* 2 *Can*⁺) was developed. The canavanine resistance of CC255 (gene designated as *can* 1) proved to be due to two, unlinked genes (each alone conferring partial resistance and one of them centromere-linked like *can* 1), and *pyr* 1 has not been assayed in X11-17A.

2.2. General conditions of growth

The composition of the growth medium used in grams per litre was: NH₄Cl, 0.5; CaCl₂ · 2H₂O, 0.05; MgSO₄ · 7H₂O, 0.30; K₂HPO₄, 0.29; KH₂PO₄, 0.14; Trizma Base, 2.42; CH₃COONa · 3H₂O, 2.0; and a mixture of trace elements as given in (4). The pH of all the media was adjusted to 7.0. Solid media were obtained by the addition of 15 g of Difco Bacto-Agar per litre. Agar plates (9 cm in diameter) contained 25 ml medium and were sealed with Parafilm during algal growth to prevent contamination. Stock cultures were maintained on agar slants in screw cap tubes with the medium augmented by 4 g per litre Difco Bacto-Yeast extract. All media were sterilized by autoclaving. Gabaculine, when desired, was added from a stock solution after partial cooling of the medium.

The algae were cultured at 20 to 22 °C in constant light (ca. 100 foot candles meter reading) supplied by a mixture of 20W cool and warm white fluorescent tubes. Growth in liquid medium occurred in 35 ml, closed screw cap Eltang tubes containing 11 to 21 ml medium. The tubes were swirled manually or with a Whirlimixer once or twice daily during the growth period. Maximum titers of 5 × 10⁶ to 10⁷ cells per ml were routinely achieved, with the greatest density of algae resulting in tubes containing 11 ml of medium. Cells were counted with a haemocytometer.

2.3. Mutagenesis of wild type, gabaculine-sensitive (Gab^s) *Chlamydomonas* with UV light and selection of gabaculine-resistant (Gab^r) mutants

Strain 137c *mt*⁺ was cultured in liquid medium into exponential phase. Aliquots (0.1 ml, 5 × 10⁴ colony forming units) were spread on either 2 or 4 mM-gabaculine-containing plates. Six plates with 2 mM-gabaculine and 8 with 4 mM-gabaculine were used. The algae were then irradiated for 30 seconds with a Hanovia Letharay lamp (light dose rate, 1.7 W × m⁻²). The plates were maintained in darkness during the following day to prevent photoreactivation. Survival after the UV irradiation was 34%. Thus 1.7 × 10⁴ mutagenized colony forming units per plate were screened for gabaculine resistance. Thirteen days after plating there were no macroscopic colonies on the 4 mM-gabaculine plates. On the 2 mM-gabaculine plates there were 27 large dark green colonies and a very large number of microscopic colonies. When the 4 mM-gabaculine plate containing UV-irradiated cells was incubated for a longer period, 9 colonies became macroscopically visible and green. Five of the largest and 10 other colonies presumed to be Gab^r were picked from the 2 mM-gabaculine plates and transferred to a 4 mM-gabaculine plate. As controls, three colonies of wild type (137c *mt*⁺) *Chlamydomonas* were also transferred to the same plate. After 5 days, algae from all 5 of the largest and 7 of the other 10 presumed Gab^r colonies showed growth with a muddy green aspect. The control streaks failed to grow and were bleached. Thus 2 to 3 per 10⁴ colony forming units that survived mutagenesis on 2 mM-gabaculine plates showed gabaculine-resistance and were presumed to be mutants.

Two of the largest colonies found originally on the 2 mM-gabaculine plate were designated as gab 1 and gab 2, while one of the colonies on the 4 mM-gabaculine plate was designated as gab 3. These were spread on 2 mM-gabaculine plates and a single colony resulting from each was isolated and gave rise to a stock culture.

2.4. Culture and harvest of algae for examination of glutamate 1-semialdehyde aminotransferase activity in vitro

For each of the strains, 20 to 25 tubes, each containing 20 ml (Table V) or 15 ml (Figures 4 and 5) of liquid medium, were inoculated with 1 ml from algal cultures approaching stationary phase. After 4 to 6 days growth, the cell numbers were determined with a haemocytometer. The algae were then sedimented by centrifugation in a Sorvall SS34 rotor at 5000 rpm for 5 minutes. The supernatants were decanted and the pelleted cells from each strain were resuspended with a Whirlimixer, pooled and centrifuged again. Thereafter, the pellets were stored in a deep freezer until extraction.

2.5. Breaking of *Chlamydomonas* cells and isolation of the soluble protein fraction

The frozen pellets (1 to 3×10^9 cells, ca 0.5 to 1 ml packed volume) were thawed and suspended in three ml of ice cold medium containing 0.1 M-Tricine-NaOH, pH 9.0, 0.3 M-glycerol, 25 mM-MgCl₂ and 1 mM-dithiothreitol. The cell suspensions were passed through an ice cold French pressure cell at 2200 psig. Cell walls, membranes and other insoluble material were removed by centrifugation at 140000g for 30 minutes at 4 °C. The supernatant was used in the enzyme assays.

2.6. Glutamate 1-semialdehyde aminotransferase assay

Each assay mixture (one ml) contained 0.1 M-Tricine-NaOH, pH 8.0, 0.3 M-glycerol, 25 mM-MgCl₂, 1 mM-dithiothreitol, 5 mM-levulinate and 0.5 to 3.8 mg soluble protein. Appropriate volumes of stock solutions of glutamate 1-semialdehyde, synthesized as in (6), were added to each assay to give a concentration of 0.1 (Table V) or 0.2 mM (Figures 4 and 5). The semialdehyde concentrations in the stock solutions were determined using Ehrlich's reagent, as described in (6). Gabaculine, when used, was added from a 10 or 100 µM-aqueous stock solution to give the desired concentrations. Assay mixtures were incubated for either 20 or 40 minutes at 28 °C on a shaker in a water bath.

Reactions were terminated by adding 25 µl of ice cold 70% perchloric acid per assay. Precipitated protein was removed by centrifugation and the supernatant transferred into a graduated tube. The pH was adjusted to 6.9 using 0.5 M-Na₃PO₄ and in case of overshoot with 0.5 M-NaH₂PO₄. Thereafter, to each tube, 100 µl of ethyl acetoacetate was added before heating for 20 minutes in a boiling water bath to produce the δ-aminolevulinate pyrrole. The tubes were then cooled to room temperature and the volume of liquid per tube adjusted to 1.5 ml with water before mixing with 1.5 ml of modified Ehrlich's reagent (9). Twenty minutes later the absorption spectra were recorded from 400 to 650 nm (Table V) or from 400 to 750 nm (Figures 4 and 5) with an Aminco DW2 spectrophotometer. The amounts of δ-aminolevulinate formed were determined taking the molar extinction coefficient at 553 nm as $7.2 \times 10^4 \text{ l} \times \text{M}^{-1}$ for a light path length of one cm (9).

Unincubated, complete assay mixtures served as the spectrophotometric references for obtaining the data in Table V. Results in Figures 4 and 5 rest on spectrophotometry with water as the reference. Accordingly, the absorbances at 553 nm had to be corrected to eliminate nonspecific contributions from Ehrlich's reagent, the algal extracts and glutamate 1-semialdehyde pyrrole. This was achieved by using equation 5, as derived below:

$$1) A_{553}^{\text{obs}} = A_{553}^{\text{ALA}} + A_{553}^{\text{ns}}$$

$$2) A_{525}^{\text{obs}} = A_{525}^{\text{ALA}} + A_{525}^{\text{ns}}$$

$$3) A_{553}^{\text{ALA}} = (1.54) A_{525}^{\text{ALA}}, \text{ (reference 9)}$$

$$4) A_{553}^{\text{ns}} = (1.1) A_{525}^{\text{ns}}, \text{ (see below)}$$

where the superscript obs refers to the observed absorbances, and ALA and ns refer to the contributions of δ-aminolevulinate pyrrole and of non-specific compounds, respectively, to the absorbances; the subscripts indicate wavelengths in nanometers.

Combining and simplifying the 4 simultaneous equations yields:

$$5) A_{553}^{\text{ALA}} = 3.50 A_{553}^{\text{obs}} - 3.85 A_{525}^{\text{obs}}$$

The third ciphers of the constants in equation 5 are not significant.

The constant, 1.1, in equation 4 is based on results of control assays run simultaneously with those shown in Figures 4 and 5. Assays with algal extracts (0.2 to 0.4 ml additions) and no gluta-

mate 1-semialdehyde gave absorbances at 553 nm ranging from 0.04 to 0.08 and A_{553}/A_{525} ranging from 0.95 to 1.03. An assay with glutamate 1-semialdehyde at the concentration used throughout (and no algal extract) gave an absorbance of 0.06 at 553 nm and A_{553}/A_{525} equal to 1.22. (We note, in passing, that these parameters are not constant for glutamate 1-semialdehyde in crude, acidic preparations stored at -18 °C; both A_{553} and A_{553}/A_{525} decrease with time). Thus, 1.1 is a reasonable approximation of the mean A_{553}/A_{525} for the demonstrated sources of nonspecific absorption, whose contributions to A_{553} , when each is assayed alone, are similar.

2.7. Genetic analyses

Strains were crossed and zygospores matured and germinated by methods that in all essential details are standard (7), except that mature zygospores sometimes were stored in darkness at 4 °C for up to 6 months. Dissections of tetrads were performed with a micromanipulator on agar slabs with complete medium. The resultant colonies were transferred as streaks to a master plate.

Segregations of genetic markers were determined after replication to various media. The media were supplemented, as appropriate, with the following compounds in mg per litre : L(+)-arginine · HCl, 50; nicotinamide, 1; L-methionine D,L-sulphoximine, 500; cycloheximide, 10; and 3-acetylpyridine, 15 to antagonize nicotinamide and sharpen the Nic⁺ phenotype. Gabaculine was used at various concentrations.

3. RESULTS AND DISCUSSION

3.1. Phenotypes of gabaculine-resistant mutants

3.1.1. Initial characterization of two *Gab*^r mutants

Liquid cultures of mutant strains *gab 1*, *gab 2* and wild type (137c *mt*⁺) were diluted and aliquots spread on sectors of 0, 2, 3 and 4 mM-gabaculine plates. Five and 14 days after plating, the number of visible colonies on the plates were counted (Table I). No colonies of wild type cells appeared up to 14 days after

Table I. The growth of wild type and two gabaculine-resistant mutants of *Chlamydomonas* on agar plates containing different gabaculine concentrations.

Dilute cell suspensions were spread on sectors of the plates.

Strain	Number of visible colonies 5 days after spreading cells on plates containing gabaculine*			
	0 mM	2 mM	3 mM	4 mM
Wild type (137c <i>mt</i> ⁺)	13	0	0	0
<i>gab 1</i>	30	22 (4)	1 (1)	0
<i>gab 2</i>	16	9	6	0 (2)

*In parentheses are given the number of additional colonies found two weeks after plating.

plating on gabaculine-containing agar, while *gab 1* and *gab 2* cells gave rise to colonies on 2 and 3 mM-gabaculine-agar within 5 days, confirming their resistance. The mutant cells produced fewer colonies on 3 and 4 mM than on 2 mM-gabaculine-agar, indicating that they are not fully resistant to high concentrations of the compound. The growth of *Gab*^r colonies on gabaculine-containing plates was retarded in comparison with gabaculine-free plates.

3.1.2. Effect of gabaculine on increase in cell number and chlorophyll synthesis of wild type and *Gab*^r strains in liquid cultures

Growth of *Gab*^r and *Gab*^s cells in 1 l liquid cultures with various concentrations of gabaculine was studied. *Gab*^r segregants from crosses of the *gab 1* and *gab 2* strains with X11-17A (section 3.2) were compared with wild type (137c *mt*⁺), *Gab*^s algae. The number of cells per ml of culture after 0, 2, 3 and 4 days of growth and chlorophyll concentrations (1) after 4 days were determined. The results are presented in Figure 1 (wild type, *Gab*^s), Figure 2 (*gab 1* segregant, *Gab*^r), and Figure 3 (*gab 2* segregant, *Gab*^r). Gabaculine up to 2 mM (or even 4 mM, data not shown) did not affect the increase in cell number (one to three doublings) during the first two days of culture in any of the three strains.

Figure 1. Inhibition of cell division and chlorophyll synthesis in wild type cells of *Chlamydomonas reinhardtii* by gabaculine.

One ml of cells of the strain 137c *mt*⁺ approaching stationary phase were added to 10 ml liquid culture media to give 3.8×10^5 cells per ml and 0, 0.5, 1.0 or 2.0 mM-gabaculine. The concentration of cells in the cultures were determined after 2, 3 and 4 days of growth. These are shown as bars with numbers corresponding to the days after time zero (t_0). Chlorophyll concentrations (stippled bars) were determined four days after inoculation. The concentration of chlorophyll at t_0 (hatched bar) was calculated by assuming that the chlorophyll content per cell was the same as in cells of the experiment grown for 4 days without gabaculine.

Thereafter, the increase in cell number of Gab^s was inhibited progressively by increasing gabaculine concentrations, reaching complete inhibition at 2 mM-gabaculine. This suggests that cell doublings during the first two days already were potentiated in the inocula and thereby insensitive to inhibition by gabaculine. Compared with wild type the cell division of the *gab 1* and *gab 2* segregants was less severely inhibited by increasing gabaculine concentrations. Increase in chlorophyll in wild type cultures was inhibited 70% by 0.5 mM and 100% by 1.0 and 2.0 mM-gabaculine. Gabaculine at 0.5 mM had little

Figure 2. Effect of gabaculine on cell division and chlorophyll synthesis in mutant cells of *Chlamydomonas* carrying the *gab 1* gene.

Details as in the legend to Figure 1, except that cultures contained 2.1×10^5 cells per ml at t_0 .

or no inhibitory effect on chlorophyll synthesis in the *gab 1* and *gab 2* segregants. However, at 2 mM-gabaculine, chlorophyll synthesis was inhibited 60 to 75% in these two Gab^r mutants.

3.2. Genetic analysis of gabaculine-resistant mutants *gab 1* and *gab 2*

The mutant *gab 1* strain was crossed with *arg 7-5 mt* and 32 tetrads were dissected. After a period of growth, the meiotic products were replicated onto non-supplemented, arginine, and arginine plus 2 mM-gabaculine-containing plates. None of the tetrads gave rise to 2 Arg⁺ and 2 Arg⁻ colonies. Most of the meiotic products which did not form colonies or did not survive replication were, by deduction, Arg⁻. Growing adequately for classification were 52 Arg⁺, 25 of which were resistant to gabaculine, and 16 Arg⁻, none of which showed gabaculine resistance. Fifteen tetrads were dissected from the cross *gab*

Figure 3. Effect of gabaculine on cell division and chlorophyll synthesis in mutant cells of *Chlamydomonas* carrying the *gab2* gene.

Details as in the legend to Figure 1, except that cultures contained 3.3×10^5 cells per ml at t_0 .

$2 \times \arg 7-5 \text{ mt}^-$. Only one tetrad gave 2 Arg^+ and 2 Arg^- colonies. Among the meiotic products that grew well, 27 were Arg^+ and 7 Arg^- . Of the arginine prototrophs, 9 were resistant to 3 mM-gabaculine ($\chi^2=3.0$; $P>0.05$ for $1/2 \text{ Gab}^+$ among the Arg^+ progeny). None of the arginine auxotrophs showed gabaculine resistance. From these experiments we concluded that both *gab1* and *gab2* are Mendelian genes conferring gabaculine resistance. Among plausible explanations for the absence of diagnosed $\text{Arg}^- \text{ Gab}^+$ progeny are: 1) cells bearing *gab1* or *gab2* mutant genes are blocked in arginine uptake and 2) *arg 7-5 gab1* or *gab2* cells, for complex reasons, do not display the Gab^+ phenotype.

Tetrad analyses were also performed after crossing the *gab1* and *gab2* strains with the strain X11-17A. The latter strain carried marker genes *nic13*, *msr1* and *act2*. The linkage groups and the map distances of these genes from their centromeres are: *nic13*, X, 4; *msr1*, I, >50; *act2*, VI, >50. The results (Table II) showed 85% ditype tetrads for the *nic13 - gab1* and *nic13*

Table II. Tetrad analyses of progenies from crosses of *gab1* and *gab2* with the strain X11-17A.

Only tetrads with 4 surviving products and with 2:2 segregations of all the markers are represented.

Loci	The number of different types of tetrad		
	Parental ditype	Tetra-type	Non-parental ditype
<i>gab1 - nic13</i>	8	2	3
<i>gab1 - msr1</i>	3	9	1
<i>gab1 - act2</i>	1	12	0
<i>gab2 - nic13</i>	8	2	3
<i>gab2 - msr1</i>	2	10	1
<i>gab2 - act2</i>	1	9	3

– *gab2* segregations. Eight to 31% ditype tetrads were found for segregation of *msr1* or *act2* with the *gab*⁺ genes. In addition, a *nic13 gab1 mt*⁻ segregant from the *gab1* times X11-17A cross was backcrossed to wild type (*137c mt*⁺). The ratio of types of tetrad for the *gab1 - nic13* segregation from this cross was 3PD: 1T: 2NPD. These results indicate that both gabaculine-resistance genes are strongly centromere-linked.

A *gab1 mt*⁻ segregant from the progeny of the cross *gab1* times X11-17A was crossed with the strain CC631 (*mt*⁺ *nic17*). The products of the dissected tetrads were replicated to diagnostic media. The replica plate containing 3 mM-gabaculine and 1 mg per l nicotinamide was examined 6, 12 and 33 days after replication (Table III). Thereby, a time course for observable macroscopic growth of gabaculine-resistant segregants was obtained. By six days after replication many gabaculine-resistant streaks had not grown sufficiently to be recognizable as such, whereas after 12 days, classification was more reliable. Almost all streaks that were judged as possibly, but not surely, resistant 12 days after replication later proved to be resistant by growing more, while sensitive streaks remained essentially static. This cross gave further evidence for a 2 Gab^+ to 2 Gab^+ segregation and, thereby, that *gab1* segregates as a single nuclear gene. Similar results were obtained upon crossing CC631 with a *gab2 mt*⁻ segregant from the cross of *gab2* with X11-17A (data not shown). Here 9 of the 10 dissected tetrads with all 4 products

Table III. Development of the gabaculine-resistant phenotype of *gab 1*: variations in descriptions of tetrads with time after replication.

A meiotic segregant carrying *gab 1 mt⁻* was crossed with the gabaculine-sensitive strain CC631. Only tetrads with 2:2 segregation for *nic 17* are included here. r = good, dark green growth (Gab^r phenotype). s = very slow or no growth sometimes with bleaching (Gab^s phenotype). i = growth characteristics intermediate between r and s classes.

Tetrad diagnosis	Distribution of tetrad classes following replication to 3 mM-gabaculine plus 1 mg per l nicotinamide after:		
	6 days	12 days	33 days
0 r+4 s	4	0	0
1 i+3 s	2	0	0
1 r+3 s	5	3	0
1 r+1 i+2 s	0	5	0
1 r+2 i+1 s	0	0	1
2 i+2 s	2	0	0
2 r+2 s	0	5	10
2 r+1 i+1 s	0	0	2

surviving were classified 33 days after replication to 3 mM-gabaculine as containing 2 Gab^r and 2 Gab^s segregants. The remaining tetrad gave one or possibly two Gab^r segregants.

For allele tests three pairs of segregants were chosen from the progenies of the crosses of *gab 1* and *gab 2* with X11-17A or *arg 7-5 mt⁻*. Crosses were made with these pairs, as designated in Table IV. Only tetrads giving 2 Nic⁻: 2 Nic⁺ or 2 Act^r: 2 Act^s products are included in the table;

combining the data from the three crosses gave 32 tetrads fulfilling these criteria. Considering the *gab 1* – *gab 2* segregations, 13 of these tetrads were parental ditypes, 10 were tetratypes and 9 were non-parental ditypes. The presence of gabaculine-sensitive meiotic products in the tetratype and non-parental ditype tetrads established that *gab 1* and *gab 2* are not allelic. Furthermore, the results indicate that *gab 1* and *gab 2* are not linked and are consistent with both *gab* genes being centromere-linked. Calculations by the method of PERKINS (8) using the data in Tables II and IV, suggest that *gab 1* and *gab 2* lie somewhere between 4 and 16 map units from their respective centromeres.

3.3. Glutamate 1-semialdehyde amino-transferase activity in gabaculine-resistant mutants and wild type *Chlamydomonas*

The extracts of soluble proteins from wild type *Chlamydomonas* (137c *mt⁺*) showed low levels of glutamate 1-semialdehyde aminotransferase activity. These extracts catalysed the synthesis of from less than 1 up to 1.7 nmoles of δ -aminolevulinate per mg protein in 20 minute assays at 28 °C (Table V and Figure 4). The gabaculine-resistant mutants all showed elevated levels of glutamate 1-semialdehyde amino-transferase activity compared to the wild type on a per mg protein basis. The mutant *gab 1* and the *gab 1* segregant had ca 4-fold higher activity than the wild type, while the mutants *gab 2* and *gab 3* showed 2- to 3-fold increased activity, respectively, in 20 minute assays. Results of some 40

Table IV. Tetrad analyses of the progenies of 3 crosses between *gab 1* and *gab 2*. The tetrads are classified according to segregation of gabaculine resistance in tetrads with all four products surviving and with 2:2 segregations of the marker genes *nic 13* or *act 2*.

Cross	The number of different types of tetrad		
	Parental ditype	Tetra-type	Non-parental ditype
<i>gab 1 mt⁺ × gab 2 act 2 mt⁻</i>	5	4	5
<i>gab 2 mt⁻ × gab 1 nic 13 mt⁺</i>	4	4	3
<i>gab 2 mt⁺ × gab 1 nic 13 mt⁻</i>	4	2	1
Total	13	10	9

Table V. Glutamate 1-semialdehyde aminotransferase activities from wild type and gabaculine-resistant mutants of *Chlamydomonas*.

δ -Aminolevulinate was determined after condensing to a pyrrole with ethyl acetoacetate and treating with Ehrlich's reagent according to MAUZERALL and GRANICK (9). An unincubated complete assay mixture served as reference during spectroscopy. The amount of soluble protein in 0.2 ml extract used per assay corresponded to ca 10^8 cells.

Glutamate 1-semialdehyde aminotransferase activity

Source of enzyme	nmoles δ -aminolevulinate formed in:			
	20 min assay		40 min assay	
	Per assay	Per mg protein	Per assay	Per mg protein
wild type	1.0	1.7	2.3	3.8
<i>gab 1</i>	6.2	6.5	10.4	10.8
<i>gab 3</i>	1.2	2.6	2.3	5.0

minute assays (Table V) indicated that the formation of δ -aminolevulinate continued unabated in the latter 20 minute parts of these incubations. Increasing amounts of the extracts added to the assays led to approximately proportional increases in the formation of δ -aminolevulinate (Figure 4). From these observations it was concluded that the gabaculine-resistant mutants are overproducers of glutamate 1-semialdehyde aminotransferase activity. The elevated activity in the mutants could result from an increased transcription and/or translation of the aminotransferase gene (or genes) or from a more efficient, mutated enzyme.

Gabaculine strongly inhibited the glutamate 1-semialdehyde aminotransferase activity in the extracts from the wild type, *gab 1*, *gab 1* segregant, *gab 2* and *gab 3* strains (Figure 5). Since the actual amounts of enzyme in the crude preparations assayed are not known, it is hazardous to make detailed, quantitative comparisons on the extents of inhibition by the various concentrations of gabaculine. The slopes of the inhibition curves do not differ markedly, and we infer, therefore, that enzyme from wild type and from gabaculine-resistant strains has similar sensitivity to gabaculine. No good evidence for inhibition by the lowest assayed concentration of gabaculine (0.25 μ M) was obtained, but above 0.5

Figure 4. Elevated glutamate 1-semialdehyde aminotransferase activities in extracts from gabaculine-resistant mutants as compared with the activity in an extract from wild type (137c *mt*⁺) *Chlamydomonas*.

Varying amounts of the extracts, in steps of 0.1 ml, were added to the assays which were terminated after 20 min incubation at 28 °C. The nmoles of δ -aminolevulinate (ALA) formed were calculated from corrected absorbances at 553 nm, as described in section 2.6. No significant difference between the results obtained with the *gab 2* and *gab 3* extracts was observed. Because the gabaculine resistance genes in the *gab 1* and *gab 1* segregant algae are the same, a single line is also drawn through the points representing those extracts.

μ M-gabaculine, the δ -aminolevulinate formed decreased in all the assays by what appears to be a constant amount per unit increase in gabaculine concentration.

Because the glutamate 1-semialdehyde aminotransferase activities of the *Gab*^r mutants are sensitive to inhibition by very low concentrations of gabaculine in vitro, resistance of this enzyme is not likely to be the basis for the phenotypes of the mutants. The moderate elevations of extractable enzyme activities in the *Gab*^r mutants as compared with wild type algae may be sufficient per se to explain the gabaculine

Figure 5. Inhibition by gabaculine of glutamate 1-semialdehyde aminotransferase activities in extracts of wild type (137c *mt*⁺) and gabaculine-resistant mutants of *Chlamydomonas*.

The following amounts (mg) of protein were added in 0.2 ml of extracts to the assays: wild type, 1.3; gab 1, 1.5; *gab 1* segregant, 1.2; gab 2, 1.4 and gab 3, 1.4. The ordinate represents absorbance at 553 nm attributed to δ -aminolevulinic acid (ALA) after correction as described in section 2.6. The values are proportional to amounts of ALA formed in the 20 minute assays.

resistance of the mutants. However, other possibilities should be considered.

Alternative bases for gabaculine resistance of the *Gab*^r mutants could be either a reduction in permeability to gabaculine or an increased capacity to destroy the compound. The latter suggestions do not account for the elevated levels of glutamate 1-semialdehyde aminotransferase activities of the mutants. We have, of course, entertained the possibility that *gab 1*, *gab 2* and *gab 3* are regulatory genes whose products have multiple roles, one of which is to control the level of activity of glutamate 1-semialdehyde aminotransferase.

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