Synechococcus PCC6301 mutants possessing resistance to the tetrapyrrole biosynthesis inhibitor gabaculine

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The central role of glutamate as the progenitor of 5-aminolaevulinic acid for tetrapyrrole formation in oxygenic photosynthetic cells [1-4] is now well-established. The initial reaction in the conversion is the activation of glutamate by ligation to tRNA\textsubscript{Glu}. The ester-linked glutamate moiety is then reduced to glutamate-semialdehyde which in turn undergoes structural rearrangement to give 5-aminolaevulinic acid (ALA). This last step is catalysed by glutamate semialdehyde aminotransferase [1]. This enzyme is inactivated by the pyridoxal phosphate antagonist gabaculine [5], a potent inhibitor of \textit{in vivo} tetrapyrrole (chlorophyll) formation in higher plants [6] and cyanobacteria [7].

A representative of the two groups that were distinguished by their response to gabaculine in batch cultures is the cyanobacterium Synechococcus PCC 6301 (Anacystis nidulans) has been established \[8\] from the gene sequence, as has the structure of the enzyme in a gabaculine-resistant strain. Synechococcus 6301-GR6 [9] we isolated previously \[10\]. Here we report the isolation and biochemical characterisation of a new family of Synechococcus 6301 strains possessing a graded tolerance to gabaculine.

The gabaculine-tolerant strains were obtained by firstly adding gabaculine to 5pM to a 800 ml continuous culture maintained under photoautotrophic conditions and at low dilution rate (0.015 h\textsuperscript{-1}). This addition resulted in a transition over 7 days to a low culture density; measured as in [7] the decrease was from 145 to 6 Klett units. There was a concomitant change in chlorophyll content which decreased from 8.65 to 0.25 pg ml\textsuperscript{-1}. Thereafter the cell density and pigment content increased steadily and the original levels were re-attained by 14 days. Cells sampled at this point gave the strain designated as Synechococcus 6301-DC5; purity was confirmed by standard microbiological procedures.

The continuous culture was then adjusted to 10pM gabaculine and the selection procedure repeated to give strain DC10. In turn exposures to 20, 40 and finally 80pM gabaculine yielded when the steady states of growth of the cultures were re-attained, strains DC20, DC40 and DC80. Following these additions the fall in culture density was not as severe as on the first exposure to gabaculine.

The degree of gabaculine tolerance possessed by the individual strains was assessed in terms of cell growth and chlorophyll content in a series of batch cultures supplemented with gabaculine over a range 5 - 100pM. The data for chlorophyll formation in strains DC5 and DC40 (Fig.1) are representative in that cells were less sensitive to gabaculine than the wild-type; the 50\,\% inhibition (I\textsubscript{50}) values for wild-type, DC5 and DC40 were 5, 15 and > 90pM, respectively. By this criterion DC5, DC10 and DC20 formed one group of mutants, and DC40 and DC80 a second group.

The basis of the gabaculine resistance of each of the mutants strains has been investigated by screening their glutamate semialdehyde aminotransferase for sensitivity to the inhibitor. Each of the mutant strains possessed a glutamate semialdehyde aminotransferase which was less sensitive to gabaculine than the wild-type. Cell-free extracts were prepared from wild-type and mutant strains and aminotransferase activity measured as described elsewhere \[11\] except assays were performed at pH 6.9 in bis-Tris buffer. The aminotransferase in the extract from the wildtype was inhibited almost completely by 5pM gabaculine. For the mutants the data for DC10 and DC40 were representative of the two groups that were distinguished at the step to gabaculine tolerance possessing a graded tolerance to gabaculine. DC10 and DC40 giving the much higher inhibitor tolerance characteristic of these phenotypes. The nature of the changes is currently being determined by sequencing the aminotransferase gene in DC10 and DC40. It is anticipated that these data will give insight into the amino acid residues implicated in interaction of gabaculine with the aminotransferase and the mechanism of action of the enzyme itself.