SHORT COMMUNICATION

Isolation of Original Amino Acid, Vitamin and Carbon Source Mutants in the Green Alga *Scenedesmus obliquus*

By R. F. MATAGNE* AND C. VINCENZOTTO

Laboratory of Molecular Genetics, Department of Botany,
University of Liège, B-4000 Liège, Belgium

(Received 5 October 1979; revised 29 January 1980)

Scenedesmus obliquus, grown under culture conditions which led to unicell populations, was treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and then cells were plated on rich medium to recover auxotrophic mutants. Carbon source and vitamin (*p*-aminobenzoic acid, thiamin and nicotinamide)-requiring mutants were characterized. Amino acid-requiring mutants, some of types not previously isolated from green eukaryotes, were also obtained: 11 had a requirement for arginine, one for valine, two for threonine or isoleucine, and one for tryptophan, tyrosine or phenylalanine. The possible metabolic blocks of these amino acid-requiring mutants are discussed.

INTRODUCTION

The isolation of biochemical mutants in higher and lower plants is of major importance for the analysis of metabolic pathways as well as for the understanding of molecular mechanisms in gene function. Unfortunately, obligate auxotrophs are scarce in photosynthetic eukaryotes and various hypotheses, such as extensive duplication of genetic material (Li *et al.*, 1967), coexistence of alternative metabolic pathways possibly related to photosynthesis (Li *et al.*, 1967) or inadapted composition of the selective media (Loppes, 1969; Kirk & Kirk, 1978), have been proposed to explain the narrow spectra of mutations in plants.

We report here the isolation of auxotrophs in the alga *Scenedesmus obliquus*, among them three types of amino acid mutants which have never previously been isolated from green eukaryotes. This novel observation indicates the interest of this organism for the isolation and study of new types of biochemical mutants in algae.

METHODS

Strain. *Scenedesmus obliquus* strain EL 19 was obtained from Dr F. R. Trainor (University of Connecticut, U.S.A.).

Media and growth conditions. Cells were grown under continuous white light (8000 lux, 25 °C) in liquid K medium buffered at pH 8.7 to 8.9 (Trainor & Roskowsky, 1967) or on agar medium solidified with 15 g Difco agar l⁻¹. Where indicated, K medium was enriched with 0.4 % (w/v) Merck yeast extract (KY medium) or supplemented with casein hydrolysate, sodium acetate, vitamins or L-amino acids (Merck) at the concentrations specified.

Mutagenesis test. Cells were grown in 500 ml K medium and collected during exponential growth. A sample containing 35 × 10⁶ cells was centrifuged and the cells were resuspended in 10 ml *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) solution (50 µg ml⁻¹ in 0.03 M-sodium phosphate buffer, pH 7.0). After 30 min treatment, the cells were washed twice in the phosphate buffer and plated on agar KY medium at a dilution subsequently yielding 50 to 100 colonies per plate. Under these conditions, survival was about 10 %.

RESULTS AND DISCUSSION

Scenedesmus species are characterized by the formation of colonies (coenobia) of 4, 8 or 16 cells. However, several species exhibit a variable morphology and form unicell populations under certain conditions (Trainor & Roskowsky, 1967). We found that strain EL 19, used in the present study, formed unicells when grown axenically in liquid alkaline medium containing NH_4^+ (K medium, pH 8.7 to 8.9). As unicell populations are better than coenobia for forward mutation experiments, the cells were grown in liquid K medium before treatment with NTG.

After growth for 10 d in the light, a total of 13 650 colonies produced from the mutagenized cells were replicated on to poor (K) and rich (KY) medium; 27 colonies were isolated that grew only on KY medium. The mutants were tested for their ability to grow on agar K medium supplemented with 0.05 % (w/v) casein hydrolysate, 0.1 % (w/v) sodium acetate or various amino acid and vitamin supplements prepared according to Holliday (1956). From these growth tests, and after confirmation of the auxotrophic requirements by using specifically supplemented media, seven different types of auxotrophs were found (Table 1). As in *Chlamydomonas reinhardtii* (Loppes, 1970), thiamin-, nicotinamide-, acetate- and arginine-requiring mutants were isolated but, in addition, three new types of amino acid mutants were obtained.

The 11 strains requiring arginine were tested on K medium supplemented with ornithine or citrulline (100 mg l^{-1}), two known precursors of arginine. Six of the mutants could grow on both media whereas the other five grew only on arginine; thus, it seems that at least two different metabolic blocks are present among these mutants.

One mutant could grow on K medium containing 20 to $100 \text{ mg valine l}^{-1}$ but optimal growth was obtained on a medium containing $100 \text{ mg valine l}^{-1}$ plus $25 \text{ mg isoleucine l}^{-1}$. This mutant can be compared to the many isoleucine-valine mutants of bacteria (Bachmann *et al.*, 1976) or fungi (Fincham *et al.*, 1979): in these micro-organisms, as in higher plants (Mifflin & Lea, 1977), the last steps in the synthesis of these two amino acids are considered to be catalysed by a common set of enzymes, a deficiency in one enzyme leading to failure of both pathways. However, it has been recently shown (Guardiola, 1977) that in *Escherichia coli* the valine and isoleucine transaminases (the last enzymes of the pathway) are in fact products of separate genes and that mutants defective in only one of these two enzyme activities could be isolated.

Two other auxotrophs were able to grow on K medium supplemented with either threonine (5 to 25 mg l^{-1}) or isoleucine (25 to 100 mg l^{-1}), but they failed to respond to homoserine at 5 mg l^{-1} , a concentration which was non-inhibitory for the wild-type. A combination of valine (100 mg l^{-1}) and isoleucine (25 mg l^{-1}) inhibited the growth of these two mutants (but not of the wild-type). From the scheme in Fig. 1(a), a possible explanation could be a partial inactivation of enzymes (1) or (2); the positive effect of isoleucine and the negative effect of isoleucine/valine would derive from their regulatory action on threonine deaminase (3).

Also puzzling is the mutant partially repaired by any one of the amino acids tryptophan, tyrosine or phenylalanine (25 to 50 mg l^{-1} each). Shikimate (50 to 200 mg l^{-1}) did not allow the growth of this mutant. In micro-organisms, as in higher plants, the three amino acids are synthesized from chorismate through two independent pathways (Fig. 1b). The partial inactivation of enzyme (1) related to the regulatory (= inhibitory) effects of tyrosine and phenylalanine on the isoenzymes of chorismate mutase (2) could perhaps explain the observed phenotype.

The present results indicate that *Scenedesmus obliquus*, recently used for isolating mutants with abnormal carotenoid composition (Powls & Britton, 1977) or resistant to antibiotics (Mahanty, 1979), can also be used for the isolation of auxotrophs. Of particular interest are the three new types of amino acid mutants not previously found in plants or in the widely

Table 1. *Types of biochemical mutants induced by NTG in Scenedesmus obliquus, compared with those induced by NTG or ethyl methanesulphonate in Chlamydomonas reinhardtii* (Loppes, 1970)

Auxotrophic requirement*	No. of mutants	
	<i>Scenedesmus obliquus</i>	<i>Chlamydomonas reinhardtii</i>
<i>p</i> -Aminobenzoic acid (1)	0	7
Thiamin (1)	2	7
Nicotinamide (0.25)	2	16
Arginine (100)	11	8
Valine (100)	1	0
Threonine or isoleucine (25)	2	0
Tryptophan, tyrosine or phenylalanine (25)	1	0
Carbon source (1000)	8	23
Total	27	61
Percentage of mutants induced	0.2	0.2-0.5

* Numbers in parentheses indicate the concentrations in mg l⁻¹ used for growth of *Scenedesmus obliquus* auxotrophs.

Fig. 1. (a) Pathway of biosynthesis of threonine and isoleucine in higher plants (simplified from Mifflin & Lea, 1977). Activation or inhibition of threonine deaminase (3) is indicated by the dashed lines.

(b) Pathway of biosynthesis of the aromatic amino acids in higher plants (Mifflin & Lea, 1977): (1) anthranilate synthase; (2) chorismate mutase (several isoenzymes).

investigated alga *Chlamydomonas reinhardtii*. A recent study (Kirk & Kirk, 1978) in *Chlamydomonas* as in *Volvox* showed that both algae possess a specific carrier for arginine transport but lack detectable carriers for other amino acids; this led the authors to postulate that such selectivity might account for the failure to recover auxotrophs for amino acids other than arginine. *Scenedesmus obliquus* could have a different amino acid uptake system but it is puzzling that here also 11 of the 15 amino acid mutants required arginine. Whatever the explanation, the new amino acid mutants isolated and others that might be isolated in the future could constitute useful tools for studying the control of amino acid synthesis in photosynthetic organisms.

Dr Max Mergeay is acknowledged for his interesting discussion of the manuscript. We thank Miss Vandeloise for technical assistance. One of us (C.V.) is a bursar of I.R.S.I.A. (Belgium).

REFERENCES

- BACHMANN, B. J., LOW, K. B. & TAYLOR, A. L. (1976). Recalibrated linkage map of *Escherichia coli* K-12. *Bacteriological Reviews* **40**, 116-167.
- FINCHAM, J. R. S., DAY, P. R. & RADFORD, A. (1979). *Fungal Genetics*, 4th edn. Oxford: Blackwell Scientific Publications.
- GUARDIOLA, J. (1977). Requirements of the newly recognized *ilvJ* gene for the expression of valine transaminase activity in *E. coli* K-12. *Molecular and General Genetics* **158**, 157-164.
- HOLLIDAY, R. (1956). A new method for the identification of biochemical mutants in microorganisms. *Nature, London* **178**, 987.
- KIRK, D. L. & KIRK, M. M. (1978). Carrier-mediated uptake of arginine and urea by *Chlamydomonas reinhardtii*. *Plant Physiology* **61**, 556-560.
- LI, S. L., RÉDEI, G. P. & GOWANS, C. S. (1967). A phylogenetic comparison of mutation spectra. *Molecular and General Genetics* **100**, 77-83.
- LOPPES, R. (1969). A new class of arginine requiring mutants in *Chlamydomonas reinhardtii*. *Molecular and General Genetics* **104**, 172-177.
- LOPPES, R. (1970). Selection of arginine-requiring mutants in *Chlamydomonas reinhardtii* after treatment with three mutagens. *Experientia* **26**, 660-661.
- MAHANTY, H. K. (1979). Kanamycin-resistant *Scenedesmus obliquus* showing simultaneous resistance to other antibiotics and polychlorinated biphenyls. *Nature, London* **277**, 562-563.
- MIFLIN, B. J. & LEA, P. J. (1977). Amino acid metabolism. *Annual Review of Plant Physiology* **28**, 299-329.
- POWLS, R. & BRITTON, G. (1977). A series of mutant strains of *Scenedesmus obliquus* with abnormal carotenoid compositions. *Archives of Microbiology* **113**, 275-280.
- TRAINOR, F. R. & ROSKOWSKY, F. G. (1967). Control of unicell formation in a soil *Scenedesmus*. *Canadian Journal of Botany* **45**, 1657-1664.