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Altered Methionyl-tRNA Synthetase in a *Spirulina platensis* Mutant Resistant to Ethionine

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Compared with the parental strain, a *Spirulina platensis* mutant that is resistant to ethionine incorporated methionine into protein at a reduced rate, whereas ethionine incorporation was practically nil. The methionyl-tRNA synthetase present in crude extracts from the resistant strain showed a reduced affinity for methionine and ethionine.

In a previous communication, we reported the isolation and partial characterization of *Spirulina platensis* mutants that are resistant to different amino acid analogs (10). A number of those mutants appeared to overproduce amino acids, whereas others did not. Subsequent in vivo studies indicated that two mutants resistant to ethionine, an analog of methionine, were probably characterized by different modes of resistance (9). One such mutant, ET7, presumably carries a mutation affecting the mechanisms regulating the biosynthesis of amino acids. Indeed, as compared with the wild-type strain, this mutant overproduces methionine and other amino acids and does not take up significant quantities of methionine from the medium. On the other hand, mutant ET17 grows at the same rate as the parental strain and does not overproduce methionine but shows a reduction in the amount of amino acids incorporated into the protein.

We report here evidence that the mutation responsible for ethionine resistance in strain ET17 most probably involves an altered methionyl-tRNA synthetase (EC 6.1.1.10).

The wild-type strain, the analog-resistant mutants, and the growth conditions have already been described (10). The uptake and incorporation into protein of radioactive L-[^{35}S]methionine (New England Nuclear Corp., Boston, Mass.) or L-[*ethyl*-^{14}C]ethionine (Sorin Biomedica, Saluggia, Italy) were measured by a method already reported (9). Briefly, 2-ml cultures from cells grown to the mid-log phase were collected by centrifugation at room temperature and resuspended in the same volume of fresh medium. To each suspension, 0.5 μCi of radioactive methionine or ethionine (adjusted to a specific activity of 44 mCi/mmol) was added, and the suspension was incubated in the light with agitation. At different intervals, 0.1-ml samples were withdrawn to measure the amount of amino acids taken up by the cells or incorporated into protein (9). Crude extracts to be assayed for the activity of aminoacyl-tRNA synthetase were prepared essentially as previously reported (2). Cells were collected by filtration, washed with 0.02 M Tris-hydrochloride (pH 7.8)–0.05 M magnesium acetate–0.02 M 2-mercaptoethanol–0.01 M KCl–40 μg of spermine per ml–0.1 mM reduced gluthatione–0.1 mM GTP (extraction buffer), and, if not used immediately, stored frozen at −20°C. Cell-free extracts were made by grinding the cells with 1 volume of sand in a prechilled mortar, followed by extraction with 1 volume of extraction buffer. After centrifugation to remove unbroken cells, sand, and cellular debris (30 min at 30,000 × *g*), the supernatant was centrifuged again for 180 min at 100,000 × *g*. The proteins in the supernatant were precipitated with solid ammonium sulfate (0 to 80% saturation at 0°C). The precipitated proteins were recovered by centrifugation (15 min at 20,000 × *g*) and dissolved in a few milliliters of extraction buffer. After dialyzing once each hour for 3 h against 300 ml of extraction buffer containing 10% of the original magnesium acetate concentration, the enzyme preparation was recovered and stored at −20°C. Protein content was determined spectrophotometrically (6).

As depicted in Fig. 1B and 1E, mutant ET7 did not take up exogenously supplied methionine or ethionine, most probably because it has a large intracellular pool of methionine (10). The mutant, therefore, is resistant to the analog because uptake of the analog is reduced to negligible amounts (less than 2.5% of the amount taken up by the parental strain [Fig. 1A and 1D]). In contrast, mutant ET17 did take up methionine and ethionine at rates comparable to those observed for the wild-type strain, but the incorporation of methionine into protein was reduced, and the incorporation of ethionine into

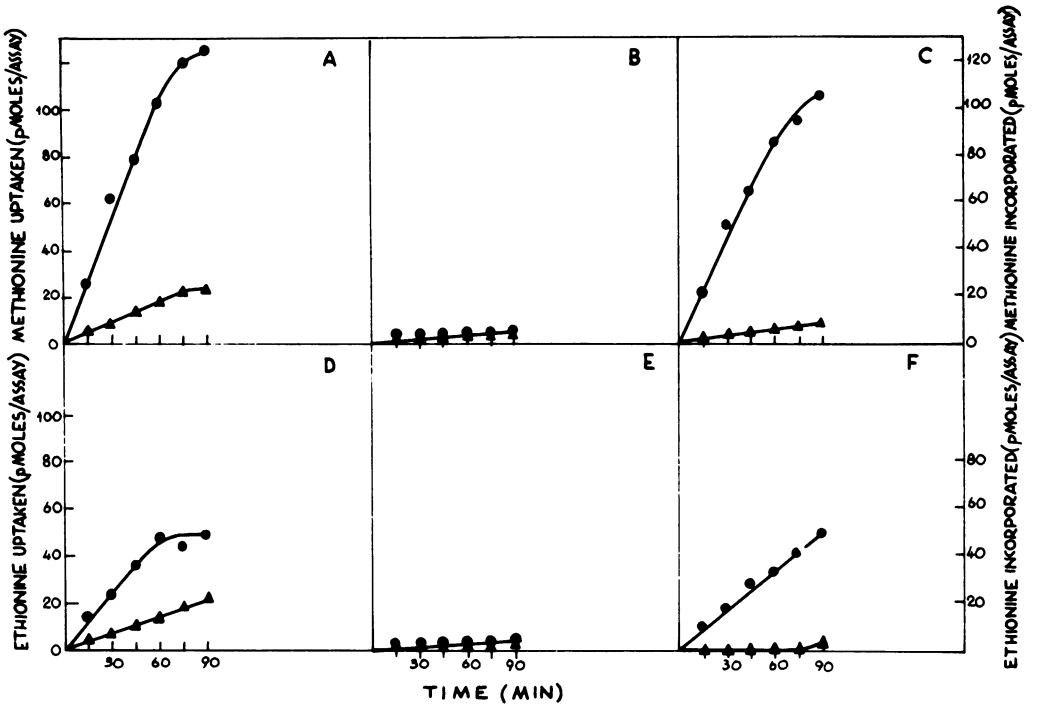

FIG. 1. Uptake (●) and incorporation (▲) of methionine and ethionine by the parental strain of *S. platensis* and by two ethionine-resistant mutants. Uptake and incorporation of methionine by the wild type (A), mutant ET7 (B), and mutant ET17 (C); uptake and incorporation of ethionine by the wild type (D), mutant ET7 (E), and mutant ET17 (F). Uptake and incorporation of methionine or ethionine were measured as described in the text.

protein was practically negligible (Fig. 1C and 1F). Therefore, this mutant must either selectively inactivate the analog or possess a discriminatory mechanism at the level of the incorporation of the analog into protein. The latter possibility seems more probable since it has already been reported that microorganisms such as *Salmonella typhimurium* (1), *Escherichia coli* (1), *Coprinus lagopus* (7), and *Neurospora crassa* (5) become resistant to ethionine by a mutation in methionyl-tRNA synthetase. The most common mutations in aminoacyl-tRNA synthetase involve the amino-acid-binding site (11). The altered enzymes have, in general, a reduced affinity for the analogs, resulting in a decrease in the incorporation of the analogs into protein. Such an assumption was verified in experiments measuring the affinity for methionine and ethionine of the methionyl-tRNA synthetase present in crude extracts of the wild-type strain and of the two mutants (Fig. 2). The enzyme from the parental strain and that from mutant ET7 seemed to have the same affinity in the transfer of methionine to *E. coli* tRNA and a somewhat decreased affinity for ethionine. On the other hand, as compared with the enzyme from the parental strain, the enzyme from mutant ET17 had decreased affinity for methionine

TABLE 1. Incorporation data and kinetic parameters for aminoacyl-tRNA synthetases in the wild-type and in two mutant strains

Strain	Apparent K_m[a] (μM) of:			(pmol) Amino acid incorporated per 10^6 cells in 1 h		
	Methionine	Ethionine	Phenylalanine	Methionine	Ethionine	Phenylalanine[b]
Parent	3.30	3.90	0.90	15	13	6
ET7	3.30	3.90	0.90	1	1	1
ET17	8.20	16.6	0.90	6	0	6

[a] Values reported are the average of at least two determinations with different enzyme preparations.
[b] Values calculated from the data reported by Riccardi et al. (9).

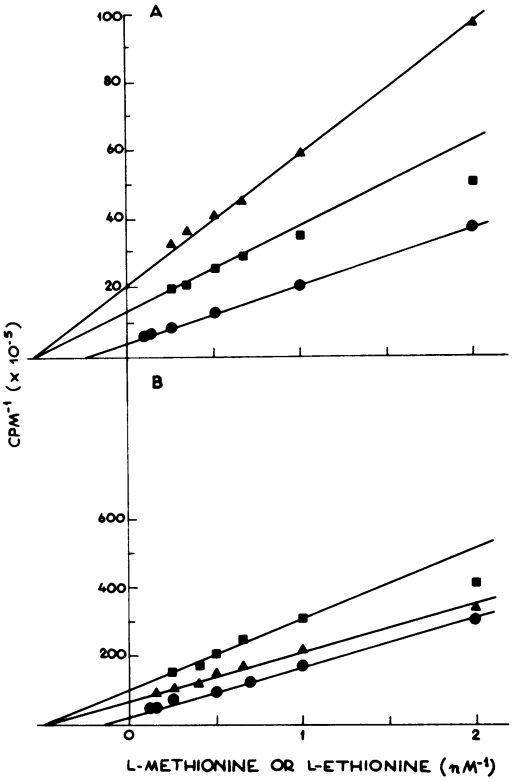

FIG. 2. Lineweaver-Burk plots for determination of K_m values for methionine (A) and ethionine (B) with crude extracts from wild-type *S. platensis* (■) mutant ET7 (▲), and mutant ET17 (●). The assay was performed in 500-μl reaction mixtures containing 200 mM Tris-hydrochloride buffer (pH 7.8), 20 mM $MgCl_2$, 20 mM ATP, 1,400 μg of unfractioned tRNA from *E. coli* (Boehringer Mannheim Corp., New York, N.Y.), various concentrations of labeled methionine or ethionine, 0.04 mM concentrations of 19 unlabeled amino acids (excluding methionine), and an enzyme fraction corresponding to 600 μg of protein. After incubation for 20 min at 37°C, the reaction was stopped with an equal volume of a 20% solution (wt/vol) of perchloric acid and kept in the cold for 10 min. The precipitate was collected by filtration (filters from Schleicher & Schuell Co., Keene, N.H.) filters and washed with two 2-ml volumes of 10% perchloric acid. The filters were dried with ethanol, and radioactivity was measured in a liquid scintillation counter (Packard Instrument Co., Inc., Rockville, Md.).

(ca. 2.5-fold) and somewhat more decreased affinity for ethionine. The specificity of the mutation was assessed by demonstrating that the apparent K_m for an unrelated amino acid, phenylalanine, was the same for the three strains (data not shown).

The results (Table 1) demonstrated for the first time the existence in cyanobacteria of mutants that have alterations in the mechanisms responsible for the incorporation of amino acids into protein. Thus, cyanobacteria may become resistant to amino acid analogs not only by alterations in the mechanisms controlling the production of amino acids (3, 4, 8, 10) or cell permeability (9) but also by mutations in specific aminoacyl-tRNA synthetases.

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