Absence of Accumulation of ppGpp and RNA during Amino Acid Starvation in Rhizobium meliloti*

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Lack of three different amino acids or treatment with the analogue DL-serine hydroxamate does not induce the accumulation of ppGpp and ppGpp, the 3′-pyrophosphates of GDP and GTP, respectively, in Rhizobium meliloti strain 41. Surprisingly, RNA accumulation is controlled under the above mentioned conditions stringently. Moreover, no significant RNA accumulation was found during chloramphenicol, tetracycline, and streptomycin treatment, suggesting that R. meliloti, unlike any other bacteria investigated so far, is not able to accumulate RNA without ongoing protein synthesis. On the other hand, lack of carbon source and ammonium starvation result in a significant ppGpp accumulation.

In wild type bacteria, amino acid starvation induces the accumulation of two unusual guanosine polyphosphates, ppGpp and ppGpp, respectively. This is accompanied by the reorientation of the whole cellular metabolism, termed the stringent response (1). Some of the metabolic processes, like ribosome synthesis, glucose uptake, etc. are inhibited, while others, such as protein breakdown, are stimulated under this condition. This pleiotropic control is governed by the product of the relA gene (2, 3).

We have recently isolated relA mutants of Klebsiella pneumoniae and shown that these mutants behave very similarly to their Escherichia coli counterparts (4). In addition, they are defective in nitrogen fixation after exhaustion of ammonium from the culture medium (5). Klebsiella is able to fix nitrogen in its free living state, while Rhizobia are known to convert dinitrogen to ammonia in symbiosis with higher plants and thus contribute more significantly to this process in nature (6). Therefore, our aim was to investigate whether ppGpp is positively involved in symbiotic nitrogen fixation as well. As the first step of this study, the stringent response was investigated in a genetically well characterized Rhizobium strain, R. meliloti 41.

RESULTS AND DISCUSSION

In order to trigger stringent response, amino acid starvation was induced by removing the required amino acid from the culture medium. Fig. 1 shows that during phenylalanine deprivation both protein and RNA accumulations were almost completely inhibited. Surprisingly, no (pp)ppGpp accumulation was detected under this condition (Fig. 2). Similarly, no (pp)ppGpp accumulation was found during histidine (strain AK74) and arginine (AK753) starvation in either of the respective auxotrophs, although both protein and RNA accumulation showed a typical stringent pattern (data not shown); for the isolation, characterization, and map position of the auxotrophic mutants, see Refs. 13, 14, and 15). The same results were obtained when amino acid starvation was induced by exhaustion of the limiting amount of required amino acids from the culture medium (data not shown). Therefore, the absence of ppGpp accumulation and the stringent RNA control during amino acid deprivation cannot be due to any possible artifacts caused by filtration and washing. Similar results were obtained when the wild type prototrophic strain was treated with the serine analogue DL-serine hydroxamate (1 mg x ml⁻¹; Sigma), a compound known to be a potent inhibitor of seryl-tRNA synthetase (7).

More surprisingly, very small or no RNA accumulation was found when either the mutants or the wild type R. meliloti was treated with the translation inhibitors chloramphenicol (Fig. 3) or tetracycline and streptomycin. As expected, no ppGpp pool expansion was detected during the above mentioned treatments; however, the nucleoside triphosphate contents increased under these conditions (data not shown).

On the other hand, Rhizobium was able to accumulate ppGpp during carbon source starvation (Figs. 2 and 4). Similarly, high ppGpp accumulation was found during ammonium deprivation, too (data not shown). This starvation was accompanied by a significant decrease in ATP and GTP contents (Fig. 4). Again, no RNA accumulation was found under either of these two conditions.

Wild type bacteria are known to accumulate ppGpp and ppGpp during amino acid starvation or under aminoacyl-tRNA limitations (1). Only one exception has been found to this general observation so far, i.e. Caulobacter crescentus did not respond with guanosine polyphosphate accumulation to amino acid starvation (8). In this respect, R. meliloti behaves in a way similar to Caulobacter, since our results clearly show that in Rhizobium neither lack of three different amino acids nor serine hydroxamate treatment triggers ppGpp accumulation. On the other hand, the two organisms respond differently to carbon source downshift. While only a moderate, 2-3-fold increase is detected in the ppGpp content of C. crescentus (8), a much higher, 10-20-fold, expansion of the ppGpp pool can be found in R. meliloti under this condition. However, the most dramatic difference becomes apparent between the two organisms when the RNA accumulation is analyzed during amino acid starvation. C. crescentus continued to accumulate RNA under this condition similar to relaxed mutants of E. coli or of other microorganisms; thus, it can be considered as a relaxed strain by its nature (8). On the other hand, R. meliloti was able to restrict its RNA synthesis in a stringent-like manner during amino acid starvation (Fig. 1).

Moreover, unlike any other bacteria investigated so far, no significant RNA accumulation was detected during chloramphenicol treatment in R. meliloti (Fig. 3). Thus, it appears that R. meliloti has developed a regulatory system which prevents net RNA synthesis during protein synthesis inhibi-
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Fig. 1. Effect of phenylalanine starvation on protein and RNA accumulation in R. meliloti 41 phe (strain GY43). Cells were grown at 30°C in GTS minimal medium (13), containing 50 μg x ml⁻¹ of L-phenylalanine as a necessary supplement. The culture in the logarithmic phase (Aₘ₀ = 0.2-0.3) was filtered and washed twice on a Sartorius membrane filter (pore size, 0.45 μ). The cells were then resuspended in the same prewarmed medium containing (○) or not containing phenylalanine (●). Simultaneously with the resuspension, they were double labeled with 4,5-[^3]H-leucine (Amersham) and 2-[^14]C-uracil (final specific activities were 4 pCi x 5 μg⁻¹ x ml⁻¹ and 0.25 pCi x 3 μg⁻¹ x ml⁻¹, respectively). The incorporated radioactivity was determined as described earlier (4).

Fig. 2. Autoradiograms of 32P-labeled cold formic acid extracts of R. meliloti after thin layer chromatography on polyethyleneimine cellulose. Bacteria were grown in GTS medium containing 3 x 10⁻³ M inorganic phosphate. The culture was labeled with 100 μCi x ml⁻¹ of 32P (carrier-free; Isotope Institute, Budapest) for one generation time to equilibrate the pool and then starved either for phenylalanine (A) or carbon source (B) by filtration, washing, and resuspension in prewarmed medium containing the same specific activity of 32P. At appropriate time intervals, samples were removed and processed as described (10).

Fig. 3. Effect of chloramphenicol treatment on RNA accumulation in R. meliloti 41. A GTS-grown culture in the logarithmic phase was double labeled and distributed into two parts. One of them was treated with chloramphenicol (○, 100 μg x ml⁻¹), while the other one was not (●). For simplicity, only the uracil incorporation is shown; the protein synthesis was inhibited by this concentration of chloramphenicol by more than 95%. For experimental details, see Fig. 1.

Fig. 4. Effect of carbon source starvation on ATP, GTP, and ppGpp pools in R. meliloti 41. Bacteria were grown, labeled with 32P, and starved for carbon source as described in Fig. 2. ATP (○), GTP (△, ▲), and ppGpp (□, ■) pools were measured and calculated as described earlier (9, 10). Open symbols, control culture; closed symbols, carbon source-starved culture.
ppGpp degradation, too (10). On the other hand, carbon source starvation enhances the ppGpp content by inhibiting its degradation without increasing the rate of its synthesis (11). Similarly, any other treatments which intervene with energy production stabilize ppGpp and thus result in significant expansion of its pool size (12). Our results clearly show that in Rhizobium ppGpp accumulation can be triggered only by the second type of treatment. Similar to E. coli, a fall in the ATP and GTP content can be detected during the above mentioned downshifts. However, the rapid accumulation of ppGpp under these conditions (Fig. 4) suggests that, in addition to stabilization, the rate of ppGpp production should be increased, too.

Since ppGpp is positively involved in nitrogen fixation in K. pneumoniae, one may ask whether the unusual ppGpp metabolism of R. meliloti has some significance in the inability of this organism to fix nitrogen in the free living state. Similarly, the question remains to be answered whether other strains of Rhizobia, some of which are able to fix nitrogen without symbiosis with higher plants, as well as the closely related Agrobacterium exhibit similar ppGpp metabolic pattern as R. meliloti.

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