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Adenylosuccinate lyase of *Bacillus subtilis* regulates the activity of the glutamyl-tRNA synthetase

(*purB*/adenylosuccinate AMP-lyase/glutamate-tRNA ligase)

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ABSTRACT **In *Bacillus subtilis*, the glutamyl-tRNA synthetase [L-glutamate:tRNA^{Glu} ligase (AMP-forming), EC 6.1.1.17] is copurified with a polypeptide of M_r 46,000 that influences its affinity for its substrates and increases its thermostability. The gene encoding this regulatory factor was cloned with the aid of a 41-mer oligonucleotide probe corresponding to the amino acid sequence of an NH_2-terminal segment of this factor. The nucleotide sequence of this gene and the physical map of the 1475-base-pair fragment on which it was cloned are identical to those of *purB*, which encodes the adenylosuccinate lyase (adenylosuccinate AMP-lyase, EC 4.3.2.2), an enzyme involved in the *de novo* synthesis of purines. This gene complements the *purB* mutation of *Escherichia coli* JK268, and its presence on a multicopy plasmid behind the *trc* promoter in the *purB*$^-$ strain gives an adenylosuccinate lyase level comparable to that in wild-type *B. subtilis*. A complex between the adenylosuccinate lyase and the glutamyl-tRNA synthetase was detected by centrifugation on a density gradient. The interaction between these enzymes may play a role in the coordination of purine metabolism and protein biosynthesis.**

As catalysts of the first step of protein biosynthesis, the aminoacyl-tRNA synthetases occupy a strategic position in a process that consumes about half of all the energy used for polymerization reactions in living cells (1).

Several aminoacyl-tRNA synthetases are known to play another function in addition to the catalysis of tRNA aminoacylation: for instance, in *Escherichia coli*, the threonyl-tRNA synthetase and the alanyl-tRNA synthetase are, respectively, translational and transcriptional repressors for their own structural genes (2, 3), whereas in mitochondria of *Saccharomyces cerevisiae* and *Neurospora crassa*, respectively, the tyrosyl- and the leucyl-tRNA synthetases play a role in RNA splicing (4–6). The possibility that several other aminoacyl-tRNA synthetases are endowed with additional functions is suggested by the extensive structural diversity among the members of this family (7) and by the presence of several aminoacyl-tRNA synthetases specific for the same amino acid in various prokaryotic organisms [two lysyl-tRNA synthetases in *E. coli* (8) and two threonyl-tRNA synthetases in *Bacillus subtilis* (9)]. Additional functions of aminoacyl-tRNA synthetases could include the coordination of protein biosynthesis with other metabolic pathways.

In *B. subtilis*, the glutamyl-tRNA synthetase [GluRS; L-glutamate:tRNA^{Glu} ligase (AMP-forming), EC 6.1.1.17] is copurified with a polypeptide of M_r 46,000 that increases its affinity about 10-fold for glutamate and for ATP and stabilizes it against heat inactivation (10). This regulatory factor does not react with anti-GluRS gamma globulins, but it is precipitated by them together with the GluRS from a crude cell extract (10). We report here the cloning and analysis of the gene encoding the regulatory factor of the *B. subtilis* GluRS and its identification as *purB*, encoding the adenylosuccinate lyase (ASL; adenylosuccinate AMP-lyase, EC 4.3.2.2).

Table 1. Bacterial strains used

Strain	Genotype	Ref.
E. coli DH5α	φ80d*lacZ*ΔM15, *endA1*, *recA1*, *hsdR17* (R_{K-}, M_{K+}), *supE44*, *thi-1*, λ$^-$, *gyrA*, *relA1*, F$^-$, Δ(*lacZYA-argF*) U169	11
E. coli JK268	*F*$^-$, *trpE61*, *trpA62*, *tna-5*, *purB58*, λ$^-$	12
E. coli JM101	*supE thi-1* Δ(*lac-proAB*) F'[*traD36 proAB*$^+$ *lacI*^{q} *lacZ*ΔM15]	13
B. subtilis 1A1*	*trpC2*	14

MATERIALS AND METHODS

B. subtilis GluRS was purified as described by Proulx *et al.* (10). The bacterial strains used are described in Table 1. *B. subtilis* 1A1 (previously named 168T) was grown in APT medium (15), which efficiently prevents sporulation. Chromosomal DNA was isolated as described (16). Oligonucleotide synthesis and labeling, Southern blot hybridization, DNA cloning, and nucleotide sequence analysis were performed as described (13, 16). Computer analyses were conducted with the programs of the Genetics Computer Group (Madison, WI) (17).

Liquid-Phase Amino Acid Sequence Analysis. In automated liquid-phase sequence analysis, a Beckman 890C sequencer was used with 10 nmol of the protein, and sequential Edman degradation was performed with the use of a 0.5 M-Quadrol program (Beckman program 111978). The phenylthiohydantoin derivatives of amino acids were identified by HPLC on a Varian Vista-56 liquid chromatograph with an IBM-cyano column (4.5 mm × 25 cm) (IBM, Meriden, CT) maintained at 37°C in 5% (vol/vol) tetrahydrofuran in 30 mM sodium acetate buffer (pH 5.1) and eluted with a 0–80% linear gradient of acetonitrile, constituted from equal volumes of this buffer and of 100% acetonitrile.

Enzyme Assays. ASL activity was measured as described by Woodward (18) from the rate of decrease of the absorbance at 280 nm of a solution containing 0.1 M Tris·HCl (pH 7.5) and 0.1 mM adenylosuccinic acid at 20°C. One unit of ASL catalyzes the cleavage of 1 μmol of adenylosuccinic acid per min under the above-mentioned conditions. The GluRS was assayed as described (19). One unit of GluRS catalyzes

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Abbreviations: ASL, adenylosuccinate lyase; GluRS, glutamyl-tRNA synthetase.

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NH2-TERMINAL   1M  I  E  R  K  S  R  P  E  M  S  A12

               13I  W  T  D  E  N  R  F  Q  A  W  L  E  V  E  I  L  A30
PROBE-β        ATt TGG ACg GAt GAa AAt cGg TTt CAg GCa TGG cTg GAa GT
               ::: ::: ::: ::: ::: ::  :  ::: ::  ::: ::: :  ::  ::
purB           ATT TGG ACG GAT GAA AAC AGA TTT CAA GCA TGG TTA GAG GT
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FIG. 1. Amino acid sequence of an NH_2-terminal segment of the regulatory factor of the *B. subtilis* GluRS, and structure of the oligonucleotides used to clone its gene. This probe "β" has 83% identity with the stretch of *B. subtilis* DNA encoding the segment from Ile-13 to Val-26 of the ASL (20). Lowercase letters are used for nucleotides that we chose among several possibilities on the basis of the codon usage typical of moderately expressed genes in *B. subtilis* (see *Results*).

the formation of 1 nmol of glutamyl-tRNA in 5 min at 30°C (10).

Ultracentrifugation on Density Gradient. The 0.2-ml enzyme solutions were layered on 5–25% sucrose gradients containing 0.1 M Tris·HCl, pH 8.0/0.05 M KCl/10 mM 2-mercaptoethanol in 4.4-ml tubes. The tubes were centrifuged at 60,000 rpm in a SW 60 T_i rotor in a Beckman ultracentrifuge model L5-65 for 10 hr at 4°C. Fractions of 0.25 ml were collected.

RESULTS

Design of a Probe for Cloning the Gene Encoding the Regulatory Factor of the *B. subtilis* GluRS. The regulatory factor of the *B. subtilis* GluRS was purified as described (10) and submitted to sequential Edman degradation. The sequence of the first 30 residues from its NH_2-terminal end is shown in Fig. 1. Considering that the amount of this factor was present in an amount similar to that of the GluRS (10) and that some *B. subtilis* genes encoding aminoacyl-tRNA synthetases (9, 21) have a codon usage typical of moderately expressed genes in this microorganism (22, 23), we chose these codons to reverse-translate the 14-residue segment from Ile-13 to Val-26, which contains two tryptophans and six residues corresponding to two codon sets, to design a single long oligonucleotide probe, "β" (Fig. 1), likely to hybridize to the gene encoding this factor (24).

Cloning of the Gene Encoding the Regulatory Factor. A Southern transfer of *B. subtilis* chromosomal DNA samples digested by various restriction endonucleases and submitted to electrophoresis on agarose gel was hybridized with the ^{32}P-labeled probe β (Fig. 2). In each digest, a single strong signal was obtained, indicating specific hybridization to a single gene on the *B. subtilis* chromosome. The *Hin*dIII fragments of about 1.5 kilobase pairs (kbp), including that hybridizing with the probe, were ligated into the corresponding site of pBSM13+. After transformation of *E. coli* DH5α with this mixture of recombinant plasmids and growth in the presence of ampicillin, isopropyl β-D-thiogalactopyranoside and 5-bromo-4-chloro-3-indolyl β-D-galactoside, recombinant plasmids from 100 white colonies were isolated. Three of them hybridized with probe β, and one of them, pLQB981, was characterized (see below).

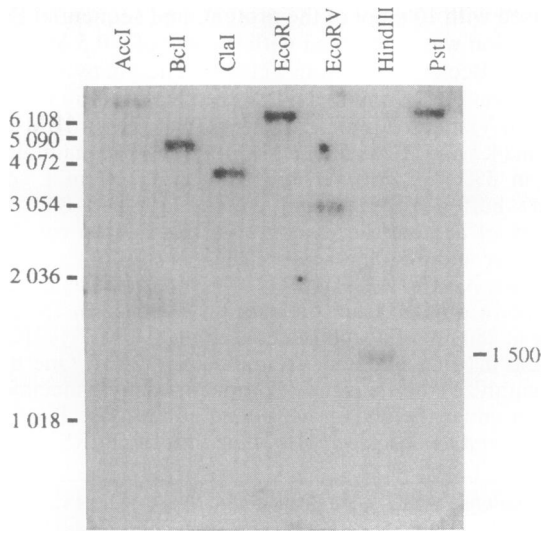

FIG. 2. Autoradiogram of a Southern transfer of various digests of *B. subtilis* DNA hybridized with probe β.

The *B. subtilis* GluRS Regulatory Factor Is the ASL. The nucleotide sequence of a part of the pLQB981 insert was determined and compared with the nucleic acid data banks. It is identical with a part of the *B. subtilis pur* operon (20), including the end of *purK* and the first 120 nucleotides of *purB* (Fig. 3). The sequence of 30 residues determined from the NH_2 terminus of the regulatory factor of the *B. subtilis* GluRS (Fig. 1) is identical to that deduced by Ebbole and Zalkin (20) for the NH_2 terminus of the adenylosuccinate lyase encoded by *purB*. The physical map of the *B. subtilis pur* operon shows that the sizes of *B. subtilis* chromosomal DNA fragments that should hybridize with probe β are respectively 1475, 3299, 4145, and 5428 bp for *Hin*dIII, *Eco*RV, *Cla* I, and *Bcl* I digests (20), in agreement with the hybridization pattern shown in Fig. 2. Moreover, the two *Hin*cII sites found on the *Hin*dIII insert of pLQB981 correspond to those of *purB* (Fig. 3).

The *Hin*dIII insert of pLQB981 was transferred into the corresponding site of the expression vector pTrc99A (25). One resulting vector, pLQB9915, containing the insert in the proper orientation for transcription from the *trc* promoter was used to transform the *purB* strain *E. coli* JK268. This strain does not grow on minimal medium in the absence of adenosine, whereas the transformants carrying pLQB9915 do. Moreover, cell extracts of this strain contain no detect-

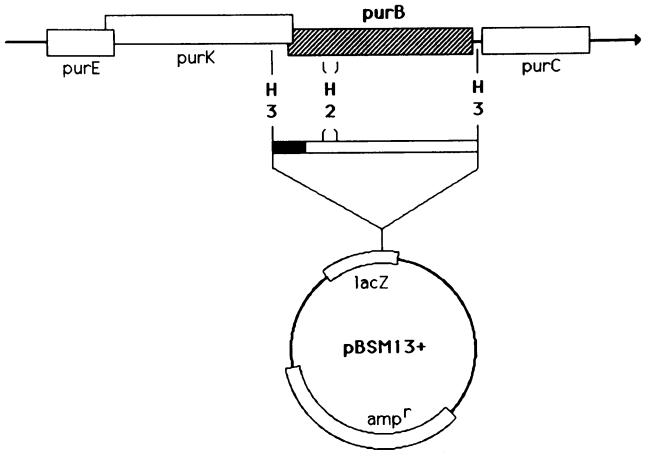

FIG. 3. Comparison of the physical maps of a segment of the *B. subtilis pur* operon (20), including all *purB* and a part of *purK*, with that of the 1.5-kbp *Hin*dIII (H3) insert of pLQB981 isolated from a transformant that hybridizes strongly with probe β. This insert is in the *Hin*dIII site of vector pBSM13+. The segment of this insert, whose nucleotide sequence was determined, is shown in black and is identical to the 3′ end of *purK* and 5′ end of *purB*. Two *Hin*cII (H2) sites observed by physical mapping of this insert correspond to those present in *purB*. amp^r, Ampicillin resistance gene.

able level of ASL, but those of the same strain transformed with pLQB9915 contain an ASL level (62 units/mg of protein) comparable to those of *B. subtilis* 1A1 grown in minimal medium (77 units/mg of protein).

The GluRS and its regulatory factor purified together from *B. subtilis* were separated by phosphocellulose chromatography as described (10). The ASL activity was eluted after the GluRS (Fig. 4) at the same position as previously shown for the regulatory factor (10). The regulatory factor obtained by this procedure is pure, since it was possible to determine the sequence of its first 30 residues from the NH_2 terminus (Fig. 1). Therefore, we conclude that the same polypeptide is endowed with ASL activity and the property of regulating GluRS activity.

In the presence of ASL, the sedimentation rate of the GluRS is increased (corresponding to a 15% increase of its sedimentation coefficient), and the width of its peak is increased (Fig. 5), indicating that these two enzymes rapidly associate and dissociate under these conditions. The existence of such a complex was previously indicated by the protection of the GluRS by its regulatory factor against heat inactivation and by the coprecipitation of these two proteins from a crude cell extract by anti-GluRS gamma globulins (10).

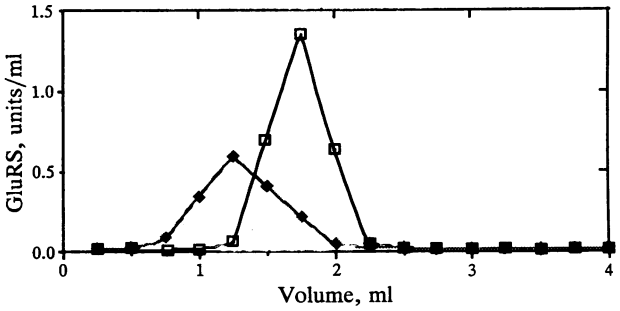

FIG. 5. Detection of a complex between the GluRS and the ASL by sedimentation on a density gradient. In one of the tubes (◆), a mixture of 10 mg of GluRS and 10 mg of ASL was layered on the gradient, whereas the control tube (□) contained 20 mg of GluRS.

DISCUSSION

The presence of a polypeptide copurified with the GluRS and able to influence its stability and activity *in vitro* was initially observed in *E. coli* (26, 27). Several attempts at repeating this copurification yielded pure monomeric GluRS (28, 29), possibly because the interaction between these proteins depends on yet unknown factors not controlled during the purification. In *B. subtilis*, a polypeptide of similar size and endowed with the same regulatory properties was found to copurify routinely with GluRS (10). The identity of its N-terminal sequence with that predicted from the *purB* gene (Fig. 1), the agreement between the nucleotide sequence of a part of the gene cloned by using an oligonucleotide designed from an NH_2-terminal segment, the presence of ASL activity in the GluRS regulatory factor purified to homogeneity, the complementation by pLQB9915 of the Pur^- phenotype of *E. coli* JK268 (*purB*), and the presence of a high level of ASL activity in extracts of *E. coli* JK268/pLQB9915 show that ASL interacts with GluRS and influences its activity *in vitro*. The existence of an interaction between these two enzymes was directly detected (Fig. 5). The relative weakness of this GluRS–ASL complex corresponds to that previously suggested by the width of the stained protein band after native gel electrophoresis of complexes between GluRS and its regulatory factor from both *B. subtilis* (10) and *E. coli* (26). The previously reported immunoprecipitation of such a complex from a crude *B. subtilis* extract (10), where the concentrations of these enzymes are high, is compatible with the strength of the GluRS–ASL interaction observed *in vitro* (Fig. 5).

This interaction may mediate a coordination of purine metabolism and protein biosynthesis. Considering that tRNA aminoacylation generates a substantial amount of AMP and that one of the two steps catalyzed by ASL is the *de novo* formation of AMP and fumaric acid from adenylosuccinic acid, the interaction of these two proteins may regulate *de novo* AMP biosynthesis. If this model is correct, would the GluRS be the only aminoacyl-tRNA synthetase involved in this process? There are hints that another aminoacyl-tRNA synthetase interacts with ASL. Indeed, in *E. coli*, a polypeptide of M_r 46,000 was found copurifying with the glutaminyl-tRNA synthetase (30), which has a close evolutionary linkage with several GluRSs (16).

Finally, in view of the medical importance of the enzymes involved in *de novo* purine nucleotide biosynthesis (31), illustrated for example by the apparent ASL deficiency in several severely affected autistic children (32, 33), it is important to understand better the physiological importance of the GluRS·ASL interaction and to identify the parameters that modulate it.

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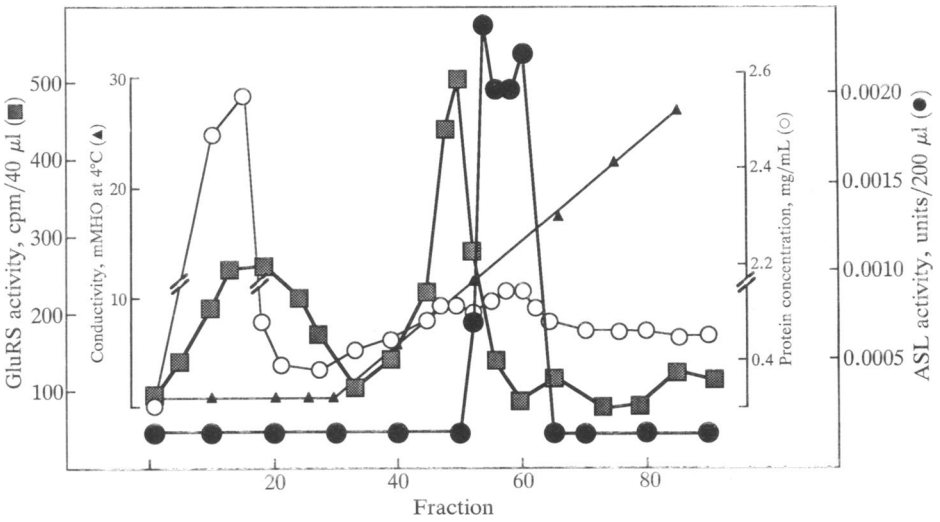

FIG. 4. Separation of the GluRS and its regulatory factor by phosphocellulose chromatography and identification of the latter as ASL.

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