Overproduction of the Bacillus subtilis glutamyl-tRNA synthetase in its host and its toxicity to Escherichia coli

Martin Pelchat, Lucille Lacoste, Fu Yang, and Jacques Lapointe

Abstract: The Bacillus subtilis glutamyl-tRNA synthetase (GluRS), encoded by the gltX gene, aminoacylates its homologous tRNA^{Glu} and tRNA^{Gln} with glutamate. This gene was cloned with its σ^{70} promoter and a downstream region including a ρ-independent terminator in the shuttle vector pRB394 for Escherichia coli and B. subtilis. Transformation of B. subtilis with this recombinant plasmid (pMP411) led to a 30-fold increase of glutamyl-tRNA synthetase specific activity in crude extracts. Transformation of E. coli with this plasmid gave no recombinants, but transformation with plasmids bearing an altered gltX was successful. These results indicate that the presence of B. subtilis glutamyl-tRNA synthetase is lethal for E. coli, probably because this enzyme glutamylates tRNA^{Gln}_{1} in vivo as it does in vitro.

Key words: glutamyl-tRNA synthetase overproduction, Bacillus subtilis, toxicity, Escherichia coli.

Résumé : La glutamyl-ARNt synthétase (GluRS) de Bacillus subtilis, codée par le gène gltX, aminoacyle l’ARNtGlu et l’ARNtGln avec le glutamate. Ce gène a été cloné avec son promoteur σ^{70} et une région en amont incluant un terminator ρ-indépendant dans le vecteur navette pRB394 pour Escherichia coli et B. subtilis. La transformation de B. subtilis avec ce plasmide recombinant (pMP411) mène à une augmentation de 30 fois de l’activité spécifique en glutamyl-ARNt synthétase dans l’extrait cellulaire. La transformation d’E. coli avec ce plasmide ne donne aucun recombinant, cependant sa transformation avec des plasmides incluant le gène gltX altéré permet la croissance. Ces résultats montrent que la présence de la glutamyl-ARNt synthétase de B. subtilis est létale pour E. coli, probablement à cause de la glutamylation de l’ARNtGln_{1} in vivo, comme observé in vitro.

Mots clés : surproduction de la glutamyl-ARNt synthétase, Bacillus subtilis, toxicité, Escherichia coli.

Aminoacyl-tRNA synthetases (aaRS) play an important role in protein biosynthesis by catalyzing the accurate charging of tRNAs with amino acids. There are significant functional similarities among several aaRSs of Bacillus species and the corresponding ones in E. coli, as indicated by several examples of complementation by Bacillus synthetases of E. coli hosts whose corresponding synthetase is temperature-sensitive (Barker 1982; Brakhage et al. 1989; Brand and Fersht 1986; Gagnon et al. 1994; Jones et al. 1986). One of the most significant differences among aaRSs of Gram-positive bacteria and those of E. coli is the lack, in the former, of an enzyme capable of charging a tRNA with glutamine (Schön et al. 1988; Wilcox and Nirenberg 1968; Wilcox 1969a, 1969b). In those organisms tRNA^{Gln} is first charged with glutamate, which is subsequently converted to glutamine by an amidotransferase while it is attached to tRNA. In Bacillus subtilis, a single glutamyl-tRNA synthetase (GluRS) is responsible for aminoacylating both tRNA^{Glu} and tRNA^{Gln} with glutamate (Lapointe et al. 1986). In vitro this enzyme can efficiently attach glutamate to E. coli tRNA_{1}^{Gln} but not to E. coli tRNA_{2}^{Glu} or tRNA^{Glu}_{1}.

As previous attempts at cloning the B. subtilis gltX gene (encoding GluRS) on a single DNA fragment in E. coli were unsuccessful (Breton 1990), it was cloned on two fragments (Breton et al. 1990). On the first (pLQB205), the gltX promoter is immediately followed by the 5′-terminal part of gltX. On the second (pLQB206), the 3′-terminal part is followed by the genes cysE (encoding the serine acetyltransferase) and the beginning of cysS (encoding the cysteinyl-tRNA synthetase). Owing to low transformation frequencies using B. subtilis as the primary host, we conducted the initial cloning step with an E. coli – B. subtilis shuttle vector (pRB394; Brückner 1992) in E. coli and then transferred the recombinant plasmid for the last step to B. subtilis. We began by forming plasmids pMP205 and pMP206 by transferring the inserts of pLQB205 and pLQB206 in the vector pRB394 and pHM4, respectively. By combining fragments of pMP205 and pMP206, we constructed pMP211, which contains the gltX gene interrupted by the 1-kb SacII segment shared by pMP206 and pMP205 (Fig. 1). Stable transformants of E. coli DH5α carrying pMP211 were obtained. By deleting this SacII fragment, plasmid pMP411 containing the intact gltX gene, its σ^{70} promoter with a 77-bp region upstream from the –35 hexamer, and a 1.3-kbp downstream region, including a ρ-independent terminator, was constructed in vitro (Fig. 1) and was used to transform B. subtilis 168p.

SDS–PAGE analysis of the clear supernatant of a cell extract of B. subtilis 168p carrying pMP411 (about 100 copies/cell, as...
measured by dot blot hybridization) reveals the overproduction of a polypeptide of about 55 kDa, which is not overproduced by the same cells in the absence of pMP411 (Fig. 2). To confirm that this polypeptide is B. subtilis GluRS, whose molecular mass is 55.7 kDa (Breton et al. 1990), we measured GluRS aminoacylation activity (Lin et al. 1992) of both extracts and found a 30-fold higher specific activity in cells car-
Table 1. Purification of the \textit{B. subtilis} glutamyl-tRNA synthetase from an overproducing strain.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume of fraction (mL)</th>
<th>Concentration (mg/mL)</th>
<th>Total amount (mg)</th>
<th>Specific activity (units/mg)</th>
<th>Total amount (units)*</th>
<th>Yield (%)</th>
<th>Purification factor (n-fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell extract</td>
<td>35</td>
<td>53</td>
<td>1,855</td>
<td>14</td>
<td>26,600</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>Two-phase system</td>
<td>39</td>
<td>15</td>
<td>585</td>
<td>12</td>
<td>7,300</td>
<td>27</td>
<td>0.9</td>
</tr>
<tr>
<td>Q-sepharose</td>
<td>20</td>
<td>2</td>
<td>40</td>
<td>153</td>
<td>6,100</td>
<td>23</td>
<td>10.9</td>
</tr>
<tr>
<td>Blue-sepharose</td>
<td>10</td>
<td>3</td>
<td>30</td>
<td>93</td>
<td>2,800</td>
<td>10.5</td>
<td>6.7</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>10</td>
<td>2</td>
<td>20</td>
<td>160</td>
<td>3,200</td>
<td>12</td>
<td>11.4</td>
</tr>
</tbody>
</table>

Note: The enzyme was purified from 11.6 g (wet weight) of \textit{B. subtilis} 168(pMP411) cells harvested during exponential growth in Luria–Bertani medium containing 5 \( \mu \)g chloramphenicol/mL.

*One unit of GluRS catalyzes the formation of 1 nmol of Glu-tRNA/min at 37°C under the conditions described by Lin et al. (1992).

Fig. 4. SDS–PAGE analysis of the fractions enriched for GluRS at various steps of its purification from \textit{B. subtilis} 168p(pMP411). Lane A, 12 \( \mu \)g of \textit{E. coli} GluRS; lane B, 400 \( \mu \)g of \textit{B. subtilis} 168p(pMP411) cell extract; lane C, 35 \( \mu \)g of the Q-Sepharose fraction; lane D, 25 \( \mu \)g of the Blue-Sepharose fraction; lane E, 12 \( \mu \)g of the hydroxyapatite fraction; lane F, 12 \( \mu \)g of \textit{E. coli} GluRS.

rning pMP411. As SDS–PAGE revealed the presence of a large amount of a polypeptide of about 55 kDa in the pellet obtained by centrifugation of the crude extract of \textit{B. subtilis} 168p(pMP411) but not in that from \textit{B. subtilis} 168p (data not shown), we measured the amount of soluble and insoluble overproduced GluRS protein in the crude extract, the clear supernatant, and the resuspended cell debris by Western blot analysis and microdensitometry, using anti-\textit{B. subtilis} GluRS antiserum. This analysis shows that the cell debris contained twice as much GluRS protein as the soluble supernatant (Fig. 3), but this protein has no aminocyaylation activity and is not soluble in the absence of detergent, apparently because it is in inclusion bodies. We concluded that the total overproduction of GluRS is about 100-fold, as the cell debris in inclusion bodies contained about two-thirds of the total GluRS present in cell extracts. Attempts to lower the proportion of inclusion bodies by lowering or elevating the growth temperature were unsuccessful. Furthermore, as pMP411 is present at about 100 copies/cell in \textit{B. subtilis} 168p, we concluded that the observed GluRS overproduction reflects \textit{gltX} gene dosage and suggests that \textit{B. subtilis} GluRS does not regulate its own biosynthesis. We also found that the 30-fold increase in GluRS specific activity does not reduce the growth rate of \textit{B. subtilis}, since these cells transformed with pM205 (which does not contain an intact \textit{gltX}) grow at the same rate as those transformed with pMP411 (data not shown). We purified the overproduced GluRS as described by Proulx and Lapointe (1985) with an additional step, a chromatography on hydroxyapatite. Table 1 summarizes the purification of GluRS from \textit{B. subtilis} 168p(pMP411). By this procedure, we obtained 15 mg of enzyme from 13 g of wet cells, with a 50-fold purification. This GluRS was pure to homogeneity as shown by SDS–PAGE (Fig. 4). It has a similar specific activity, in the aminocyaylation reaction, as that of the enzyme purified from a wild-type strain (Proulx et al. 1983; Proulx and Lapointe 1985).

To determine if the previous failure at cloning the complete \textit{gltX} gene of \textit{B. subtilis} in \textit{E. coli} was due to the toxicity of \textit{B. subtilis} GluRS to \textit{E. coli}, we deleted a part of the \textit{gltX} gene carried by pMP411, without destroying its reading frame; the resulting plasmid, pMP400, lacks the 105-bp EcoRV–SacII segment encoding the \({\text{91L}}^{–125}\text{R}\) region of GluRS, which is a substantial part of the tRNA acceptor stem binding domain (For alignments of this region with \textit{Thermus thermophilus} GluRS and \textit{E. coli} GlnRS, whose three-dimensional structures are known (Nureki et al. 1995; Rould et al. 1989), see Liu et al. (1995).) By transforming \textit{E. coli} DH5\(\alpha\) with pMP400, pMP211, and pMP411, we found about 10\(^5\) transformants/\(\mu\)g DNA with pMP400 and pMP211 (containing, respectively, a deletion or an insertion in the \textit{gltX} gene), but none with pMP411 (containing an intact \textit{gltX} gene), which proves the toxicity of this \textit{B. subtilis} protein to \textit{E. coli}. We used thermosensitive \textit{E. coli} JP1449 (Russell and Pittard 1971; Lapointe and Delcuve 1975), whose altered \textit{gltX} gene encoded a GluRS whose activity was undetectable in cell extracts, to test the activity of the product of the above-mentioned altered \textit{gltX} gene. Extracts of JP1449 transformed with pMP400 or pMP211 had no detectable GluRS activity (results not shown).

Furthermore, no \textit{E. coli} transformants were obtained with plasmids carrying \textit{B. subtilis} \textit{gltX} under the control of the following promoters, in the absence of an inducer: (i) the \textit{trc} promoter repressed by a high level of lac repressor owing to the presence of the \textit{lacI}\(^q\) gene (Amann et al. 1988), (ii) the \(\lambda\text{p}_{21}\) promoters repressed by the temperature-sensitive repressor \(\lambda\text{i}857\), and (iii) a T7 RNA polymerase promoter repressed by the T7 lysozyme (Studier et al. 1990).

The \textit{B. subtilis} GluRS aminocyalates with glutamate both tRNA\textit{Glu} and tRNA\textit{Gln} in \textit{B. subtilis} (Lapointe et al. 1986); the resulting mischarged Glu-tRNA\textit{Gln} is then transformed into the correct Glu-tRNA\textit{Gln} by a specific amidotransferase using...
ATP and a donor of amino group such as glutamine or NH3 (Wilcox and Nirenberg 1968). Because E. coli does not contain such an amidotransferase, Glu-tRNA\textsubscript{Gln} may lead to the production of altered proteins. The observed lethality of the B. subtilis GluRS to E. coli is then probably due to the misacylation of tRNA\textsubscript{Gln} of E. coli with glutamate, as observed in vitro (Lapointe et al. 1986). Cells that synthesize Gln-tRNA\textsubscript{Gln} via a mischarged Glu-tRNA\textsubscript{Gln} intermediate could avoid the misincorporation of glutamate into proteins by a direct transfer of Glu-tRNA\textsubscript{Gln} from GluRS to the specific amidotransferase or by having an elongation factor EF-Tu, which does not recognize the mischarged tRNA\textsubscript{Gln} as is the case for misacylated tRNA\textsubscript{Se} (reviewed by Baron and Böck 1995). The second strategy appears to have been chosen by chloroplasts of 	extit{Pisum sativum}, as its misacylated Glu-tRNA\textsubscript{Gln} does not interact in vitro with its homologous EF-Tu (Stanzel et al. 1994). The fact that 	extit{B. subtilis} 168p(pMP411), which overproduces GluRS, grows normally is consistent with this model.

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**References**


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