A dual-specific Glu-tRNA^{Gln} and Asp-tRNA^{Asn} amidotransferase is involved in decoding glutamine and asparagine codons in *Acidithiobacillus ferrooxidans*

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Abstract The *gatC*, *gatA* and *gatB* genes encoding the three subunits of glutamyl-tRNA^{Gln} amidotransferase from *Acidithiobacillus ferrooxidans*, an acidophilic bacterium used in bioleaching of minerals, have been cloned and expressed in *Escherichia coli*. As in *Bacillus subtilis* the three *gat* genes are organized in an operon-like structure in *A. ferrooxidans*. The heterologously overexpressed enzyme converts Glu-tRNA^{Gln} to Gln-tRNA^{Gln} and Asp-tRNA^{Asn} to Asn-tRNA^{Asn}. Biochemical analysis revealed that neither glutaminyl-tRNA synthetase nor asparaginyl-tRNA synthetase is present in *A. ferrooxidans*, but that glutamyl-tRNA synthetase and aspartyl-tRNA synthetase enzymes are present in the organism. These data suggest that the transamidation pathway is responsible for the formation of Gln-tRNA and Asn-tRNA in *A. ferrooxidans*.

Key words: tRNA-dependent amidation; Heterotrimeric amidotransferase; Acidophilic bacterium

1. Introduction.

Correct formation of aminoacyl-tRNA (AA-tRNA) is crucial for faithful decoding of messenger RNA and thus for faithful translation of the genetic information. Two pathways are known for the formation of Gln-tRNA and Asn-tRNA: the direct aminoacylation of tRNA with the glutaminyl- or asparaginyl-tRNA synthetase, and the indirect transamidation pathway in which the mischarged Glu-tRNA^{Gln} and Asp-tRNA^{Asn} are converted to their cognate AA-tRNA species by a tRNA-dependent amidotransferase [1]. This pathway has been shown to be present in a wide array of organisms [2]. The mischarging process requires the presence of non-discriminating aminoacyl RNA synthases for glutamate (GluRS) and aspartate (AspRS) [3]. Recently, it has been shown that bacterial amidotransferases have both Glu-tRNA^{Gln} and Asp-tRNA^{Asn} amidotransferase activities [4,5]. The absence in the genome of the genes encoding the enzymes for the direct pathway (i.e. GlnRS or AsnRS) is another indication of the function of amidotransferase in the organism under study.

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The *Bacillus subtilis* Glu-tRNA^{Gln} amidotransferase (Glu-AdT) has been shown to be a heterotrimer encoded by the *gatC*, *gatA* and *gatB* genes organized in the *gatCAB* operon [6]. These genes have been found in the genome of almost all microorganisms. Here we report that *Acidithiobacillus ferrooxidans*, an acidophilic bacterium that participates in bioleaching of minerals, possesses the genes encoding AdT, which are organized in a *gatCAB* operon as in *B. subtilis*. Genomic and biochemical analysis revealed that *A. ferrooxidans* AdT can amidate both Glu-tRNA^{Gln} and Asp-tRNA^{Asn} and that this is the only route to the formation of amide AA-tRNAs.

2. Materials and methods

2.1. Bacterial cultures

A. ferrooxidans ATCC 19859 was cultured in Mackintosh medium [7]. *Escherichia coli* BL21 DE3, the recipient of cloned DNA, was cultured in Luria–Bertani medium supplemented with ampicillin 0.1 mg/ml.

2.2. DNA cloning

Primers (gatCF1 5′-CACCCACACCCGGTGAAGCCT-3′, *gatC*F2 5′-CTATGGCGGCGCCATCAAAGT-3′) were designed for PCR based on the preliminary sequence of *A. ferrooxidans gatCAB* from the Institute of Genome Research (TIGR) Microbial Database. A 3554-bp PCR product was amplified from *A. ferrooxidans* chromosomal DNA and cloned in the *Eco*RV restriction site of pT7 Blue 3 (Novagen), generating the recombinant plasmid pAAf. The cloned DNA was purified by standard protocols [8]. Automatic sequencing (Centro de Síntesis y Análisis de Biomoléculas, Universidad de Chile) was used to confirm the identity and sequence of the genes.

2.3. Formation of misacylated Glu-tRNA^{Gln} and Asn-tRNA^{Asn}

B. subtilis tRNA^{Gln} was misacylated with [^{14}C]glutamate as described by Curnow et al. [6]. *Chlamydia trachomatis* tRNA^{Asn} cloned and overexpressed in *E. coli* was misacylated with [^{14}C]aspartic acid (G. Razcniak, unpublished results).

2.4. Assay of AdT activity

This assay was carried out essentially as described by Curnow et al. [6]. Either [^{14}C]Glu-tRNA^{Gln} or [^{14}C]Asp-tRNA^{Asn} were substrates for AdT obtained from *A. ferrooxidans gatCAB* overexpressed in *E. coli* or from an *A. ferrooxidans* S-100 fraction. The extracts were prepared essentially as described by Salazar et al. [9]. *B. subtilis* AdT was used as positive control [6].

2.5. Aminoacylation activity

GluRS, GlnRS, AspRS and AsnRS activities were assayed as described by Salazar et al. [9]. 25 μM ^{14}C-labeled amino acids (210, 213, 228 and 260 mCi/mmol for Gln, Asp, Asn and Glu, respectively),

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10 mM ATP and 8 mg/ml of total tRNA from *E. coli* (Sigma) or 1.6 mg/ml total tRNA from *A. ferrooxidans* were used as substrates in a 50 μl reaction. At the desired time, aliquots of the reaction mixture were spotted on Whatman filter papers, precipitated with cold 5% trichloroacetic acid and dried. Acid-precipitable radioactivity (AA-tRNA) was measured by scintillation counting.

3. Results

3.1. A. ferrooxidans AdT genes

The predicted protein sequences of *B. subtilis gatC*, *A* and *B* genes were used for a similarity search of the *A. ferrooxidans* genome database (TIGR). The search revealed the presence of all three genes organized in an operon-like arrangement similar to that present in the *B. subtilis* genome (Fig. 1). The predicted GatC, GatA and GatB proteins are, respectively, 95, 490 and 476 amino acids long. TblastN searches revealed that the *A. ferrooxidans gat* gene products are most similar to those of *Pseudomonas putida* (overall similarity of 68%, 70%, and 68% for *gatC*, *gatA* and *gatB* respectively). Upstream of the *gatC* gene is *vacJ* (encoding a lipoprotein precursor); only two nucleotides separate the coding regions of both genes. A putative bacterial transcriptional promoter (5′-CTGCAGAAAGATGGAGATTTCTGGCGTATCGTCA-3′, −35 and −10 are underlined) was identified upstream of the coding region of *vacJ*. No rho-independent transcription terminator was found as far as 500 nucleotides downstream of the 3′-terminus of *gatB*. These findings suggest that *A. ferrooxidans gatCAB* is expressed from a complex transcription unit.

Based on the DNA sequence, oligonucleotide PCR primers were designed to amplify the entire operon. The 3554 bp amplification product was cloned and expressed in *E. coli*. The sequence of the genes was confirmed by DNA sequence analysis.

3.2. Activity of A. ferrooxidans AdT

The activity of cloned *gatCAB* genes from *A. ferrooxidans* was tested by its ability to transform mischarged *B. subtilis* [^{14}C]Glu-tRNA^{Gln} into Gln-tRNA^{Gln} or *C. trachomatis* [^{14}C]Asp-tRNA^{Asn} into Asn-tRNA^{Asn}. The conversion of both Glu-tRNA^{Gln} (Fig. 2A, lane 2) and Asp-tRNA^{Asn} (Fig. 2B, lane 6) was carried out by *A. ferrooxidans* AdT overexpressed in *E. coli*. The amidation of Glu-tRNA^{Gln} was also detected with the *A. ferrooxidans* S-100 enzyme preparation (Fig. 2A, lane 4). Under these conditions, amidation of Asp-tRNA^{Asn} was not detected with the *A. ferrooxidans* S-100 (data not shown). Mixtures of ammonium and asparagine or ammonium and glutamine were used as ammonium donor for Glu-tRNA^{Gln} or Asp-tRNA^{Asn} AdT activities respectively. Thus, *A. ferrooxidans gatCAB* encodes a functional dual-specificity AdT able to form Asn-tRNA and Gln-tRNA.

3.3. Biochemical context of A. ferrooxidans AdT

As the *A. ferrooxidans* AdT is able to transform both Glu-tRNA^{Gln} and Asp-tRNA^{Asn} into the correctly charged tRNAs, we addressed the question: what is the function of the enzyme in vivo in *A. ferrooxidans*? A Blast similarity search of the TIGR *A. ferrooxidans* genome database revealed no sequence matching either GlnRS or AsnRS from *E. coli*. This result might indicate that the genes encoding these proteins are not present in the genome; alternatively the genes might not be sequenced yet. To test the hypothesis that *A. ferrooxidans* lack the genes encoding GlnRS and AsnRS, aminoacylation analysis was carried out. Extracts from *A. ferrooxidans* and *E. coli* were tested for the ability to directly acylate total tRNA from either *E. coli* or *A. ferrooxidans* with Gln and Asn. As seen in Fig. 3, the *A. ferrooxidans* extract was unable to charge total tRNA from either *E. coli* (Fig. 3A) or *A. ferrooxidans* (Fig. 3C) with Gln. In addition, charging of *E. coli* tRNA with Asn was not detected when an *A. ferrooxidans* S-100 preparation was used as the source of enzyme (Fig. 3B). However, total *A. ferrooxidans* tRNA was aminoacylated with Glu and Asp when *A. ferrooxidans* extract was used as the enzyme source (data not shown). The dual specificity of *A. ferrooxidans* AdT as well as the absence of GlnRS and AsnRS in *A. ferrooxidans* led us to conclude that AdT provides Gln-tRNA^{Gln} and Asn-tRNA^{Asn} for protein biosynthesis by the indirect pathway in *A. ferrooxidans*.

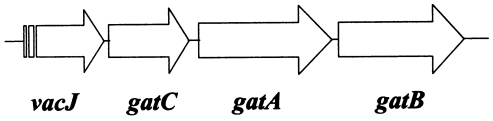

Fig. 1. Schematic representation of *A. ferrooxidans* genes encoding AdT. *GatC* (288 bp), *gatA* (1473 bp) and *gatB* (1437 bp). *VacJ* (839 bp) is also represented.

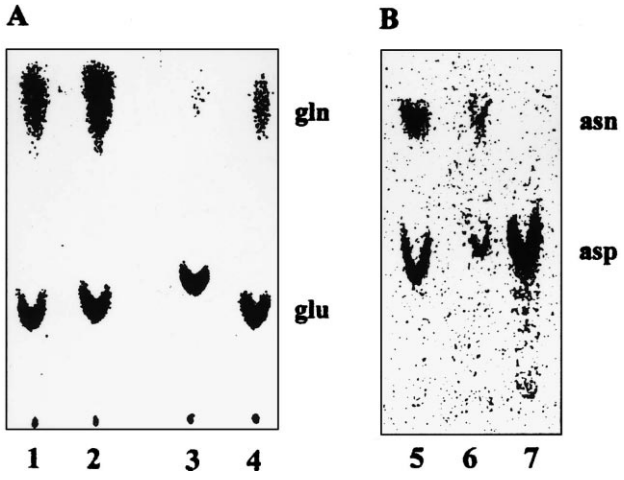

Fig. 2. AdT activity. Phosphorimage of thin-layer chromatographic separation of [^{14}C]glutamine and [^{14}C]glutamic acid (A) or [^{14}C]asparagine and [^{14}C]aspartic acid (B) recovered from transamidation assays in which *B. subtilis* tRNA^{Gln} was aminoacylated by *B. subtilis* GluRS or *C. trachomatis* tRNA^{Asn} was aminoacylated by *C. trachomatis* AspRS respectively. *B. subtilis* AdT (lanes 1 and 5), *E. coli* BL21 DE3 transformed with pAAf containing *A. ferrooxidans gatCAB* (lanes 2 and 6), *E. coli* BL21 DE3 (lanes 3 and 7) and *A. ferrooxidans* S-100 (lane 4) were used as AdT sources.

4. Discussion

tRNA-dependent AdTs are present in all archaea and in most bacteria. The bacterial enzymes are heterotrimeric; the role of each subunit is still unclear. Sequence comparisons suggest that GatA is probably the catalytic subunit of the holoenzyme, since its amino acid sequence contains an amidase signature. The C subunit is not always required for the enzyme function. *B. subtilis* AdT is a heterotrimeric enzyme [6], but *Thermus thermophilus* AdT has been isolated as an active AB dimer [3]. It has been suggested that the C subunit

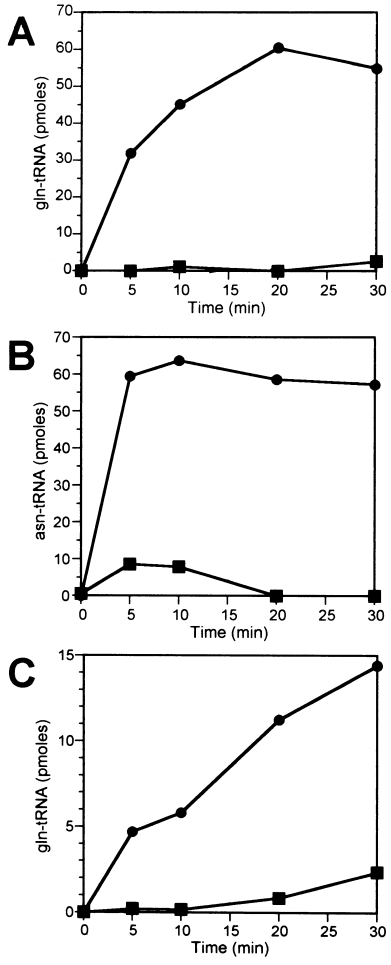

Fig. 3. Direct aminoacylation activities of GlnRS (A, C) and AsnRS (B) of *E. coli* (●) or *A. ferrooxidans* (■) S-100 with total tRNA from *E. coli* (A, B) or *A. ferrooxidans* (C).

is involved in the correct synthesis of the A subunit [6]. No function has been assigned yet to GatB.

At the genomic level, the *B. subtilis* AdT subunit genes are organized as an operon [6]. However, the three *T. thermophilus gat* genes are dispersed in the genome. Whether the role of the C subunit is related to the genomic organization and expression of the other *gat* genes remains to be elucidated. The *A. ferrooxidans* AdT is encoded by a *gatCAB* operon similar to *B. subtilis*. Preliminary data on the purification of the overexpressed AdT indicate that the three subunits are tightly bound to each other.

A. ferrooxidans AdT, as well as the enzymes from *B. subtilis*, *Deinococcus radiodurans* and *T. thermophilus* are able to catalyze in vitro tRNA-dependent amidation of both Glu and Asp [3–5]. This explains the lack of AsnRS and GlnRS in *A. ferrooxidans* and suggests that this dual-specificity Asp/Glu-AdT is responsible for in vivo formation of Gln-tRNA^{Gln} and Asn-tRNA^{Asn}. It will be interesting to determine the reason for the enzyme's selectivity for two tRNAs out of the organism's 40–50 tRNA species. Additional information on tRNA recognition and *gatCAB* gene organization and expression is required to understand the molecular basis and evolution of this pathway.

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