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Expression, purification, and characterization of a new heterotetramer structure of leucyl-tRNA synthetase from *Aquifex aeolicus* in *Escherichia coli*

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Abstract

Aminoacyl-tRNA synthetases are key players in the interpretation of the genetic code. They constitute a textbook example of multi-domain proteins including insertion and terminal functional modules appended to one of the two class-specific active site domains. The non-catalytic domains usually have distinct roles in the aminoacylation reaction. *Aquifex aeolicus* leucyl-tRNA synthetase (LeuRS) is composed of a separated catalytic site and tRNA anticodon-binding site, which would represent one of the closest relics of the primordial aminoacyl-tRNA synthetase. Moreover, the essential catalytic site residues are split into the two different subunits. In all other class-I aminoacyl-tRNA synthetases, those two functional polypeptides are nowadays fused into a single protein chain. In this work, we report the isolation and the characterization, in *Escherichia coli*, of a novel oligomeric form $(\alpha\beta)_2$ for *A. aeolicus* LeuRS, which is present in addition to the $\alpha\beta$ heterodimer. *A. aeolicus* $(\alpha\beta)_2$ LeuRS has been characterized by biochemical and biophysical methods. Native gel electrophoresis, mass spectrometry, analytical ultracentrifugation, and kinetic analysis confirmed that the $(\alpha\beta)_2$ enzyme was a stable and active entity. By mass spectrometry we confirmed that the heterodimer $\alpha\beta$ can bind one $tRNA^{Leu}$ molecule whereas the heterotetramer $(\alpha\beta)_2$ can bind two $tRNA^{Leu}$ molecules. Active site titration and aminoacylation assays showed that two functional active sites are found per heterotetramer, suggesting that this molecular species might exist and be active in vivo. All those data suggest that the existence of the heterotetramer is certainly not an artifact of overexpression in *E. coli*.

Keywords: Leucyl-tRNA synthetase; Quaternary structure

Aminoacyl-tRNA synthetases (AARSs) [1] are a family of RNA-binding proteins which catalyze the attachment of specific amino acids to their cognate tRNA in a crucial step of protein biosynthesis. AARSs are key players in the interpretation of the genetic code by providing the interface between nucleic acid triplets in mRNA and the corresponding amino acids in proteins. Due to their fundamental importance for cell life, AARSs are likely to be one of the most ancient families of enzymes and have been therefore extensively analyzed. In most organisms there are 20 distinct AARSs, each responsible for the correct loading of a tRNA with its corresponding amino acid. Aminoacylation

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[1] *Abbreviations used:* AARS, aminoacyl-tRNA synthetase; ESI, electrospray ionization; ESI-MS, electrospray mass spectrometry; LeuRS, leucyl-tRNA synthetase.

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generally proceeds in a two-step catalytic reaction. The first step, called amino acid activation, leads from amino acids, ATP, and magnesium ions to formation of an intermediate called aminoacyl–adenylate. In the second step, the aminoacyl moiety is transferred to one of the two hydroxyl groups of the 3′-terminal adenosine of the tRNA to form an aminoacyl-tRNA.

Based on sequences homologies, the AARSs are divided into two classes, which correspond to two different architectures of the active site controlling mechanistic features such as ATP conformation, mode of tRNA binding, and region specificity of the transfer reaction (for recent reviews [1–3]). The catalytic core is responsible for both aminoacyl–adenylate formation and the transfer of the amino acid to the 3′ end of the tRNA. Prodigious efforts spanning more than 20 years led to the determination of crystal structures of nearly all AARSs, either in the free state or engaged in complexes with the other partners of the aminoacylation reaction. AARSs constitute nowadays one of the best textbook examples of multi-domain proteins including insertion and terminal functional modules appended to one of the two class-specific active site domains. The non-catalytic domains may have distinct roles in the aminoacylation reaction. In several cases, the specificity of AARSs for their cognate substrates has been analyzed in terms of chemical and physical interactions at the atomic level, revealing a remarkable diversity in the strategies employed for amino acid and tRNA recognition. The specificities of AARSs for their substrates have been shown to be achieved by a variety of ways, including shape complementarity of binding pockets or surfaces, hydrogen bonding, chelation by metals, and hydrolysis of eventually mischarged tRNAs.

However, recent developments in structural biology and exploration of fully sequenced genomes provided many surprises which enlarged the well-known function of AARSs. Novel pathways and enzymes for the synthesis of several aminoacyl-tRNAs have been discovered. Moreover, 'non-aminoacylation' functions of AARSs have been revealed and further characterized (see for recent review [3–5]). Non-canonical and non-catalytic functions of AARSs now include RNA processing and trafficking, apoptosis, rRNA synthesis, angiogenesis, and inflammation. The modular organization of AARSs is thought to be the result of a patchwork assembly of different functional modules during evolution. It has been proposed that ancestral AARSs only contained a catalytic core devoted to the aminoacylation of primitive minihelices [6]. Gradually, as minihelices became L-shaped tRNAs, extra domains were recruited by the catalytic cores to increase efficiency and accuracy of the aminoacylation reaction [6]. At the present time, typical class-I AARSs exhibit all the catalytic elements on a single polypeptide chain. An exception to this scheme was found in the primitive thermophilic bacterium *Aquifex aeolicus*. Phylogenetic analysis places the *A. aeolicus* genus very close to the root in the tree of life, suggesting that it is one of the first bacteria to have diverged from the common ancestral bacterium. The exceptions and deviations found in this organism can therefore be considered as relics from an ancestral metabolic machinery. Genome sequencing revealed that the gene encoding *A. aeolicus* LeuRS was split into two pieces, making this usually monomeric enzyme a heterodimer of αβ type [7–9]. The α subunit of *A. aeolicus* LeuRS (634 amino acids) contains most part of the class-I active site (the so-called Rossmann-fold) and a large insertion domain (often called CP1, connective peptide 1) which is usually involved in the editing activity of LeuRS (Fig. 1). The β subunit of *A. aeolicus* LeuRS (289 amino acids) is responsible for recognition of the anticodon loop of the $tRNA^{Leu}$ substrate. A more striking feature concerns the location of the two conserved

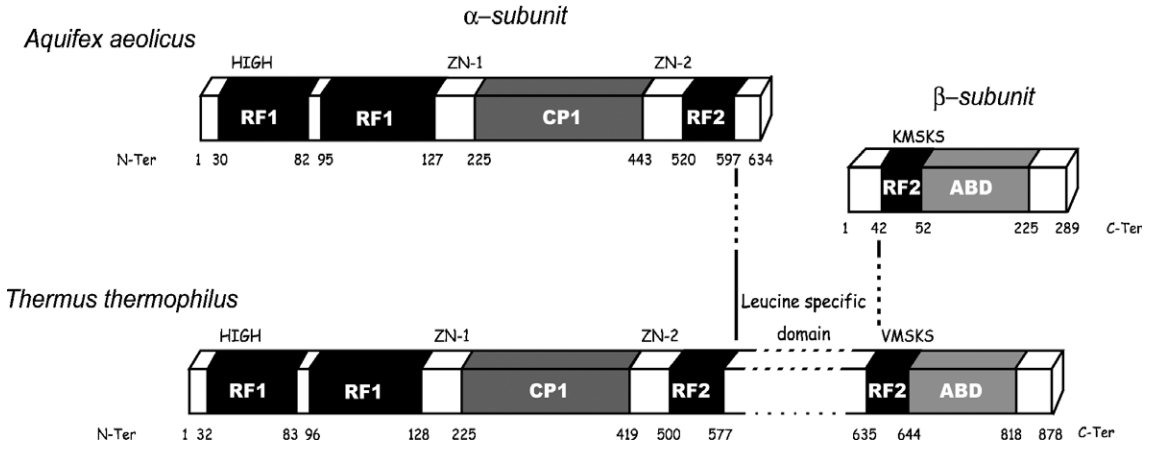

Fig. 1. Schematic diagram of the modular organization of *A. aeolicus* and T. *thermophilus* LeuRS. Four structural defined domains are highlighted: the active site which forms the scaffold of the Rossman fold is composed of two halves RF1 and RF2; the so-called connective peptide 1 (CP1), inserted between RF1 and RF2 and which is usually involved in the editing activity of LeuRS; the so-called "prokaryote leucyl-specific domain" [10]; the anticodon-binding domain (ABD) responsible for the binding of the anticodon loop of the $tRNA^{Leu}$ substrate. The location of the two class-I conserved motifs (HIGH and MSKS) and of the two zinc-binding modules is also indicated. The borders of each domain are indicated for both LeuRS schematic diagrams. The "leucyl-specific domain" is split and is also larger in *A. aeolicus* compared to *T. thermophilus* LeuRS.