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Synthetic and editing reactions of aminoacyl-tRNA synthetases using cognate and non-cognate amino acid substrates

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ABSTRACT

The covalent coupling of cognate amino acid-tRNA pairs by corresponding aminoacyl-tRNA synthetases (aaRS) defines the genetic code and provides aminoacylated tRNAs for ribosomal protein synthesis. Besides the cognate substrate, some non-cognate amino acids may also compete for tRNA aminoacylation. However, their participation in protein synthesis is generally prevented by an aaRS proofreading activity located in the synthetic site and in a separate editing domain. These mechanisms, coupled with the ability of certain aaRSs to discriminate well against non-cognate amino acids in the synthetic reaction alone, define the accuracy of the aminoacylation reaction. aaRS quality control may also act as a gatekeeper for the standard genetic code and prevents infiltration by natural amino acids that are not normally coded for protein biosynthesis. This latter finding has reinforced interest in understanding the principles that govern discrimination against a range of potential non-cognate amino acids. This paper presents an overview of the kinetic assays that have been established for monitoring synthetic and editing reactions with cognate and non-cognate amino acid substrates. Taking into account the peculiarities of non-cognate reactions, the specific controls needed and the dedicated experimental designs are discussed in detail. Kinetic partitioning within the synthetic and editing sites controls the balance between editing and aminoacylation. We describe in detail steady-state and single-turnover approaches for the analysis of synthetic and editing reactions, which ultimately enable mechanisms of amino acid discrimination to be determined.

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Abbreviations: aaRS, aminoacyl-tRNA synthetase; aa-AMP, aminoacyl-adenylate; aa-tRNA, aminoacyl-tRNA; aaRS:aa-AMP, noncovalent complex of aaRS and aa-AMP; aa-Ap, amionoacyl-adenosine-5′-monophosphate; Ap, adenosine-5′-monophosphate; ArgRS, arginyl-tRNA synthetase; GlnRS, glutaminyl-tRNA synthetase; GluRS, glutamyl-tRNA synthetase; His-tRNA^{His}, histidyl-tRNA^{His}; Ile-tRNA^{Ile}, isoleucyl-tRNA^{Ile}; IleRS, isoleucyl-tRNA synthetase; IPPase, inorganic pyrophosphatase; Leu-AMP, leucyl-AMP; Leu-tRNA^{Leu}, leucyl-tRNA^{Leu}; LeuRS, leucyl-tRNA synthetase; LysRS, lysyl-tRNA synthetases; Ni^{2+}-NTA, nickel-nitrilotriacetic acid; PP_i, pyrophosphate; RPC, reverse-phase chromatography; ThrRS, threonyl-tRNA synthetase; TLC, thin-layer chromatography; $tRNA_{ox}$, tRNA with the oxidised vicinal diol of the terminal adenosine; $tRNA_{2'H/3'H}$, tRNA lacking the 2′-OH or 3′-OH at the terminal adenosine; $tRNA_{CC}$, tRNA lacking the terminal adenosine; 3′dtRNA^{Ile}, tRNA^{Ile} lacking the 3′-OH at the terminal adenosine; Val-tRNA^{Ile}, valyl-tRNA^{Ile}; ValRS, valyl-tRNA synthetase.

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1. Introduction

Aminoacyl-tRNA synthetases are the key players in the translation of the genetic code (reviewed in [1,2]). They use ATP to covalently couple amino acids with their cognate tRNAs, assembling aminoacylated tRNAs for ribosomal protein synthesis. Aminoacylation proceeds through two consecutive steps; activation of the amino acid to an aminoacyl-adenylate (aa-AMP) intermediate paves the way for the transfer of the aminoacyl moiety to the tRNA (Fig. 1). The majority of aaRSs can activate amino acids at the expense of ATP in the absence of tRNA, making it possible to kinetically isolate and independently follow the first step of aminoacylation. The exceptions are glutaminyl-, glutamyl-, arginyl- and class I lysyl-tRNA synthetases (GlnRS, GluRS, ArgRS and class I LysRS) that require tRNA for the amino acid activation step. In addition to this, many aaRSs appear to use their tRNA to modulate amino acid recognition and activation [3–7]. The second step of aminoacylation comprises the nucleophilic attack of either the 2′- or 3′-OH group of the terminal adenosine of the tRNA on the carbonyl carbon atom of the aa-AMP intermediate, allowing transfer of the aminoacyl moiety and formation of the aminoacyl-tRNA (aa-tRNA) with a concomitant release of AMP. The kinetics of the aminoacyl transfer step can be tested by mixing an isolated preformed aaRS:aa-AMP complex with tRNA [8]. Both steps take place at the same synthetic active site within the catalytic module of the enzyme. Based on the different topologies of the catalytic domains and characteristic sequence motifs, aaRSs can be divided into two evolutionary distinct classes: class I and class II [9,10].

Besides the cognate amino acid, some aaRSs may activate structurally and/or chemically similar but non-cognate amino acids, and transfer them to tRNA. To limit errors in protein synthesis and consequently cellular lethality [11–14], these enzymes have evolved sophisticated proofreading or editing mechanisms to clear mistakenly activated amino acids and/or erroneously formed aa-tRNAs (Fig. 1; reviewed in [15,16]). The majority of aminoacyl-tRNA synthetases that use proofreading to enhance their fidelity do so by a post-transfer editing mechanism, which involves the rapid hydrolysis of misaminoacylated tRNAs in the domain dedicated to the deacylation activity (Fig. 1 pathways 4 and 5). Post-transfer editing may operate *in cis* and *in trans*; the *in cis* pathway (Fig. 1 pathway 4) accounts for the translocation of the misaminoacylated 3′-end of the tRNA from the synthetic to the distant editing site, while editing *in trans* (Fig. 1 pathway 5) acts on the misacylated tRNA fraction released in solution and includes rebinding to the enzyme and deacylation [17]. Non-cognate aa-AMP intermediates can be also proofread (pre-transfer editing) through several pathways all of which take place in the confines of the synthetic site (Fig. 1, pathways 1–3). In the case of tRNA-independent pre-transfer editing, two distinct mechanisms are recognized: i) hydrolysis of non-cognate aa-AMP in the enzyme active site (Fig. 1, pathway 1) and ii) selective release of non-cognate aa-AMP followed by solution-based hydrolysis (Fig. 1 pathway 2). The former pathway was recognized as the prevalent pre-transfer route in many aaRSs [18–23]. If tRNA stimulates non-cognate aa-AMP hydrolysis, we define this as tRNA-dependent pre-transfer editing (Fig. 1 pathway 3), a rare pathway that is best characterized in case of isoleucyl-tRNA synthetase (IleRS) [3,24,25]. Extensive kinetic analysis showed that kinetic partitioning of the non-cognate aa-AMP between hydrolysis and aminoacyl transfer dictates the partitioning of pre- and post-transfer routes [20,26]. Similarly, the kinetic partitioning of aminoacylated tRNA between hydrolysis and dissociation from the enzyme dictates the contribution of *in cis* and *in trans* post-transfer editing [21].

There is increasing interest in understanding the amino acid discrimination by aaRSs. Besides canonical non-cognate amino acids that were recognized as powerful editing substrates (i.e. valine editing by IleRS) as far back as in 1966 [27], we have recently become aware that non-canonical amino acids, which accumulate under particular stress conditions, may represent at least as important substrates of aaRS proofreading as the canonical ones [28–30]. Furthermore, the characterisation and reengineering of the synthetic and editing pathways may be of interest for synthetic biology and the incorporation of unnatural amino acids into proteins [31]. To achieve a mechanistic understanding of amino acid discrimination, an extensive kinetic methodology in the analysis of the aaRS synthetic and proofreading reactions must be employed using both cognate and non-cognate amino acids. Here we present an overview of kinetic assays established for monitoring synthetic and editing reactions, combined with a detailed description of the steady-state and single-turnover approaches developed in our recent work on three editing class I aaRSs *E. coli* isoleucyl-, leucyl- and valyl-tRNA syntehtases (IleRS, LeuRS and ValRS, respectively; ILV enzymes) [3,20,21,24,25,29]. Two papers have previously been published in *Methods* on topics related to

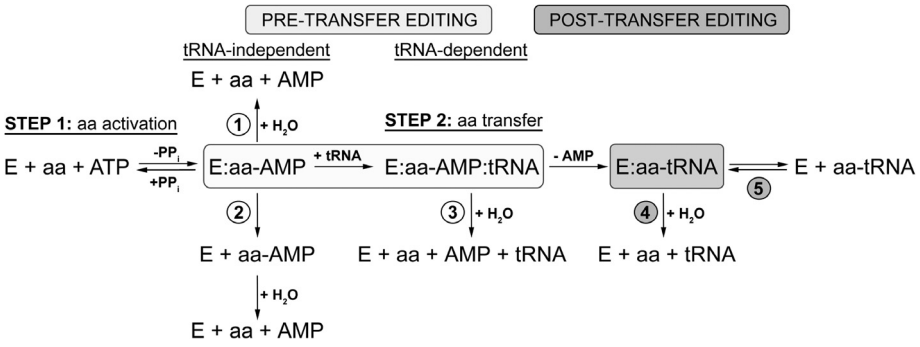

Fig. 1. Schematic presentation of the aaRS editing pathways. Pre-transfer editing occurs within the synthetic site via enhanced dissociation of non-cognate aminoacyl-adenylate (2) or its enzymatic hydrolysis (1, 3) in the absence of tRNA (tRNA-independent, 1) or stimulated by tRNA's presence (tRNA-dependent, 3). Misaminoacylated tRNA can be deacylated through hydrolysis in a post-transfer editing domain (4, 5) directly after the translocation of the misaminoacylated 3′-end of the tRNA's (post-transfer editing *in cis*, 4) or after dissociation and rebinding (post-transfer editing *in trans*, 5).