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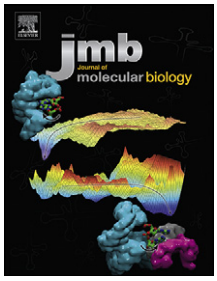

Flipping of the Ribosomal A-Site Adenines Provides a Basis for tRNA Selection

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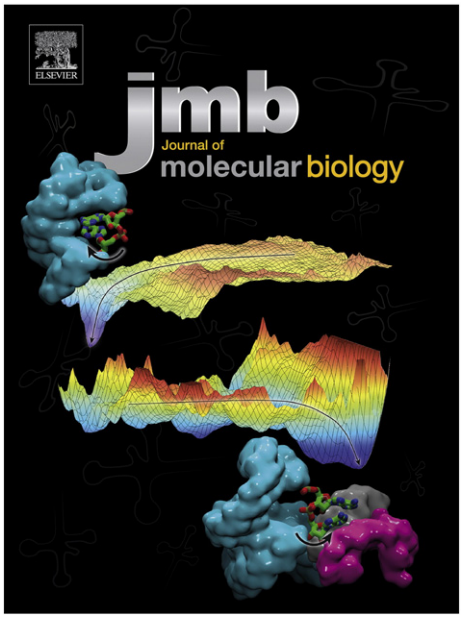
Abstract

Ribosomes control the missense error rate of ~10^{-4} during translation though quantitative contributions of individual mechanistic steps of the conformational changes yet to be fully determined. Biochemical and biophysical studies led to a qualitative tRNA selection model in which ribosomal A-site residues A1492 and A1493 (A1492/3) flip out in response to cognate tRNA binding, promoting the subsequent reactions, but not in the case of near-cognate or non-cognate tRNA. However, this model was recently questioned by X-ray structures revealing conformations of extrahelical A1492/3 and domain closure of the decoding center in both cognate and near-cognate tRNA bound ribosome complexes, suggesting that the non-specific flipping of A1492/3 has no active role in tRNA selection. We explore this question by carrying out molecular dynamics simulations, aided with fluorescence and NMR experiments, to probe the free energy cost of extrahelical flipping of 1492/3 and the strain energy associated with domain conformational change. Our rigorous calculations demonstrate that the A1492/3 flipping is indeed a specific response to the binding of cognate tRNA, contributing 3 kcal/mol to the specificity of tRNA selection. Furthermore, the different A-minor interactions in cognate and near-cognate complexes propagate into the conformational strain and contribute another 4 kcal/mol in domain closure. The recent structure of ribosome with features of extrahelical A1492/3 and closed domain in near-cognate complex is reconciled by possible tautomerization of the wobble base pair in mRNA–tRNA. These results quantitatively rationalize other independent experimental observations and explain the ribosomal discrimination mechanism of selecting cognate *versus* near-cognate tRNA.



Legend: *The free energy landscapes that determine the role of key ribosomal A-site residues A1492/3 in the decoding process were explored for model systems comprising the apo and paromomycin bound A-sites, as well as in the context of the ribosomal decoding center with cognate and near-cognate tRNA bound, using all-atom molecular dynamics simulations. The free energy changes calculated between the different states provide a quantitative description of ribosomal tRNA selection and the mechanistic influence of A-site drug interference on the codon recognition process. The residues A1492/3 were found to actively monitor the binding of the mRNA–tRNA minihelix through specific extrahelical base flipping, selecting cognate tRNA against near-cognate tRNA; however, the binding of paromomycin eliminates the discrimination power of the A-site by stabilizing the states with A1492/3 flipped out, thereby facilitating interactions with both cognate and near-cognate partners in the mRNA–tRNA minihelix.*

Introduction

Ribosomes translate mRNAs and synthesize proteins with high fidelity (error rate of ~10^{-4}) [1,2] and high efficiency (~22 codons per second) [3] in order to sustain cellular functions. Ribosomes achieve 10^{-3} to 10^{-2} missense frequencies in discrimination of near-cognate *versus* cognate tRNA [4–6] during the decoding process, in which quantitative contributions of individual mechanistic steps remain to be fully determined. Biochemical studies together with NMR and X-ray structures of the ribosomal A-site reveal that the extrahelical flipping of the universally conserved adenine nucleobases at the ribosomal A-site, A1492 and A1493 (A1492/3), is a fundamental aspect of codon recognition [7–18]. The prevailing model posits that A1492/3 actively monitor the conformation of the minihelix formed between the mRNA codon and the tRNA anticodon [19–22]. Formation of canonical base pairs between cognate mRNA and tRNA induces extrahelical flipping of A1492/3, which form A-minor interactions with the minor groove of the mRNA–tRNA minihelix, thereby stabilizing the tRNA and promoting GTP hydrolysis through mechanisms that are thought to involve global conformational changes, including 30S domain closure, tRNA distortion, and EF-Tu rearrangement [15,16,22]. By contrast, binding of near-cognate or non-cognate tRNA results in non-Watson–Crick base pairing in the minihelix, which disrupts the A-minor interactions and consequently fails to induce A1492/3 flipping and subsequent GTPase activation [15]. In this manner, near-cognate and non-cognate tRNAs are discriminated against due to a combination of destabilized binding affinity and reduced GTP hydrolysis rate. This model also explains the effects of aminoglycoside antibiotics that bind the ribosomal A-site and induce the extrahelical flipping of A1492/3 and 30S domain closure [12,15,23–25].

However, the above decoding model, and particularly the specificity of A1492/3 flipping, was recently questioned by X-ray structures of the 70S ribosome with cognate and near-cognate tRNAs [26]. For both cognate and near-cognate complexes, A1492/3 were observed in extrahelical conformations, forming A-minor interactions with the minihelix. The mRNA–tRNA minihelix adopts a Watson–Crick-like geometry even for the near-cognate tRNA, possibly through nucleobase tautomerization that allows a G:U mismatch to adopt a Watson–Crick-like base pair [26]. Moreover, both structures also feature 30S domain closure. These structures were interpreted as evidence that the extrahelical flipping of A1492/3 is a non-specific response to tRNA binding and thus has no active function in tRNA selection.

Using all-atom molecular simulations of both oligonucleotide and ribosomal A-site models, as well as fluorescence and NMR experiments, we have computed the energetics of microscopic events during decoding, including the free energy cost of A1492/3 flipping and the strain in domain closure of the decoding center. We demonstrate that the A1492/3 flipping in the ribosomal A-site is indeed a specific response to the binding of cognate tRNA, contributing 3 kcal/mol to the specificity of tRNA selection. In addition, we show that the different A-minor interactions in cognate and near-cognate complexes propagate into the strain of domain closure in the decoding center and contribute another 4 kcal/mol in discriminatory free energy. Furthermore, our thermodynamic integration (TI) calculations characterize the free energies associated with the possible tautomerization of the wobble base pair in mRNA–tRNA minihelix. We find that the Watson–Crick-like conformation of the G:U tautomer is only about 3 kcal/mol higher than the normal wobble pair conformation in the closed form of ribosomal decoding center; thus, the cognate-like conformation of the near-cognate complex is conjectured to be a transient state captured in the crystallographic experiments. We demonstrate the validity of these computed free energy values by building a complete thermodynamic model for the initial selection stage that quantitatively accommodates other independent experimental observations and explains the ribosomal decoding error rate of 10^{-3} to 10^{-2} in selecting cognate *versus* near-cognate tRNA.

Results and Discussion

Intrinsically endothermic A1492/3 flipping in empty A-site

To explore the role of the A1492/3 flipping in tRNA selection, we first examined the intrinsic energetic cost of the A1492/3 flipping. The dynamics and energetics of the A1492/3 flipping in the empty A-site or oligonucleotide analogues were intensively examined in the previous experimental and theoretical studies [10–12,14,27–34], reaching a common agreement that A1492/3 disfavors the fully extrahelical conformation in the absence of tRNA or ligand. However, it is important to calculate the intrinsic free energy cost of the A1492/3 flipping in the empty A-site (apo state) here because this provides self-consistent reference states for comparison with cognate and near-cognate tRNA bound A-sites. An oligonucleotide A-site model [10–12] was employed to capture this intrinsic energetic cost before we advance to the more realistic A-site model in the context of ribosomal decoding center (Fig. S1).

In our free energy calculations, we adapted a progress variable called the center-of-mass pseudo-dihedral angle (CPD) for each adenine to characterize the extrahelical flipping (Fig. S2) [35]. The CPD was found to be an efficient progress variable to characterize similar motions studied in other nucleic