Domain Configuration Defines the Thermodynamic Landscape of Elongation Factor Tu Nucleotide Binding

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Keywords: Translation, Thermodynamics, Molecular Dynamics, Kinetics.

During protein synthesis Elongation Factor (EF) Tu plays a critical role in maintaining translational fidelity. EF-Tu functions as a molecular switch gating, in a nucleotide-dependent manner, the entrance of aminoacyl (aa)-tRNA into the translating ribosome. Correct codon-anticodon interaction stimulates EF-Tu to hydrolyze GTP to GDP and Pi which is followed by a conformational change releasing the bound aa-tRNA. To prevent premature nucleotide exchange on the ribosome EF-Tu has evolved a 60-fold higher affinity for GDP than GTP. To this end we investigate what thermodynamic and structural features of EF-Tu give rise to these differences in nucleotide affinity, investigating how this property of EF-Tu contributes to translational fidelity. Here we report a rapid kinetics analysis using the stopped-flow technique to measure the temperature dependence of the rate constants describing nucleotide association and dissociation. This has allowed us to determine the thermodynamic parameters governing these processes. We find that EF-Tus affinity to GTP and GDP differ mainly due differences in the transition state energy barriers of dissociation and not association. In addition, we observe that formation of the EF-TuGDP complex is enthalpically favored while the EF-TuGTP complex is entropically favored. This is consistent with our previous work reporting that the GTP dissociation is entropically driven. To provide a structural interpretation of the different thermodynamic contributions to nucleotide binding, we utilized Molecular Dynamic simulations of EF-Tu in its respective nucleotide bound forms. From these simulations we have identified a highly dynamic and transient hydrogen-bonding network that spans all domains of EF-Tu as the likely contributor to stabilizing the GDP conformation, whereas differences in water coordination defined by domain arrangement favor the GTP conformation. Our findings show that EF-Tu has evolutionarily modulated both the entropic and enthalpic contributions to the transition state barrier of nucleotide dissociation by modulating domain configuration in order for the fine-tuning of nucleotide-binding affinities.