

Loop and stem dynamics during RNA hairpin folding and unfolding

Sarkar K., Nguyen D.A., Gruebele M.

Department of Chemistry, University of Illinois, 600 South Mathews Ave., Urbana, IL 61801, United States; Department of Physics, University of Illinois, Urbana, IL 61801, United States; Center for Biophysics and Computational Biology, University of Illinois, Urbana, IL 61801, United States; Faculty of Chemistry, Hanoi University of Science, 19 Le Thanh Tong, Hoan Kiem, 10000 Ha Noi, Viet Nam

Abstract: 2-Aminopurine (2AP) is a fluorescent adenine analog that probes mainly base stacking in nucleic acids. We labeled the loop or the stem of the RNA hairpin *gacUACGguc* with 2AP to study folding thermodynamics and kinetics at both loci. Thermal melts and fast laser temperature jumps detected by 2AP fluorescence monitored the stability and folding/unfolding kinetics. The observed thermodynamic and kinetic traces of the stem and loop mutants, though strikingly different at a first glance, can be fitted to the same free-energy landscape. The differences between the two probe locations arise because base stacking decreases upon unfolding in the stem, whereas it increases in the loop. We conclude that 2AP is a conservative adenine substitution for mapping out the contributions of different RNA structural elements to the overall folding process. Molecular dynamics (MD) totaling 0.6 μ sec were performed to look at the conformations populated by the RNA at different temperatures. The combined experimental data, and MD simulations lead us to propose a minimal four-state free-energy landscape for the RNA hairpin. Analysis of this landscape shows that a sequential folding model is a good approximation for the full folding dynamics. The frayed state formed initially from the native state is a heterogeneous ensemble of structures whose stem is frayed either from the end or from the loop. Published by Cold Spring Harbor Laboratory Press. Copyright © 2010 RNA Society.

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Correspondence Address: Sarkar, K.; Department of Chemistry, University of Illinois, 600 South Mathews Ave., Urbana, IL 61801, United States; email: ksarkar2@scs.uiuc.edu

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Authors with affiliations:

- Sarkar, K., Department of Chemistry, University of Illinois, 600 South Mathews Ave., Urbana, IL 61801, United States
- Nguyen, D.A., Department of Chemistry, University of Illinois, 600 South Mathews Ave., Urbana, IL 61801, United States, Faculty of Chemistry, Hanoi University of Science, 19 Le Thanh Tong, Hoan Kiem, 10000 Ha Noi, Viet Nam
- Gruebele, M., Department of Chemistry, University of Illinois, 600 South Mathews Ave., Urbana, IL 61801, United States, Department of Physics, University of Illinois, Urbana, IL 61801, United States, Center for Biophysics and Computational Biology, University of Illinois, Urbana, IL 61801, United States

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