Tying the knot in the tetrahydrofolate (THF) riboswitch: A molecular basis for gene regulation

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Effective gene regulation by the THF riboswitch has shown dependencies on both ligand affinity and the kinetics of ligand association. Moreover, crystal structures of the aptamer in the ligand-bound conformation have revealed two ligand-binding sites. Knowledge of ligand-free aptamer conformations that are distinct from the ligand-bound form, therefore, can aid in determining the mechanism by which the absence or presence of ligand elicits conformational switching. We have determined a 1.9 Å-resolution crystal structure of the THF riboswitch aptamer domain in the absence of ligand that shows significant differences from previously reported apo and holo structures, particularly in the conformation of the P1 and P3 helices. The pseudoknot binding site 'unwinds' in the absence of ligand, causing rotation and misalignment of the gene-regulatory P1 helix with respect to P3. The second binding site, however, located at the three-way junction, is structurally conserved between apo and holo forms. This suggests cooperativity for the two binding sites, one which is preformed to elicit kinetic control, and the other which is directly involved in conformational switching through winding and unwinding of the pseudoknot.

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