Abstract: Riboswitches are gene-regulating mRNA segments most commonly found in bacteria. A riboswitch contains an aptamer domain that binds to a ligand, causing a conformational change in a downstream expression platform. The aptamer domain of the Class I preQ1 riboswitch from Bacillus subtilis, which consists of a stem-loop structure and an adenine-rich single-stranded tail (L3), re-folds into a pseudoknot structure upon binding of its ligand, preQ1. To study the role of L3 in ligand recognition, I inserted 2-aminopurine (2-AP), a fluorescent base analogue of adenine (A), into the riboswitch at six different positions within L3. 2-AP differs from A in the relocation of its amino group from C6 to C2, allowing us to directly probe the significance of this specific functional group. I used circular dichroism spectroscopy and thermal denaturation experiments to study the structure and stability, respectively, of the riboswitch in the absence and presence of preQ1. At all labeling positions tested, 2-AP substitution inhibited the ability of preQ1 to stabilize the pseudoknot structure, with its location impacting the severity of the effect. Structural studies of the riboswitch suggest that at the most detrimental labeling sites, 2-AP substitution disrupts noncanonical base pairs. My results show that these base pairs and tertiary interactions involving other residues in L3 play a critical role in ligand recognition by the preQ1 riboswitch, even at positions that are distal to the ligand binding pocket. They also highlight the importance of accounting for perturbations that fluorescent analogues like 2-AP may exert on the system being studied.

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