The Effect of Covalent Cross Links on the Secondary Structure of the Tetracycline Aptamer

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Tetracycline is an antibiotic that treats bacterial infections by acting as protein synthesis inhibitor through disruption of bacterial translation. Overuse of tetracycline has led to its resistance in an increasing number of pathogenic bacteria, which limits its use in disease treatment. The tetracycline aptamer is used in the detection of tetracycline, and is one way to determine if tetracycline is potentially being misused. Like most aptamers, the stability of the tetracycline aptamer is dependent on the conditions of the solution it is placed in. The structure of this aptamer, held together by weak hydrogen bonds, is susceptible to bond cleavage under high temperature, high pH, and high salt concentration. The purpose of this experiment is to determine whether replacing the hydrogen bond at a specific location on the tetracycline aptamer with a stronger covalent bond will increase the stability of the aptamer's secondary structure when encountering changes in external stimuli. Unmodified and modified (covalent group at nucleotide 70) aptamer species were obtained, and covalent crosslinking was activated in the latter through light exposure at 254 nm. Isothermal titration calorimetry (ITC) was then used to measure the binding affinity of unmodified and modified aptamer when exposed to neutral and high pH conditions. Overall, the results showed binding under normal conditions for the unmodified aptamer; however, no binding occurred for any of the other conditions set. The main reasons for these results are likely that the aptamer, even with modification, was unable to remain stable under high pH conditions, or that this specific site was not ideal for cross-linking.

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