STUDIES IN FLUORESCENCE RESONONANCE ENERGY TRANSFER IN A FAM/IOWA BLACK LABELED THROMBIN-BINDING APTAMER

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Aptamers are 15 to 50 base long, single stranded DNA or RNA that based on sequence bind strongly and specifically to a molecular target. Aptamers are increasingly important in diagnostics, research and therapeutics. Thrombin-binding aptamer (TBA) is an aptamer that is getting a lot of attention; thrombin is a protein important in blood coagulation. TBA folds into a unique “chair” structure when it binds to thrombin or certain cations. In the chair structure, TBA has two guanine-quadruplexes that are arrangements of 4 guanines stabilized by hydrogen bonds. Fluorescence resonance energy transfer (FRET) can often be used to monitor structural changes in molecules. FRET is the transfer of energy from an excited fluorescent donor group to an acceptor, and is a distance dependent process. Changes in fluorescence are a result from structural changes in TBA. Prior work, with TBA dual labeled with 6- carboxytetramethylrhodamine (TAMRA) and 6-carboxyfluorescein (FAM) was unsuccessful despite numerous attempts. We will be using a FAM/Iowa-Black FQ donor/acceptor pair labeled TBA in the presence of Li+, Na+, K+, Rb+, and Cs+. These group 1A ions provide a series of cations that differ in size, something the folding of TBA is dependent upon, but not charge. Measuring the binding constants of these monovalent cations will help in determining the structural changes of TBA. By understanding the interaction between TBA and monovalent cations we may better understand the interaction of TBA and thrombin, potentially helping its use as a therapeutic.