ELECTROCHEMICAL STUDIES OF ELECTRON TRANSFER IN DNA FILMS WITH COVALENTLY TETHERED INTERCALATORS

Thesis By

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ABSTRACT

The base stack within double-helical DNA provides an efficient pathway for charge transport. This DNA-mediated charge transport chemistry has been shown to be exquisitely sensitive to minor perturbations in DNA structure and base stacking both in solution and on surfaces. As a result, electrochemical studies on DNA-modified electrodes may provide a novel approach to the development of sensitive, but inexpensive DNA sensing devices. Using intercalated, covalently bound daunomycin as a redox probe, we have examined ground state charge transport in DNA films on gold electrodes. DNA-mediated electron transfer is found to occur over a distance as long as 100 Å in the film. Moreover, while the introduction of one or even two breaks in the sugar-phosphate backbone yields no detectable effect on electron transfer, a CA base-pair mismatch significantly attenuates the electron transfer yield. These results confirm that the base pair stack is the pathway for DNA mediated charge transfer, not the sugar-phosphate backbone. Based on these studies, we have developed a method to electrochemically monitor the trapping of double- stranded DNA with a 6-base overhang on a gold electrode modified with double- stranded DNA probes containing a complementary overhang. The trapping of the double-stranded target can be monitored by the reduction of daunomycin crosslinked to the target. A CA mismatch in the target duplex can also be detected by the diminished reduction signal.

It has been shown that the electronic coupling between an intercalator and the π-stack of DNA is critically important for the reduction of the intercalator through DNA-mediated charge transport. Using covalently tethered anthraquinone derivatives as

the redox probe, we have investigated also the influence of the binding mode of the intercalator on its reduction in DNA films. The results of these studies underscore the importance of direct interaction between the redox probe and the π-stack in order to observe efficient DNA-mediated electrochemistry through DNA films. These studies have also shown that the covalent linkage has a significant effect on the intercalation of the probe to the base stack of DNA.

In an effort to develop a redox probe that has effective electronic coupling with the π-stack while being covalently tethered to a DNA strand with a stable linkage, we have crosslinked Nile blue, a redox active DNA intercalator with high DNA binding affinities, to the 5'-end of oligonucleotides. The covalently tethered Nile blue is shown to be sensitive probe for DNA-mediated electron transfer on the gold surface. An intervening CA mismatch has been detected in both tightly packed and loosely packed films of DNA–Nile blue conjugate. We have also coupled the reduction of Nile blue to an electrocatalytic cycle involving freely diffusing ferricyanide, which significantly enhances the sensitivity to intervening mismatches. Furthermore, using Nile blue as the covalently tethered probe, we have developed a method for DNA mismatch detection that eliminates sample modification and has a potential for high throughput assays. These studies may provide a practical approach to diagnostic devices for mutation detection with high sensitivity and low expense.

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