ELECTRON TRANSFER IN DNA:

ELECTROCHEMICAL INVESTIGATION OF

DNA SINGLE BASE MISMATCH DETECTION

HETTIARACHCHIGE DHANUJA DEEPAMALI PERERA

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3.0 Abstract

A large number of genetic diseases and genetic disorders are simply caused by single base substitutions or small additions and deletions in the genome. Therefore developing efficient and cost effective techniques for routine detection of DNA mutations is of great importance. Different methods involving gel electrophoresis and Polymerase Chain Reaction have been developed to identify altered DNA. Many of these are costly, time consuming and lack high throughput capability. Detection methods based on electrochemical techniques are becoming very popular and are critical in biosensor development. In this study, new strategies for single base mismatch detection were developed using electrochemical methods. MutS protein, a member of the mis-matched repair (MMR) system, recognizes mispaired and unpaired bases in duplex DNA and initiates mismatch repair. The natural specificity of MutS mismatch repair protein for single base mismatch recognition was exploited in this study. Particular focus was given to oligonucleotides with single G:T mismatches. This method involves modification of a gold electrode surface using a bi-functional succinimidyl compound, immobilization of MutS protein on the modified gold electrode surface through amide linkages, and application of a DNA probe for mismatch recognition. Electrochemical discrimination between mismatched and complementary DNA strands was performed using redox properties of Tris-2,2'-dipyridylcobalt(III) Perchlorate Trihydrate. QCM and EMSA studies further supported the results from square wave voltammetry. Another strategy for single base mismatch detection is covalent DNA immobilization. Mismatches were detected via charge transduction through the DNA film. This method involves chemical modification by electrochemical reduction of 4-diazonium carboxylic acid tetrafluoroborate on a glassy carbon electrode and immobilization of DNA on the modified electrode surface through carbodiimide / N-hydroxy succinimide coupling. Square wave and cyclic voltammograms for different types of redox active probes showed discrimination between complementary and mismatch DNA.