

Amplified Electrochemical Detection of Biomolecules using Biometallization

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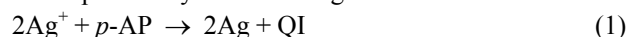
I. INTRODUCTION

There is an increasing demand for ultrasensitive methods to detect biomolecules such as DNA and protein for various applications including the genetics of disease and medical diagnosis. The electrochemical detection have attracted particular attention because it provides a simple, inexpensive, accurate and sensitive platform for the detection of biomolecules. Although several methods have been developed for the detection of DNA^{1,2} and protein,³ they have some drawbacks such as low sensitivity, long time for analysis and requirement of the complex transducing layer.

II. RESULT AND DISCUSSION

Herein we report the amplified detection of a target DNA based on the enzymatic deposition of silver. Scheme 1 depicts the construction of the sandwich-type DNA sensing electrode based on enzymatic silver deposition. First, mixed monolayer surfaces of capture probe DNA and mercaptoethanol were prepared as by immersion in a 1 μM solution of thiolated capture probe oligonucleotide in 1.0 M HEPES buffer for 1h, followed by its exposure to an aqueous solution of 1.0 mM mercaptoethanol solution for 1h. Then, the immobilized capture probes were hybridized with target DNA and then sequentially with biotinylated detection probes needed for enzyme binding in hybridization solution containing 1 mM CTAB as a cationic detergent for rapid and specific hybridization. Subsequently, avidin conjugated alkaline phosphatase (Av-ALP) was added, which binds to the

exposed biotin group on the detection probes and converts p-aminophenyl phosphate (*p*-APP) to a p-aminophenol (*p*-AP), a reducing agent that reduces silver ions, forming a metallic silver layer on the DNA backbone and electrode for 30 min of reaction time. Since the half-wave potential of *p*-AP is 0.097 V vs NHE, and that of Ag^+ is 0.80 V, *p*-AP spontaneously reduces silver ions in solution. The reduction can be explained by the following reaction:

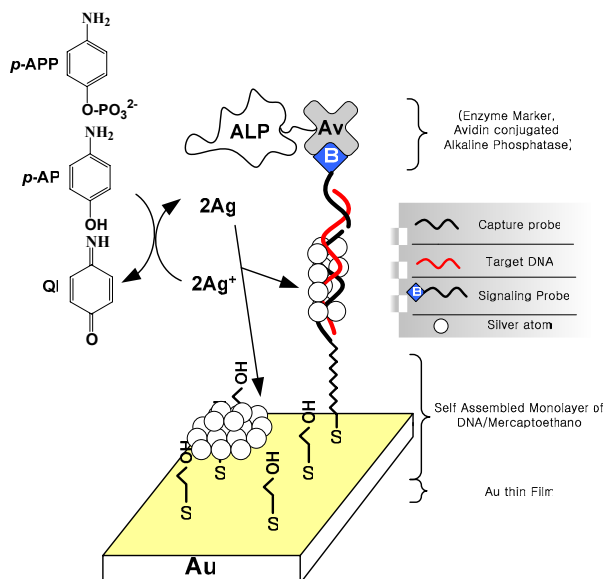


where QI (quinonimine) is the oxidation product of *p*-AP, caused by the loss of two-electrons. Therefore, only the enzymatic reaction can start the reduction of silver ions in solution and the anodic stripping current of deposited silver provides a measure of the extent of hybridization of the target oligomers.

This process, which we term biometallization, leads to a great enhancement in signal due to the accumulation of metallic silver by a catalytically generated enzyme product, and thus the electrochemical amplification of a biochemically amplified signal. The anodic stripping current of enzymatically deposited silver (Figure 1) provides a measure of the extent of hybridization of the target oligomers. This biometallization process offers a very selective detection of DNA.

III. REFERENCES

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Scheme 1. assembly of a sandwich-type DNA sensing electrode based on enzymatic silver deposition.

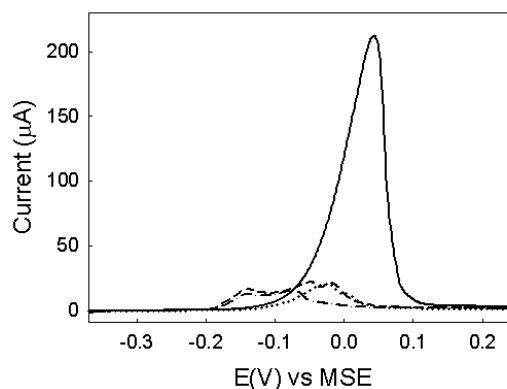


Figure 1. Linear sweep voltammogram (LSV) of enzymatically deposited electrodes in 0.1 M H_2SO_4 solution hybridized (a) with 1 μM complementary target (solid line); (b) with 1 μM noncomplementary target (dashed line); (c) in the absence of hybridization with target and detection probe (dash-dot line); (d) with 1 μM single-base-mismatched target (dotted line).

Scan rate = 0.010 V/s.