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Indirect Electrochemical Sensing of DNA Hybridization Based on the Catalytic Oxidation of Cobalt (II)

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Abstract

A new electrochemical approach for detecting target DNA is described. The scheme involves the catalytic oxidation of a cobalt bipyridine “reporter molecule” in solution. Probe/target DNA duplexes (ds-DNA), bound on an ITO electrode, selectively recruit redox catalyst molecules from solution. This surface-bound catalyst “turns on” the redox chemistry of the reporter molecule which is otherwise kinetically inert to oxidation on ITO. The mode of selective catalyst binding is intercalation between base pairs of ds-DNA. With this approach, we demonstrate detection of a 20-mer DNA target oligonucleotide at picomolar concentrations with outstanding signal-to-noise.

In little over a decade, since the concept was first introduced,[1] efforts to develop electrochemical methods for detecting nucleic acid hybridization (e.g., DNA) have mushroomed. [2-7] In 2005 alone, over 100 articles and patents appeared describing electrochemical biosensors for, or electrochemical detection of, DNA hybridization. At least three commercial products based on electrochemical methods are marketed. [8] Compared with nearly all other analytical techniques, electrochemical instrumentation is inexpensive, robust and relatively simple to operate. On this basis alone, electrochemical biosensors for DNA hybridization present attractive prospects for real-world clinical applications which represent a substantial driver to achieve reliable, sensitive quantitative detection of DNA hybridization.

Fundamentally, electrochemical DNA detection bears considerable similarity to common fluorescent nucleic acid detection. [2-7] Usually, a probe molecule consisting of a single strand oligonucleotide (ss-DNA) is first chemically attached to an electrode surface. The probe-modified electrode is then challenged with a solution containing the complementary target ss-DNA sequence to form a surface-bound duplex (ds-DNA).[2-7] At this point, fluorescence- and electrochemical-based detection approaches diverge. For electrochemical detection, the surface-bound ds-DNA must produce an electrochemical signal — usually a current. Only guanine (G) and adenine (A) bases are inherently electrochemically active at practical potentials.[7,9] At ca. 1.0 and 1.3 V vs. Ag/AgCl, respectively, G and A can undergo oxidation but only when the solution and electrode conditions are precisely controlled. [7,9] Furthermore, because these oxidation processes are chemically irreversible, the sensing event necessarily destroys the DNA. For these reasons, much effort seeking to improve electrochemical DNA sensors has pursued indirect sensing approaches.[2-7] Because the DNA is not destroyed, prospects for multiuse are a significant advantage of indirect sensing. More importantly, however, the electrochemical signal is not limited by the A and G composition of the probe/

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target complex strand. Consequently, the potential for significantly enhanced (i.e., beyond the stoichiometric G and A content) analyte detection signals are possible.

Herein we describe a new electrochemical approach to detect target DNA molecules from solution based on the catalytic oxidation of a reporter molecule, $Co(DTB)_3^{2+}$ where DTB is 4,4′-di-t-butyl-2,2′-bipyridine. Briefly, probe/target duplex DNA bound to ITO electrode surfaces selectively recruit redox catalysts that "turns on" the redox chemistry of the reporter molecule. Catalytic currents result producing signal amplification. With this approach, we demonstrate detection of a 20-mer ss-DNA target oligonucleotide at picomolar concentrations with outstanding signal-to-noise.

We have observed and previously reported that $Co(bpy)_3^{2+}$-type complexes containing sterically bulky substituents exhibit heterogeneous electron transfer properties that are exquisitely electrode-surface dependent.[10] Figure 1 shows CVs of $Co(DTB)_3^{2+}$ on both glassy carbon and ITO electrodes, along with a background scan of the same ITO electrode. For over-potentials of <350mV, $Co(DTB)_3^{2+}$ is essentially kinetically inert to oxidation on the bare ITO surface. However, in the presence of any of a number of redox-active molecules, the oxidation of $Co(DTB)_3^{2+}$ can be catalyzed via an EC' mechanism. [11] The catalyst can either be dissolved in solution (cf. Figures 1 and 2) or, as previously shown,[12] be confined to the electrode surface. This dramatic on/off behavior for the oxidation of $Co(DTB)_3^{2+}$ led us to investigate exploiting this chemistry to detect hybridization of target ss-DNA with surface-bound probe ss-DNA.

Success in this strategy requires that the chemistry used to recruit the catalyst molecule to the electrode surface reliably and strongly discriminate between ss- (e.g., the noise) and ds-DNA. Selective intercalation of planar molecules between the base pairs of ds-DNA, in principle, meets this requirement. Complex **I** was chosen as the catalyst primarily for three reasons: (1) phenothiazine is known to strongly intercalate into ds-DNA,[13] (2) $Ru(bpy)_2Cl_2$ undergoes oxidation in the desired potential region,[14] and (3) the molecule is uncharged and, thus, should be free of non-specific electrostatic interactions with ss-DNA. Probe and target ss-DNA employed were fully complementary 20-mer synthetic oligonucleotides studied previously in surface-capture fluorescence hybridization assays.[15-17]

Prior to attaching ss-DNA probe, the ITO surface was modified with a monolayer of adsorbed 11-phosphonoundecanoic acid.[18] Probe ss-DNA, modified on its 3′-end with a $-(CH_2)_6-NH_2$ moiety was then covalently attached to the monolayer carboxylic acid groups on the ITO surface using standard aqueous amide-coupling chemistry (see Supplemental Material for details).[19]

Figure 3 shows typical, representative differential pulse voltammograms (DPVs) for two ITO electrodes, each measured in the same 0.10mM solution of $Co(DTB)_3^{2+}$. Both electrodes were identically modified as described to attach probe ss-DNA. Both electrodes were immersed in the same 1×10^{-6}M acetonitrile solution of catalyst **I** for 15 minutes and rinsed with acetonitrile immediately prior to obtaining the respective DPVs. Electrodes A and B differed only in that the former electrode was stirred with gentle rocking for 24 hrs in 25 mL of a 4pM solution of the complementary target ss-DNA. Figure 3 shows that on electrode A the target/probe duplex has formed and successfully recruited catalyst **I,** whereas no catalyst activity is apparent on electrode B containing only the ss-DNA probe. Additional DPV data is provided in the Supporting Material, including controls with mismatched (i.e., non-complimentary) target.

Based on the signal-to-noise and background from data in Figure 3, sensing DNA hybridization from dilute target solutions < 1×10^{-14}M should be possible. Proof for such limits of detection are currently hampered by the solution volumes and transport kinetis required in the current electrode configuration. For example, consider measuring a 10 fM analyte solution with the

electrode used in Figure 3. Assuming approximately a limiting coverage of probe (~4×10^{12} probe molecules),[15] ca. 7L of solution would be required to contain sufficient target molecules to hybridize only 1% of the probe molecules on the ca. 0.5 cm^2 surface. Thus, from a practical perspective, investigating target concentrations significantly less than 1pM requires reducing the electrode dimensions to ~0.01 mm^2 or less, and improving target mass transport to the electrode surface during hybridization. These experiments are in progress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

(1). Millan KM, Mikkelsen SR. Analytical Chemistry 1993;65:2317–2323. [PubMed: 8238927]

(2). de-los-Santos-Alvarez P, Lobo-Castanon MJ, Miranda-Ordieres AJ, Tunon-Blanco P. Analytical and Bioanalytical Chemistry 2004;378:104–118. [PubMed: 14624325]

(3). Kerman K, Kobayashi M, Tamiya E. Measurement Science and Technology 2004;15:R1–R11.

(4). Drummond TG, Hill MG, Barton JK. Nature Biotechnology 2003;21:1192–1199.

(5). Wang J. Analytica Chimica Acta 2003;500:247–257.

(6). Wang J. Biosensors & Bioelectronics 2006;21:1887–1892. [PubMed: 16330202]

(7). Thorp HH. Topics in Current Chemistry 2004;237:159–181.

(8)a. Osmetech LLC. http://www.osmetech.comToshiba Corporation. http://dna-chip.toshiba.co.jp/eng/.GeneOhm Sciences, Inc.. http://www.geneohm.com/

(9). Meric B, Kerman K, Ozkan D, Kara P, Erdem A, Kucukoglu O, Erciyas E, Ozsoz M. Journal of Pharmaceutical and Biomedical Analysis 2002;30:1339–1346. [PubMed: 12408924]

(10). Sapp SA, Elliott CM, Contado C, Caramori S, Bignozzi CA. Journal of the American Chemical Society 2002;124:11215–11222. [PubMed: 12224970]

(11). Bard, AJ.; Faulkner, LR. Electrochemical Methods, Fundamentals and Applications. 1 st ed.. John Wiley and Sons, INC; New York: 1980.

(12). Elliott CM, Caramori S, Bignozzi CA. Langmuir 2005;21:3022–3027. [PubMed: 15779980]

(13). Wang J, Rivas G, Cai X, Shiraishi H, Farias P, Dontha N, Luo D. Analytica Chimica Acta 1996;332:139–144.

(14). Ikeda T, Leidner CR, Murray RW. Journal of Electroanalytical Chemistry and Interfacial Electrochemistry 1982;138:343–365.

(15). Gong P, Harbers GM, Grainger DW. Analytical Chemistry 2006;78:2342–2351. [PubMed: 16579618]

(16). Gong P, Lee C-Y, Harbers GM, Castner DG, Grainger DW, Gamble LJ. Anal. Chem 2006;78

(17). Lee C-Y, Gong P, Gamble LJ, Castner DG, Grainger DW. Analytical Chemistry 2006;78:3316–3325. [PubMed: 16689532]

(18). Lowe LB, Brewer SH, Krämer S, Fuierer RR, Qian G, Agbasi-Porter CO, Moses S, Franzen S, Feldheim DF. J. Am. Chem. Soc 2003;125:14258–14259. [PubMed: 14624557]

(19). Gong P, Grainger DW. Surf. Sci 2004;570:67–77.

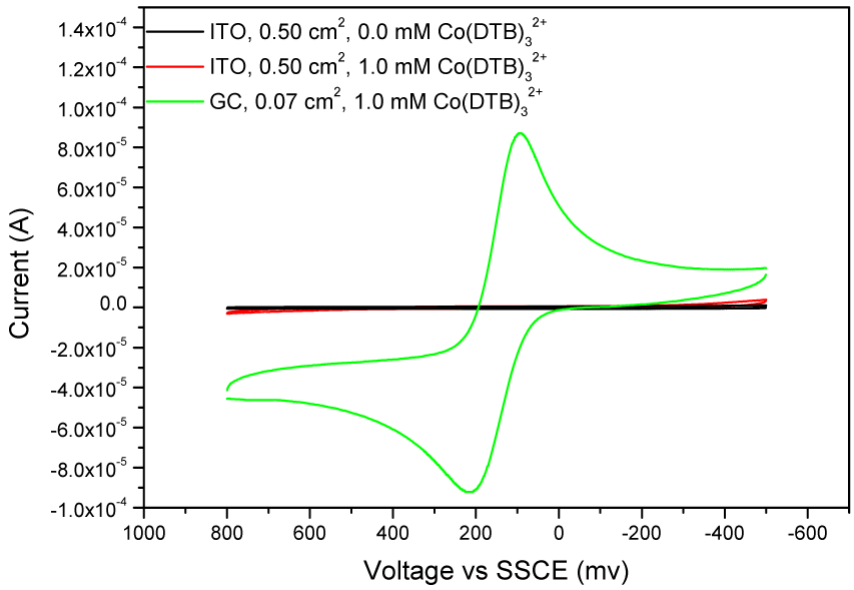
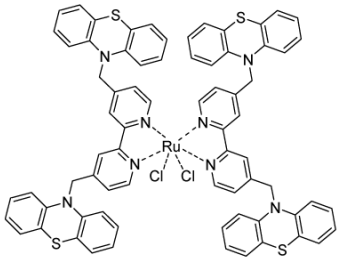


Figure 1.

Cyclic voltammograms for $Co(DTB)_3^{2+}$ on both glassy carbon (green) and ITO (red) electrodes, along with a background scan (black) of the same ITO electrode. Current for the CV on glassy carbon is normalized to that on the ITO electrode based on differences in area.



Complex I

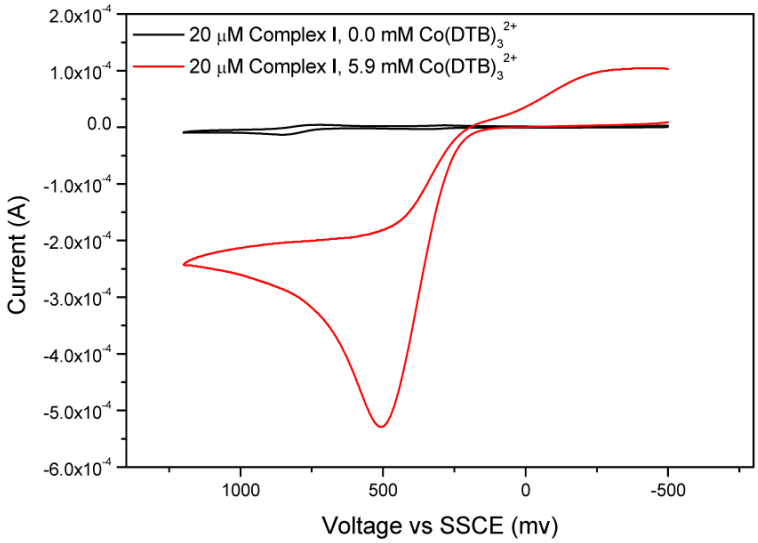


Figure 2.

Cyclic voltammograms of Complex I in 0.1M $TMAPF_6$/acetonitrile on an ITO electrode in the absence (black) or presence (red) of $Co(DTB)_3^{2+}$.

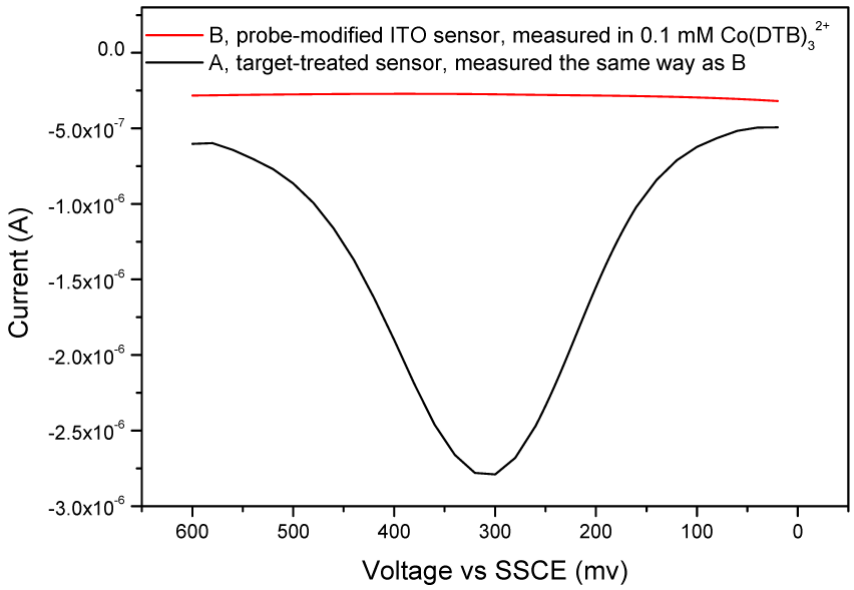


Figure 3.

DPVs of two identical probe-modified ITO sensors with (sensor A, black) and without (sensor B, red) exposure to 4 pM ss-DNA target solution. Both electrodes were exposed identically to the same catalyst **I** solution before DPV measurement (see text and Supporting Information for details).