Chapter 9: Electrical Detection of TATA-Binding Protein at DNA-Modified Microelectrodes

Adapted from: Gorodetsky, A. A., Ebrahim, A. E., Barton, J. K. (2008) *J. Am. Chem. Soc. 130*, 2924–2925.

ABSTRACT

A simple method for the electrochemical detection of TATA-binding protein is demonstrated at DNA-modified microelectrodes. The assay is general and based on the interruption of DNA-mediated charge transport to Nile Blue, a redox-active probe covalently attached to the DNA base pair stack. Nanomolar quantities of TATA binding protein can be detected on the microelectrodes even in the presence of micromolar amounts of bovine serum albumin, EndonucleaseIII, or Bam HI methyltransferase. The scheme outlined provides a basis for the sensitive electrical detection of numerous proteins on a single DNA chip.

9.1 Introduction

Sensitive methods to detect the specific binding of proteins to DNA are essential in monitoring pathways for gene expression and in the development of new diagnostics of disease states (1, 2). There are several high-throughput, array-based methodologies currently available for monitoring transcription factor binding to DNA including immunoprecipitation of reversibly crosslinked chromatin/protein complexes (3, 4), capture of fluorescent antibodies by epitope-tagged DNA-binding proteins (5, 6), and amplification of sequences methylated by enzyme/DNA adenine-methyltransferase fusions (7, 8). However, these methods rely on fluorescence detection and require DNA amplification and/or protein labeling.

Electrochemical assays based on DNA-mediated charge transport (CT) through self-assembled DNA monolayers offer an alternative approach (9). DNA electrochemistry has been useful not only in assays to detect mutations and lesions (10–17) but also for the detection of protein binding at self-assembled DNA monolayers (18–25). Moreover, DNA electrochemistry provides the opportunity for highly sensitive detection in a multiplexed format (9). However, although we have demonstrated the detection of all possible single base pair mismatches at gold microelectrodes (10), there are few reports of the electrical detection of protein binding utilizing DNA monolayers at small scales (9, 18–25).

In a typical protein detection experiment, a loosely packed DNA monolayer with a covalently appended, electroactive probe is self-assembled in the absence of Mg^{2+} and the surface is backfilled with an alkanethiol such as mercaptohexanol (9, 18, 22). Such

sparse DNA films are necessary for improved accessibility of the protein and possess a well-defined morphology; the DNA oriented in an upright orientation (26, 27). After self assembly and backfilling, voltammetry of the DNA-modified electrode is recorded in the absence and presence of protein. Detection of protein binding is confirmed by an attenuation of the yield of DNA CT to a distally-bound probe, such as DM. Overall, this electrochemical assay is highly general and based on exquisitely sensitive DNA-mediated electrochemistry, so a wide range of protein/DNA interactions and binding motifs can be interrogated in rapid fashion.

Our previous studies at DNA-modified macroelectrodes demonstrated that we could easily detect micromolar concentrations of TATA-binding protein (TBP), a transcription factor that bends duplex DNA by ~ 90º upon binding and attenuates DNA CT (18, 28–30). Importantly, this transcription factor not only plays a central role in the eukaryotic/prokaryotic transcription machinery but also possesses an extensively explored crystal structure and a simple 5'-TATAAAG-3' binding site (30). Therefore, TBP was chosen as the model system for our explorations at DNA-modified microelectrodes. Here, we demonstrate the rapid and specific detection of *nanomolar* concentrations of TBP at *microelectrodes* modified with DNA featuring Nile Blue (NB), an easily prepared and stable redox probe.

9.2 Experimental

9.2.1 Materials

All reagents for DNA synthesis were purchased from Glen Research. Mercaptohexanol and buffer preparation reagents were purchased from Sigma in the highest purity available and used as received. Nile Blue perchlorate was purchased from Acros in laser grade purity. Gold microelectrodes were purchased from Bioanalytical Systems Inc., and silver/silver chloride reference electrodes were purchased from Cypress Systems, Inc. Nanocloth and aluminum oxide slurry were purchased from Buehler, Inc. Sodium phosphate buffers were prepared with Milli-Q water and pH adjustments were made with sodium hydroxide, if necessary.

9.2.2 Synthesis of Thiol and Nile Blue-Modified DNA

Oligonucleotides were prepared on solid support using standard phosphoramidite chemistry on an Applied Biosystems 394 DNA synthesizer. The pure oligonucleotides were characterized with matrix-assisted laser desorption (MALDI) mass spectrometry, UV-visible (UV-Vis) spectrophotometry, and multiple rounds of high performance liquid chromatography (HPLC).

Thiol-terminated oligonucleotides were synthesized according to established protocols from Glen Research, Inc. using the C6 S-S thiol modifier. After deprotection and cleavage from solid support with ammonium hydroxide (60 ºC for 8 hours), the disulfide containing DNA was purified by HPLC. The disulfide was subsequently reduced with an excess of dithiothreitol in ammonium acetate buffer at pH = 8, and the

free thiol containing single-stranded DNA was then purified with a second round of HPLC.

DNA modified with NB at the 5' terminus was prepared according to ultra-mild protocols (Glen Research, Inc.) to avoid degradation of the NB moiety. Additionally, Pac-protected bases and ultra mild reagents were utilized during the synthesis to prevent undesirable capping of the protecting groups. A 17-mer sequence (5'-UGC GTG CTT TAT ATC TC-3') was prepared on solid support with a 5-[3-acrylate NHS Ester]-deoxy uridine as the terminal 5' base. The beads were then removed from the synthesizer and dried thoroughly. The solid support were reacted with a 10 mg/mL Nile Blue perchlorate solution in either 9:1 N,N-dimethylformamide/N,N-diisopropylethylamine or 9:1 dichloromethane/N,N-diisopropylethylamine for 12–48 hours. The beads were subsequently washed up to three times with dichloromethane or N,N-dimethylformamide, methanol, and acetonitrile. Subsequently, the Nile Blue-containing sequence was simultaneously cleaved from the support and deprotected with 0.05 M potassium carbonate in methanol at room temperature for 12–14 hours. The overall yields of the reaction ranged from 30% to 80%.

Both thiol- and NB-modified DNA was quantified using the extinction coefficient of the single-stranded DNA at 260 nm on a Beckman UV-Vis spectrophotometer. Equimolar amounts of each strand were combined before the resulting solution was purged with argon. Duplexes were formed by thermally annealing in deoxygenated phosphate storage buffer containing 5 mM NaP_i, pH = 7.1, 50 mM NaCl to 90 ºC, followed by cooling to ambient temperature.

9.2.3 Protein Preparation and Storage

Human TATA binding protein (TBP) was custom ordered from ProteinOne, Inc. in pH = 7, 5 mM NaP_i, 50 mM NaCl, 4 mM $MgCl_2$, 4 mM spermidine, 50 μM EDTA, 10 % glycerol buffer. Due to conflicting concentration reports from ProteinOne, Inc., the protein stock concentration (~ 16 μM) was determined using the Bradford Assay Kit from Bio-Rad Laboratories. Stock solutions of the protein were stored at -80 ºC and aliquoted under an argon atmosphere. The TATA binding protein utilized for detection experiments was taken through no more than two freeze/thaw cycles (including the aliquoting step).

In addition to TBP, bovine serum albumin (BSA), Endonuclease III (Endo III), and Bam HI methyltransferase (Bam HI) were utilized for electrochemistry experiments. Bovine serum albumin was purchased from New England Biolabs, Inc., and utilized for experiments as received. Bam HI Methyltransferase was purchased from New England Biolabs, Inc. Prior to electrochemistry experiments, Bam HI was dialyzed overnight in pH = 7, 5 mM NaP_i, 50 mM NaCl, 4 mM $MgCl_2$, 4 mM spermidine, 50 μM EDTA, 10 % glycerol buffer to remove excess dithiothreitol. Endo III was prepared according to established literature procedures and quantified by UV-visible spectroscopy (20).

9.2.4 Preparation of Backfilled DNA Monolayers

Gold microelectrodes possessing either a 10 μm or a 25 μm diameter were polished in 0.05 μm alumina slurry on nanocloth. The electrodes were subsequently briefly polished in H_2O on nanocloth, rinsed thoroughly in H_2O, and washed with 1 N sulfuric acid to remove residual alumina particles. The electrodes were then sonicated in deionized water. Finally, to remove gold oxide from the exposed surface, the

microelectrodes were etched in 1 N H_2SO_4 before being briefly rinsed in deionized water and phosphate storage buffer.

Immediately after cleaning, the microelectrodes were incubated in a 25–50 μM duplex NB-DNA solution in phosphate storage buffer. Monolayer formation was allowed to proceed in a humidified environment for a period of 24–48 hours. Upon completion of film formation, the cell was rinsed thoroughly with phosphate buffer to remove residual DNA before the DNA-modified surface was backfilled with 1.0 mM 1-mercaptohexanol in phosphate storage buffer/5% glycerol for 30–60 minutes or 0.1 mM 11-mercaptoundecylphosphoric acid in phosphate buffer/25% glycerol for 5–10 minutes. Alkanethiols rapidly displace DNA from the electrode surface, so the microelectrodes were rinsed thoroughly in buffer to remove residual 1-mercaptohexanol or 11-mercaptoundecylphosphoric acid.

9.2.5 Electrochemistry and Protein Detection Experiments

Cyclic and square wave voltammetry experiments were recorded using a CH Instruments electrochemical analyzer (Austin, TX). When necessary, the data was smoothed for clarity using the CH instruments software. Experiments were performed at ambient temperature in pH 7 phosphate buffer containing either 5 mM NaP_i and 50 mM NaCl (standard phosphate buffer) or in pH 7 phosphate buffer containing 5 mM NaP_i, 50 mM NaCl, 4 mM $MgCl_2$, 4 mM spermidine, 50 μM EDTA, 10% glycerol (TBP dialysis buffer) under an Argon atmosphere. An eppendorf tube electrochemical cell was used for all experiments with a Pt auxiliary electrode, a gold working electrode, and a silver/silver chloride (Ag/AgCl) reference electrode. In a typical detection experiment, background scans were initially recorded in TBP dialysis buffer, which was thoroughly purged with

Argon. Great care was taken to ensure that similar degassing times were utilized in all experiments. Subsequently, TBP or other proteins were added to the electrochemical cell from a stock solution in phosphate buffer at pH = 7 containing 5 mM KP_i, 50 mM NaCl, 4 mM $MgCl_2$, 4 mM spermidine, 50 μM EDTA, 10% glycerol. The solution was equilibrated via gentle pipetting, stirred with a brief argon purge, and voltammetry was recorded again.

9.3 Results

Figure 9.1 schematically illustrates the strategy utilized for electrochemistry experiments, and Figure 9.2 shows the corresponding cyclic voltammetry (CV) and square wave voltammetry (SWV) for electrodes modified with NB-DNA duplexes in TBP dialysis buffer. For macroelectrodes, the CV is reversible with a midpoint potential of -220 mV (±50) versus Ag/AgCl. We have found that the observed midpoint potentials of NB-DNA monolayers vary somewhat depending upon the type of gold surface utilized and the buffer conditions, leading to an uncertainty of ~ 50 mV in the potentials, although they have a higher certainty for any given surface. In addition, the cathodic and anodic waves are symmetric (though not overlapping), and a plot of peak current as a function of scan rate is linear, as expected for a surface-bound species (31).

For microelectrodes with critical dimensions ≥ 25 μm, the CV differs in shape from that observed for a macroelectrode in TBP dialysis buffer. For example, in pH = 7 phosphate buffer containing only 5 mM NaP_i and 50 mM NaCl, the microelectrode voltammetry more closely resembles that typically found at a macroelectrode (Figure 9.3).

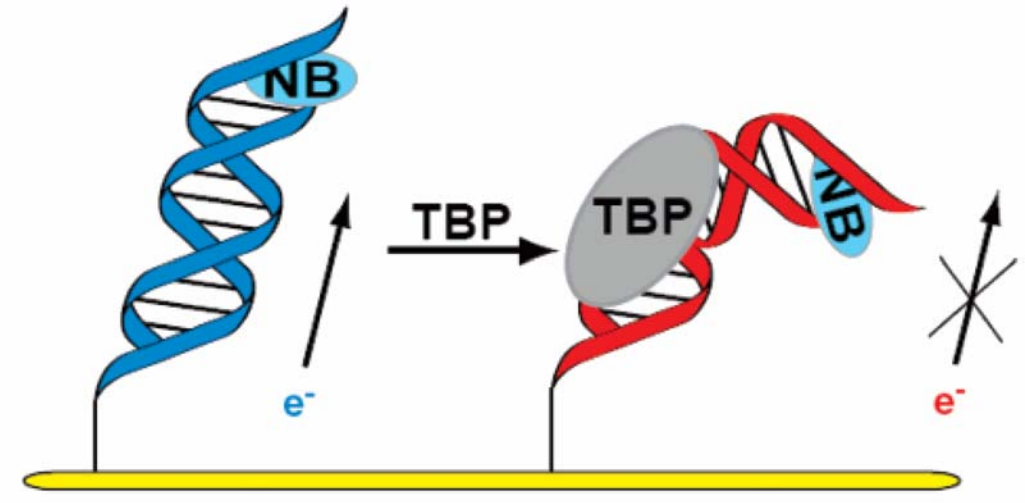

Figure 9.1: Schematic illustration of addition of TBP to a DNA monolayer modified with Nile Blue (NB), followed by DNA kinking and attenuation of DNA-mediated electrochemistry

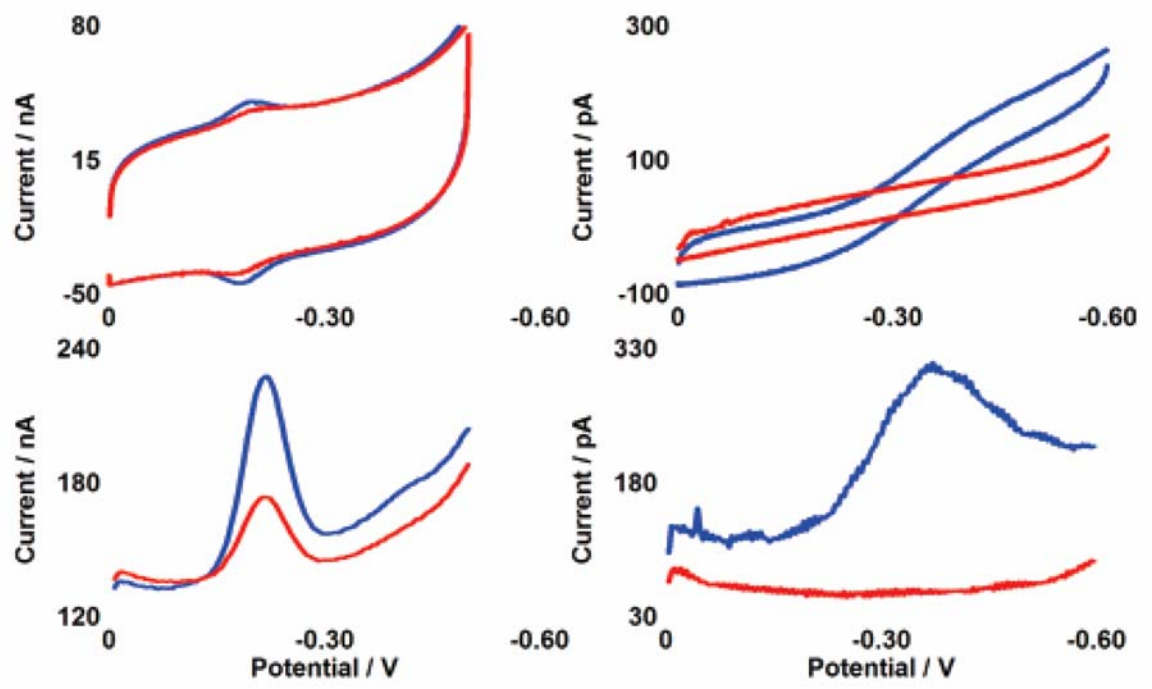

Figure 9.2: CV (top) and SWV (bottom) of NB-DNA at macroelectrodes of 2 mm diameter (left) and microelectrodes of 25 μm diameter (right). Voltammetry without TBP (blue) and with 300 nM TBP (red) is shown at pH 7 in a degassed phosphate buffer containing 5 mM NaP_i, 50 mM NaCl, 4 mM $MgCl_2$, 4 mM spermidine, 50 μM EDTA, 10% glycerol. The sequence is thiol-5'-GAGA*TATAAAG*CACGCA-3' plus NB-modified complement. The potentials are reported versus Ag/AgCl with a CV scan rate of 50 mV/s and an SWV frequency of 15 Hz.

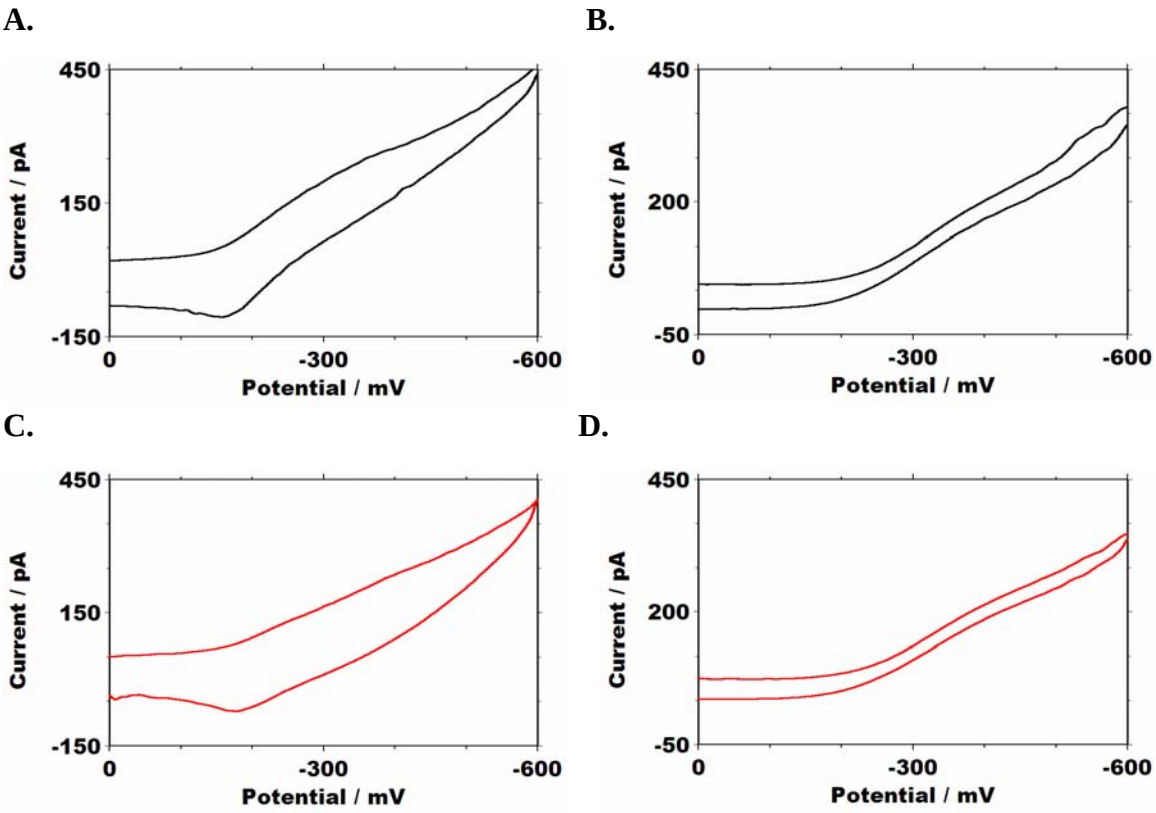

Figure 9.3: CV of a NB-DNA modified microelectrode of a 25 μm diameter for two different buffers. Voltammetry in a pH 7 degassed phosphate buffer containing 5 mM NaP_i and 50 mM NaCl, is shown in (A). The electrode is moved to a pH 7 degassed phosphate buffer containing 5 mM NaP_i, 50 mM NaCl, 4 mM $MgCl_2$, 4 mM spermidine, 50 μM EDTA, 10% glycerol with the recorded voltammetry shown in (B). The electrode is subsequently backfilled with 11-mercaptoundecylphosphoric acid. Voltammetry of the backfilled electrode in a pH 7 degassed phosphate buffer containing 5 mM NaP_i and 50 mM NaCl, is shown in (C). The subsequent voltammetry in a pH 7 degassed phosphate buffer containing 5 mM NaP_i, 50 mM NaCl, 4 mM $MgCl_2$, 4 mM spermidine, 50 μM EDTA, 10% glycerol is shown in (D). The sequence is thiol-5'-GAGA*TATAAAG*CACGCA-3' plus NB-modified complement. The potentials are reported versus Ag/AgCl with a CV scan rate of 50 mV/s.

The more sigmoidal voltammetry can be restored simply by moving the microelectrode from standard phosphate buffer to TBP dialysis buffer. In fact, similar buffer effects have been previously documented for the electrochemistry ferrocene-peptide conjugates at microelectrodes (32). Nonetheless, regardless of the buffer conditions utilized, the observed cyclic voltammetry at microelectrodes is different from the nearly ideal voltammograms found at macroelectrodes. Nile Blue has displayed catalytic activity with a number of substrates (33), and we attribute this phenomenon to a small catalytic contribution to the NB signal from trace oxygen (Figure 9.4) (34).

For microelectrodes, the midpoint potential appears to be systematically shifted by ~ 100 mV. Interestingly, the potential shifts we observe here upon moving from the macro to the micro scale are comparable to those previously observed by others (35, 36). However, this shift in potential persists even when DNA modified macroelectrodes and microelectrodes are fabricated in parallel on a single substrate and run minutes apart with a common reference (Figure 9.5). The potential shift may reflect the different coverages and therefore geometries of DNA monolayers on microelectrodes, as indicated by both radioactive labeling and ruthenium hexammine assay (36).

Upon addition of 300 nM TBP, as illustrated in Figure 9.1 and the red traces of Figure 9.2, reduction of DNA-bound NB is dramatically attenuated at both the macro- and microscales. For macroelectrodes, addition of the protein causes a signal loss of 60% with the signal slowly decreasing over a period of 15 minutes. For microelectrodes, 100% signal loss occurs in less than 30 seconds. The consistently greater signal loss and appreciably faster equilibration on the microelectrode may reflect better access of the protein to the NB-modified DNA on the smaller surface. It is also noteworthy that

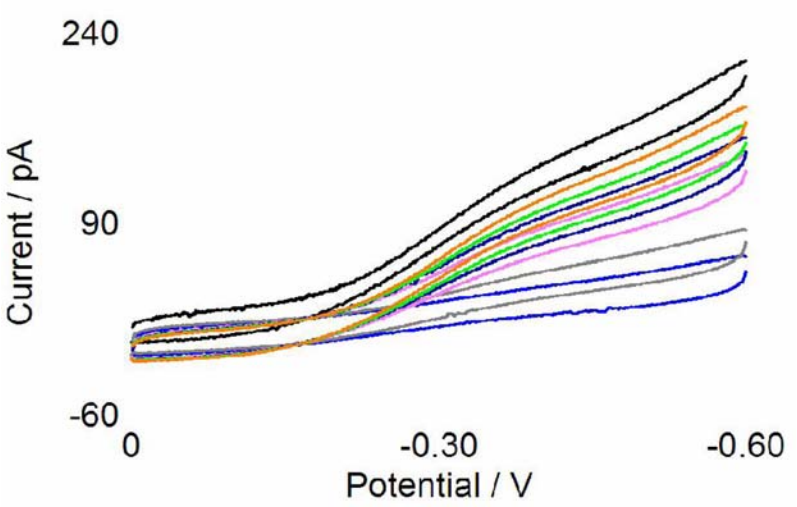

Figure 9.4: The effect of oxygen on the CV of a NB-DNA-modified microelectrode of a 10 µm diameter. Voltammetry in an oxygen saturated pH 7 phosphate buffer containing 5 mM NaP_i, 50 mM NaCl, 4 mM $MgCl_2$, 4 mM spermidine, 50 µM EDTA, 10% glycerol is shown in black with voltammetry after ten to fifteen more minutes of vigorous argon bubbling into the solution in blue. Trace amounts of oxygen have a significant effect on the voltammetry observed at microelectrodes. The gray-to-orange traces represent successive voltammograms taken on the same electrode open to air in an initially degassed solution with a slow, steady increase observed over thirty minutes. The sequence is thiol-5'-GAGA*TATAAAG*CACGCA-3' plus NB-modified complement. The potentials are reported versus Ag/AgCl with a CV scan rate of 50 mV/s.

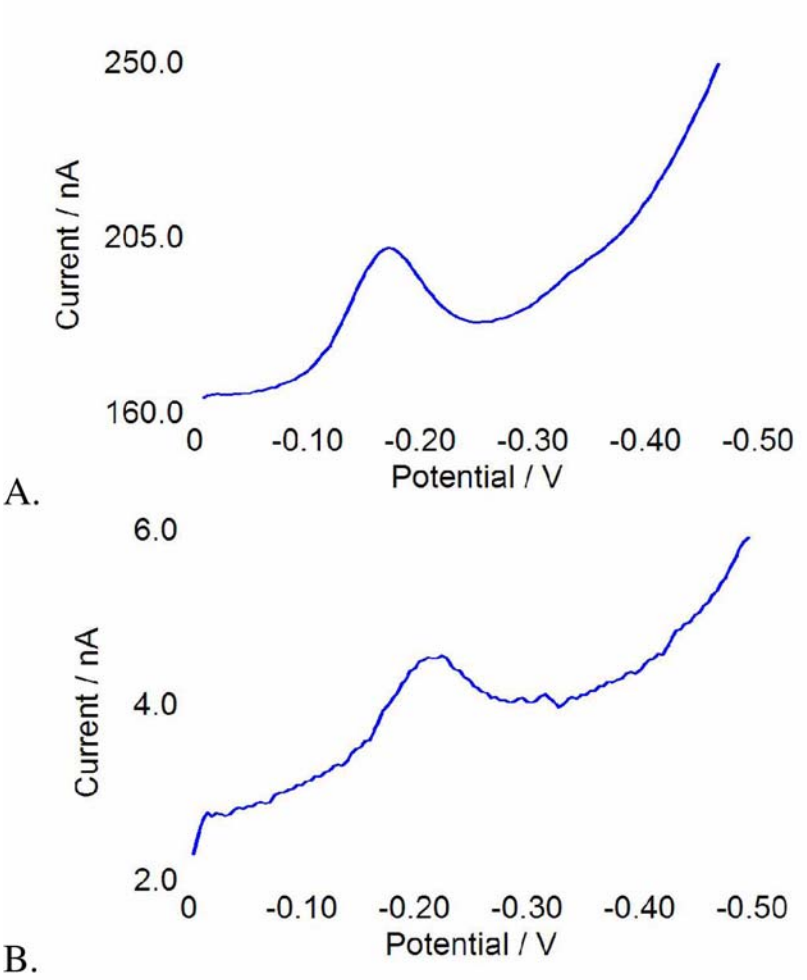

Figure 9.5: SWV of a NB-DNA-modified Au on silicon electrodes of 130 μm radius (A) and 13 μm radius (B) in pH = 7 phosphate buffer containing 5 mM P_i and 50 mM NaCl. Note that the two electrodes were fabricated together in parallel and run minutes apart yet still did not yield identical potentials. The sequence is thiol-5'-GAGA*TATAAAG*CACGCA-3' plus NB-modified complement.

binding of TBP, in kinking the DNA (19), may bring NB in closer proximity to the surface, yet a *decrease* is observed in the NB signal. This observation is fully consistent not with contact of NB to the surface but instead a DNA-mediated reduction that is inhibited by bending the intervening DNA.

TBP binding and the resulting signal attenuation are fully reversible on a microelectrode (Figure 9.6). A microelectrode incubated with TBP can be washed in 1 M KCl, promoting protein dissociation (37, 38) and rapid recovery of the NB signal. Electrodes can be taken through this cycle up to three times with no loss of response. In addition, detection of low concentrations of TBP is easily achieved (Figure 9.7). At 30 and 15 nM protein concentrations, complete NB signal loss is observed. At 3 nM protein, the NB signal is partially attenuated, as expected based upon the affinity of the protein for its target sequence (39, 40).

The detection event is furthermore specific to TBP (Figure 9.8). Bovine serum albumin (BSA), a protein that does not bind DNA, has little effect on DNA-mediated electrochemistry or on signal attenuation associated with TBP binding (18). BSA is known to adsorb nonspecifically on self-assembled alkanethiol monolayers (41). Treatment of the electrode with BSA effectively passivates against further non-specific adsorption. The inclusion of micromolar Endonuclease III (EndoIII), a glycosylase that targets damaged pyrimidines (not unlike the NB-modified thymine), also has little effect on the DNA-mediated NB signal (42–44). Finally, the addition of BamHI methyltransferase (BAM), a base-flipping methylase that targets 5'-GGATCC-3' (45), yields little change in the voltammetry or the signal decrease associated with TBP binding. Although EndoIII and BAM likely bind nonspecifically to the DNA film at these

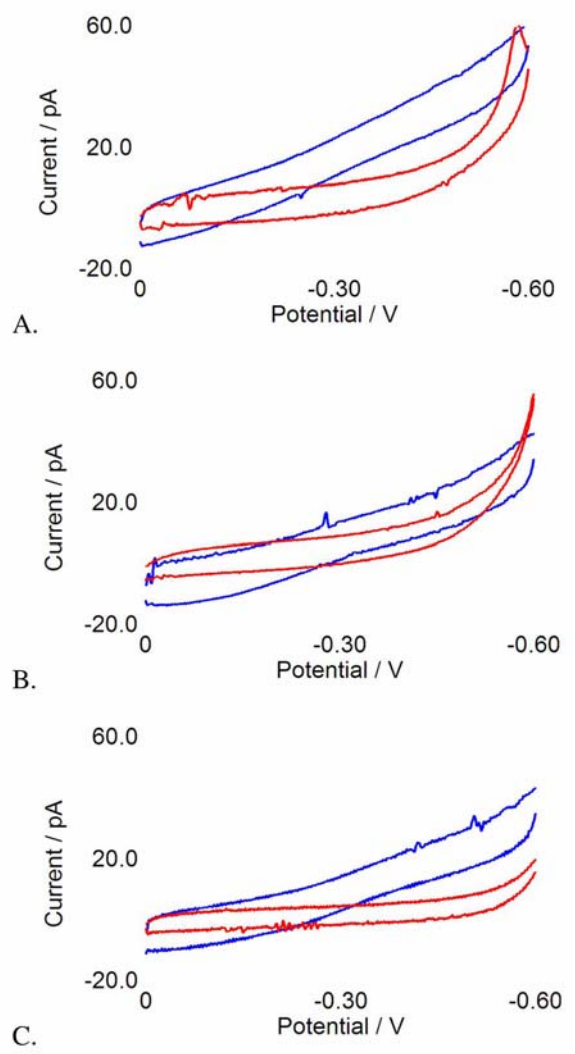

Figure 9.6: CV of a NB-DNA-modified 10 µm diameter electrode before addition of TBP in blue and after addition of 300 nM TBP in red. Initial background voltammetry of the electrode is shown in pH 7 phosphate buffer containing 5 mM NaP_i, 50 mM NaCl, 4 mM $MgCl_2$, 4 mM spermidine, 50 µM EDTA, 10% glycerol (A) with significant signal loss observed after addition of TBP. The electrode is subsequently rinsed in succession (5 minutes each) with 1 M KCl in MQ H_2O, 1 M KCl in Tris buffer, and TATA dialysis buffer. Background voltammograms are recorded for the same electrode before it is used to detect TBP again (B). The electrode can then be taken through another KCl rinse cycle before being used for detection a third time (C).

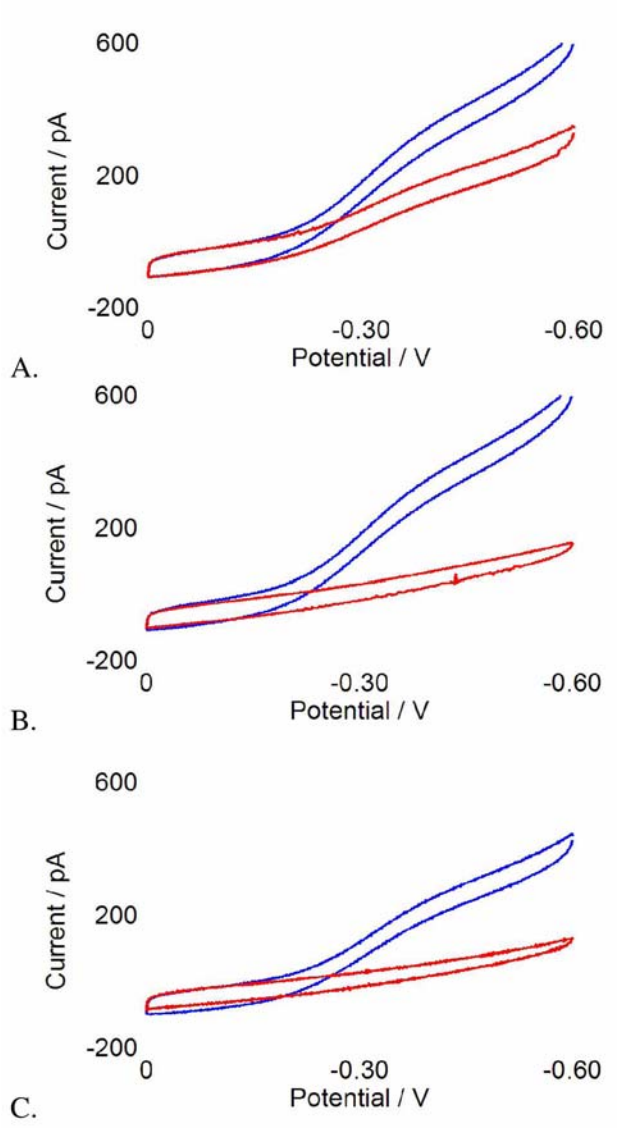

Figure 9.7: CV of a NB-DNA-modified 25 µm diameter electrode before addition of TBP in blue and after addition of TBP in red. Initial background voltammetry of the electrode is shown in pH 7 phosphate buffer containing 5 mM NaP_i, 50 mM NaCl, 4 mM $MgCl_2$, 4 mM spermidine, 50 µM EDTA, 10% glycerol. Significant signal attenuation is found after addition of (A) 3 nM, (B) 15 nM, and (C) 30 nM TBP. The sequence is thiol-5'-GAGA*TATAAAG*CACGCA-3' plus NB-modified complement, and the potentials are reported versus Ag/AgCl with a CV scan rate of 50 mV/s.

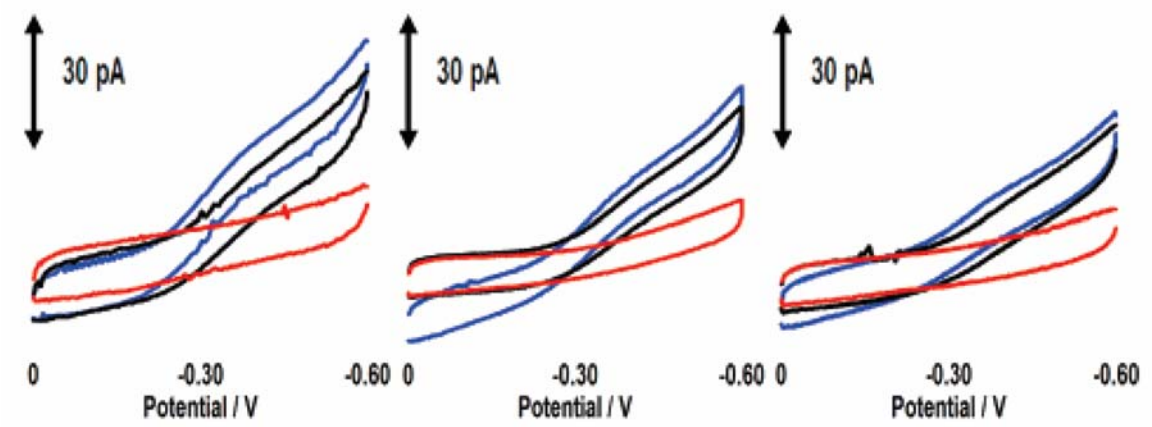

Figure 9.8: CV of three NB-DNA-modified microelectrodes (10 μm) before and after addition of proteins. CVs before protein addition are in blue. From left to right, the black CVs represent addition of BSA (2 μM); BSA (1 μM) + EndoIII (1 μM); and BSA (1 μM) + BAM (1 μM). CVs in red reflect the subsequent addition of 30 nM TBP to each electrode. The sequence is thiol-5'-GAGA*TATAAAG*CACGCA-3' plus NB-modified complement, and the potentials are reported versus Ag/AgCl with a CV scan rate of 50 mV/s.

concentrations, they do not inhibit specific binding by TBP. These results are therefore consistent with observing a sequence-specific TBP binding event.

9.4 Implications

We have thus demonstrated the electrochemical detection of nanomolar TBP binding to DNA in the presence of other proteins with a rapid and sensitive electrochemical assay. This methodology is based upon an interruption of DNA-mediated electrochemistry associated with protein binding and therefore may be generally applied in detecting other proteins that perturb the DNA base stack. Our ability to detect protein binding to DNA reliably at small scales provides a foundation for rapid, electrical assays of DNA-binding proteins on a single chip.

REFERENCES

(1) Sikder, D., Kodadek, T. (2005) *Curr. Opin. Chem. Biol. 9*, 38–45.

(2) Bulyk, M. L. (2006) *Curr. Opin. Biotechnol. 17*, 422–430.

(3) Kim, J., Iyer, V. R. (2004) *Mol. Cell. Biol. 24*, 8104–8112.

(4) Lee, T. I., Johnstone, S. E., Young, R. A. (2006) *Nature Protoc. 1*, 729–748.

(5) Berger, M. F., Philippakis, A. A., Qureshi, A. M., He, F. S., Estop, P. W., III, Bulyk, M. L. (2006) *Nature Biotechnol*. *24*, 1429–1435.

(6) Bulyk, M. L. (2007) *Adv. Biochem. Engin./Biotechnol. 104*, 65–85.

(7) van Steensel, B., Delrow, J., Henikoff, S. (2001) *Nature Genet. 27*, 304–308.

(8) Vogel, M. J., Peric-Hupkes, D., van Steensel, B. (2007) *Nature Protoc. 2*, 1467–1478.

(9) Drummond, T. G., Hill, M. G., Barton, J. K. (2003) *Nature. Biotechnol. 21*, 1192–1199.

(10) Boon, E. M., Ceres, D. M., Drummond, T. G., Hill, M. G., Barton, J. K. (2000) *Nature Biotechnol. 18*, 1096–1100.

(11) Kelley, S. O., Boon, E. M., Barton, J. K., Jackson, N. M., Hill, M. G. (1999) *Nucl. Acids Res. 27*, 4830–4837.

(12) Gorodetsky, A. A., Barton, J. K. (2006) *Langmuir 22*, 7917–7922.

(13) Boal, A. K., Barton, J. K. (2005) *Bioconjugate Chem. 16*, 312–321.

(14) Inouye, M., Ikeda, R., Takase, M., Tsuri, T., Chiba, J. (2005) *Proc. Natl. Acad. Sci. U.S.A. 102*, 11606–11610.

(15) Okamoto, A., Kamei, T., Saito, I. (2006) *J. Am. Chem. Soc. 128*, 658–662.

(16) Li, X., Song, H., Nakatani, K., Kraatz, H.-B. (2007) *Anal. Chem. 79*, 2552–2555.

(17) Wong, E. L. S., Gooding, J. J. (2007) *J. Am. Chem. Soc. 129*, 8950–8951.

(18) Boon, E. M., Salas, J. E., Barton, J. K. (2002) *Nature Biotechnol. 20*, 282–286.

(19) Boon, E. M., Livingston, A. L., Chmiel, N. H., David, S. S., Barton, J. K. (2003) *Proc. Natl. Acad. Sci. U.S.A. 100*, 12543–12547.

(20) Boal, A. K.; Yavin, E.; Lukianova, O. A.; O'Shea, V. L.; David, S. S.; Barton, J. K. (2005) *Biochemistry 44*, 8397–8407.

(21) Gorodetsky, A. A., Boal, A. K., Barton, J. K. (2006) *J. Am. Chem. Soc. 128*, 12082–12083.

(22) DeRosa, M. C., Sancar, A., Barton, J. K. (2005) *Proc. Natl. Acad. Sci. U.S.A. 102*, 10788–10792.

(23) Yeung, S. S. W., Lee, T. M. H., Hsing, I.-M. (2006) *J. Am. Chem. Soc. 128*, 13374–13375.

(24) Anne, A., Bonnaudat, C., Demaille, C., Wang, K. (2007) *J. Am. Chem. Soc. 129*, 2734–2735.

(25) Li, C.-Z., Long, Y.-T., Lee, J. S., Kraatz, H.-B. (2004) *Chem. Comm. 5*, 574–575.

(26) Zhou, D., Sinniah, K., Abell, C., Rayment, T. (2003) *Angew. Chem. Int. Ed. 42*, 4394–4397.

(27) Erts, D., Polyakov, B., Olin, H., Tuite, E. (2003) *J. Phys. Chem. B. 107*, 3591–3597.

(28) Kim, Y., Geiger, J. H., Hahn, S. H., Sigler, P. B. (1993) *Nature 365*, 512–520.

(29) Kim, J. L., Nikolov, D. B., Burley, S. K. (1993) *Nature 365*, 520–527.

(30) Patikoglou, G. A., Kim, J. L., Sun, L., Yang, S.-H., Kodadek, T. K., Burley, S. K. (1999) *Genes Dev. 13*, 3217–3230

(31) Bard, A. J., Faulkner, L. R. (2001) *Electrochemical Methods*, *2nd ed.*, John Wiley & Sons: New York.

(32) Orlowski, G. A., Chowdhury, S., Kraatz, H.-B. (2007) *Electrochim. Acta 53*, 2034–2039.

(33) Liu, H.-H., Lu, J.-L., Zhang, M., Pang, D.-W. (2002) *Anal. Sci*. *18*, 1339–1344.

(34) Ju, H., Shen, C. (2001) *Electroanalysis 13*, 789–793.

(35) Gore, M. R., Szalai, V. A., Ropp, P. A., Yang, I. V., Silverman, J. S., Thorp, H. H. (2003) *Anal. Chem. 75*, 6586–6592.

(36) Lapierre-Devlin, M. A., Asher, C. L., Taft, B. J., Gasparac, R., Roberts, M. A., Kelley, S. O. (2005) *Nano Lett. 5*, 1051–1055.

(37) Daugherty, M. A., Brenowitz, M., Fried, M. G. (1999) *J. Mol. Biol. 285*, 1389–1399.

(38) Khrapunov, S., Brenowitz, M. (2007) *Biochemistry 46*, 4876–4887.

(39) Coleman, R. A., Taggart, A. K. P., Benjamin, L. R., Pugh, B. F. (1995) *J. Biol. Chem. 270*, 13842–13849.

(40) Gilfillan, S., Stelzer, G., Piaia, E., Hofmann, M. G., Meisterernst, M. (2005) *J. Biol. Chem. 280*, 6222–6230.

(41) Holmlin, R. E., Chen, X., Chapman, R. G., Takayama, S., Whitesides, G. M. (2001) *Langmuir 17*, 2841–2950.

(42) Asahara, H., Wistort, P. M., Bank, J. F., Bakerian, R. H., Cunningham, R. P. (1989) *Biochemistry 28*, 4444–4449.

(43) Cunningham, R. P., Asahara, H., Bank, J. F., Scholes, C. P., Salerno, J. C., Surerus, K., Munck, E., McCracken, J., Peisach, J., Emptage, M. H. (1989) *Biochemistry 28*, 4450–4455.

(44) David, S. S., Williams, S. D. (1998) *Chem. Rev. 98*, 1221–1262.

(45) Lindstrom, W. M., Jr., Malygin, E. G., Ovechkina, L. G., Zinoviev, V. V., Reich, N. O. (2003) *J. Mol. Biol. 325*, 711–720.