J|A|C|S
COMMUNICATIONS

Published on Web 08/29/2006

Direct Electrochemistry of Endonuclease III in the Presence and Absence of DNA

Alon A. Gorodetsky, Amie K. Boal, and Jacqueline K. Barton*

Department of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125

Received July 5, 2006; E-mail: jkbarton@caltech.edu

In vivo, DNA is constantly being assaulted and damaged.[1] To protect the integrity of the genome, an impressive repair network has evolved. Macromolecular crowding, low repair enzyme copy number, and small structural differences in DNA base lesions are, however, challenges in detecting damage. Processive searches along DNA may represent one component of detection.[2] We have proposed DNA-mediated charge transport as the first step in damage detection since it provides a means to redistribute base excision repair (BER) proteins in the vicinity of damage rapidly and efficiently.[3,4]

Endo III is a DNA glycosylase that repairs damaged pyrimidines.[5] Much like the closely related BER enzyme MutY, Endo III features a [4Fe−4S] cluster.[5−7] In MutY, the [4Fe−4S] cluster is not required for protein folding but is crucial in vivo.[8] We have demonstrated for both proteins that the cluster is activated toward oxidation upon enzyme binding to DNA, and this DNA-dependent redox activity promotes charge transport through DNA.[3,4] Electrochemistry of MutY and Endo III on DNA-modified gold electrodes shows a redox potential of ∼60 mV versus NHE for the $[4Fe-4S]^{3+/2+}$ couple; DNA binding appears to shift the potential, so that the protein bound to DNA is more similar to a high-potential iron protein than a ferredoxin.[9]

Here we demonstrate this shift in potential associated with DNA binding directly using highly oriented pyrolytic graphite (HOPG) electrodes to compare the electrochemical properties of Endo III bound to DNA and free (Figure 1). Previous work had shown that, without DNA binding, the $[4Fe-4S]^{2+}$ cluster is not readily oxidized or reduced within a physiological range of potentials.[5] We have recently explored the electrochemical properties of HOPG modified with pyrenated DNA.[10] The DNA monolayers formed are quite similar to thiolated DNA films on gold,[11] but the accessible potential window is significantly larger. Graphite electrodes, moreover, are particularly useful for protein electrochemistry.[12]

Figure 2 shows cyclic voltammetry (CV) and square wave voltammetry (SWV) of Endo III on HOPG with and without DNA modification.[13,14] For the DNA-modified electrode, a quasi-reversible redox couple is observed with a midpoint potential of 20 ± 10 mV versus NHE. Backfilling the DNA electrode with octane has no effect on this signal, while backfilling HOPG without DNA leads to the loss of any protein signal (data not shown). To establish that this signal is DNA-mediated, we examined also an electrode modified with DNA featuring an abasic site prepared under identical conditions; DNA-mediated charge transport has been shown to be inhibited by the abasic site owing to the disruption in base stacking.[3,11] As seen in Figure 2, a complete loss of signal for Endo III is observed at the electrode modified with DNA containing an abasic site. Thus the DNA does not serve to locally concentrate the protein on the graphite surface; the duplex with an abasic site would serve a similar function. Instead it is the *DNA-bound* protein

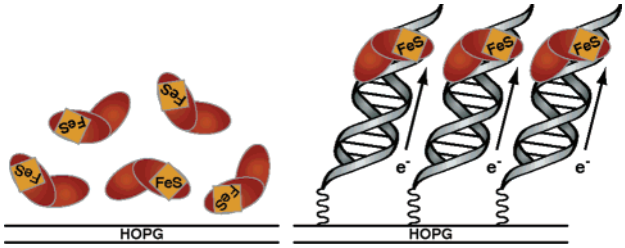

Figure 1. Schematic representation of electrochemistry for Endo III on HOPG with and without modification with DNA.

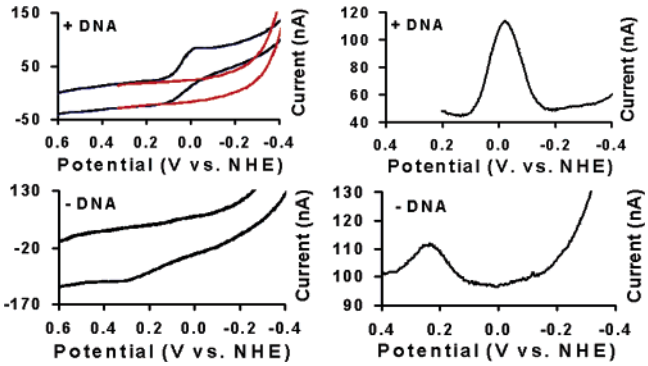

Figure 2. CV (left, 50 mV/s scan rate) and SWV (right, 15 Hz) of 50 μM Endo III in 20 mM Na phosphate, 100 mM NaCl, 1 mM EDTA, 20% glycerol, pH 7.5. The top two panels show electrochemistry of Endo III at a HOPG electrode modified with the sequence pyrene-$(CH_2)_4$-Pi-5′-AG*T* ACA GTC ATC GCG-3′ plus complement. Cyclic voltammetry of a HOPG electrode modified with DNA featuring an abasic site is in red (top left), where the abasic position corresponds to the complement of the italicized base. The bottom two panels show electrochemistry of Endo III on bare HOPG. All runs were taken using the inverted drop cell electrode configuration versus Ag/AgCl reference and Pt auxiliary.

that is probed electrochemically on HOPG in a DNA-mediated reaction, as long as the DNA duplex is well stacked.

Note that at the DNA-modified surface, we observe only one redox signal, with no other peaks evident in the range of 600 to −400 mV versus NHE. The only couple we observe features a cathodic peak at −30 ± 30 mV versus NHE whose shape and magnitude indicate slow diffusive kinetics, as found for MutY.[3] Indeed in all respects, this couple resembles that found for Endo III at a DNA modified Au surface[3] and is assigned to the $[4Fe-4S]^{3+/2+}$ couple.[15]

Significantly, on HOPG versus Au, we may explore the electrochemistry of Endo III at a larger range of applied biases,[10] and thus we may directly compare the electrochemistry of Endo III in the presence and absence of DNA. Oxidative scans of Endo III on bare HOPG reveal an irreversible anodic peak at 250 ± 30 mV versus NHE and no couple at 20 mV as with DNA (Figure 2).[16] Successive positive scans lead to new broad, irregular signals at approximately −80 and −710 mV versus NHE; additionally,

10.1021/ja064784d CCC: $33.50

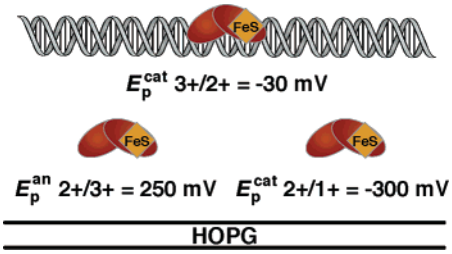

Figure 3. Illustration of the potentials versus NHE of the couples of Endo III in the presence and absence of DNA. These values are based upon SWV on HOPG and are averages of at least four trials each.

the yellow color of the protein solution is lost. These results are fully consistent with oxidative decomposition of the cluster in Endo III without DNA. Indeed, these redox signals are commonly associated with ferredoxin [3Fe−4S] clusters.[9,17] It is noteworthy that on bare HOPG, we observe also the 2+/1+ couple of the [4Fe−4S] cluster during reductive scans with a cathodic peak at approximately −300 ± 80 mV versus NHE (Supporting Information). The peak is near the edge of our potential window, and this redox signal also contains a small oxidative wave at slow scan rates.[18]

Figure 3 summarizes the potentials we have observed for Endo III on HOPG over several trials. A significant negative shift in potential occurs for the 3+/2+ couple on DNA binding; the shift in 2+/1+ couple cannot be determined. DNA binding clearly stabilizes the oxidized 3+ form of the cluster, whereas without DNA, it is $[4Fe-4S]^{2+}$ that is more stable. This shift is understandable based upon the sensitivity of [4Fe−4S] cluster potentials to their environment.[9] Crystal structures of Endo III with and without DNA reveal that the cluster is located near amino acid residues that contact DNA.[7] DNA binding takes the cluster to a more hydrophobic environment compared to the exposed and polar environment in the absence of DNA. Importantly, the resultant shift in potential is not associated with significant conformational changes in the protein; the structures of the bound and free proteins are remarkably similar. Instead, then, the ∼200 mV shift[19] in potential must correspond to a decrease in DNA binding affinity of more than 3 orders of magnitude between the 2+ and 3+ forms of the cluster. While previous evidence qualitatively indicated a lessened DNA binding affinity for the reduced protein,[3] these data provide a more quantitative estimate. In the context of our model of DNA-mediated signaling for damage detection, it is this difference in DNA binding affinity for the reduced versus oxidized state that leads to the dissociation of protein from the DNA upon reduction and thus the redistribution of BER proteins onto sites near damage.

We have, therefore, now identified the electrochemistry of Endo III both with and without DNA on HOPG electrodes. DNA binding is seen to promote a shift in redox potential, activating the protein toward oxidation; subsequent reduction of the cluster to the 2+ form leads to dissociation from the duplex. These results provide strong support for the detection strategy we have proposed for BER enzymes. Furthermore, these data underscore the importance of the outer sphere environment in regulating potentials of [4Fe−4S] proteins,[9,12] as well as the utility of DNA-modified electrodes in probing the redox characteristics of proteins that bind to DNA.

Acknowledgment. We are grateful to the NIH (Grant GM61077) for their financial support and the Parsons Foundation for a fellowship to A.K.B.

Supporting Information Available: CV and SWV for the Endo III reductive couple and CV for the oxidative couple at higher concentration on bare HOPG. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Friedberg, E. C.; Walker, G. C.; Siede, W. *DNA Repair and Mutagenesis;* ASM Press: Washington, DC, 1995. (b) Krokan, H. E.; Standal, R.; Slupphaug, G. *Biochem. J.* **1997**, *325*, 1−16.

(2) (a) Roberts, R. J.; Cheng, X. *Annu. Rev. Biochem.* **1998**, *67*, 181−198. (b) Verdine, G. L.; Bruner, S. D. *Chem. Biol.* **1997**, *4*, 329−334. (c) Francis, A. W.; David, S. S. *Biochem.* **2003**, *42*, 801−810.

(3) (a) Boon, E. M.; Livingston, A. L.; Chmiel, N. H.; David, S. S.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 12543−12547. (b) Boal, A. K.; Yavin, E.; Lukianova, O. A.; O'Shea, V. L.; David, S. S.; Barton, J. K. *Biochem.* **2005**, *44*, 8397−8407.

(4) (a) Yavin, E.; Boal, A. K.; Stemp, E. D. A.; Boon, E. M.; Livingston, A. L.; O'Shea, V. L.; David, S. S.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 3546−3551. (b) Yavin, E.; Stemp, E. D. A.; O'Shea, V. L.; David, S. S.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 3610.

(5) (a) Asahara, H.; Wistort, P. M.; Bank, J. F.; Bakerian, R. H.; Cunningham, R. P. *Biochem.* **1989**, *28*, 4444−4449. (b) 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. *Biochem.* **1989**, *28*, 4450−4455. (c) Fu, W.; O'Handley, S.; Cunningham, R. P.; Johnson, M. K. *J. Biol. Chem.* **1992**, *267*, 16135−16137. (d) O'Handley, S.; Scholes, C. P.; Cunningham, R. P. *Biochem.* **1995**, *34*, 2528−2536. (e) Xing, D.; Dorr, R.; Cunningham, R. P.; Scholes, C. P. *Biochem.* **1995**, *34*, 2537−2544.

(6) (a) Michaels, M. L.; Pham, L.; Nghiem, Y.; Cruz, C.; Miller, J. H. *Nucleic Acids Res.* **1990**, *18*, 3841−3845. (b) Tsai-Wu, J. J.; Liu, H. F.; Lu, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 8779−8783. (c) Lu, A. L.; Tsai-Wu, J. J.; Cillo, J. *J. Biol. Chem.* **1995**, *270*, 23582−23589. (d) Bulychev, N. V.; Varaprasad, C. V.; Dorman, G.; Miller, J. H.; Eisenberg, M.; Grollman, A. P.; Johnson, F. *Biochem.* **1996**, *35*, 13147−13156. (e) Porello, S. L.; Williams, S. D.; Kuhn, H.; Michaels, M. L.; David, S. S. *J. Am. Chem.* Soc. **1996**, *118*, 10684−10692. (f) David, S. S.; Williams, S. D. *Chem. Rev.* **1998**, *98*, 1221−1262.

(7) (a) Kuo, C. F.; McRee, D. E.; Fisher, C. L.; O'Handley, S. F.; Cunningham, R. P.; Tainer, J. A. *Science* **1992**, *258*, 434−440. (b) Thayer, M. M.; Ahern, H.; Xing, D.; Cunningham, R. P.; Tainer, J. A. *EMBO J.* **1995**, *14*, 4108−4120. (c) Fromme, J. C.; Verdine, G. L. *EMBO J.* **2003**, *22*, 3461−3471.

(8) Porello, S. L.; Cannon, M. J.; David, S. S. *Biochem.* **1998**, *37*, 6465−6475.

(9) (a) Cowan, J. A.; Lui, S. M. *Adv. Inorg. Chem.* **1998**, *45*, 313−350. (b) Beinert, H. *JBIC* **2000**, *5*, 2−18. (c) Carter, C. W.; Kraut, J.; Freer, S. T.; Alden, R. A.; Sieker, L. C.; Adman, E.; Jensen, L. H. *Proc. Natl. Acad. Sci. U.S.A.* **1972,** *69*, 3526−3529.

(10) Gorodetsky, A. A.; Barton, J. K. *Langmuir* **2006**, *22*, 7917−7922.

(11) (a) Kelley, S. O.; Boon, E. M.; Barton, J. K.; Jackson, N. M.; Hill, M. G. *Nucleic Acids Res.* **1999**, *27*, 4830−4837. (b) Boon, E. M.; Ceres, D. M.; Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* **2000**, *18*, 1096−1100. (c) Boal, A. K.; Barton, J. K. *Bioconjugate Chem.* **2005**, *16*, 312−321.

(12) (a) Guo, L. H.; Hill, H. A. O. *Adv. Inorg. Chem.* **1991**, *36*, 341−375. (b) Armstrong, F. A. *Adv. Inorg. Chem.* **1992**, *38*, 117−163. (c) Armstrong, F. A.; Heering, H. A.; Hirst, J. *Chem. Soc. Rev.* **1997** *26*, 169−188. (d) Camba, R.; Armstrong, F. A. *Biochemistry* **2000** *39*, 10587−10598. (e) Armstrong, F. A.; Wilson, G. S. *Electrochem. Acta* **2000**, *45*, 2623−2645. (f) Nassar, A. F.; Rusling, J. F.; Nakashima, N. *J. Am. Chem. Soc.* **1996**, *118*, 3043−3044.

(13) Endo III was expressed and purified according to procedures slightly modified from (a) Boiteux, S.; O'Connor, T. R.; Laval, J. *EMBO J.* **1987**, *6*, 3177−3183. (b) O'Connor, T. R. *Nucleic Acids Res.* **1993**, *21*, 5561−5569.

(14) In a typical protein experiment, a loosely packed DNA film is self-assembled in the absence of Mg^{2+} (see ref 3). After incubation with protein and cooling of the electrodes, electrochemical experiments are performed using the inverted drop cell electrode configuration. See Bowler, R.; Davies, T. J.; Hyde, M. E.; Compton, R. G. *Anal. Chem.* **2005**, *77*, 1916−1919.

(15) EPR experiments in solution on DNA bound MutY reveal *g* values characteristic of a 3+ cluster and support this assignment. See ref 4.

(16) At higher protein concentration, we have observed a quasireversible wave (SI).

(17) Thomson, A. J.; Breton, J.; George, S. J.; Butt, J. N.; Armstrong, F. A.; Hatchikian, E. C. *Biochem. Soc. Trans.* **1991**, *19*, 594−599. Similar peaks are seen with $FeCl_3$ controls.

(18) The potential difference between 3+/2+ and 2+/1+ couples is somewhat smaller than expected (ref 5) and may be an underestimate since we are on the edge of the potential window.

(19) SWV gives a shift of 280 mV between the cathodic DNA-bound potential and the anodic potential on bare HOPG. The shift in midpoint potentials should be slightly smaller. Given the lack of reversibility, we provide only a lower estimate in the shift.

JA064784D