

DNA binding shifts the redox potential of the transcription factor SoxR

Alon A. Gorodetsky[†], Lars E. P. Dietrich[‡], Paul E. Lee[†], Bruce Dimple[§], Dianne K. Newman^{‡,¶,||}, and Jacqueline K. Barton^{†,||}

[†]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125; Departments of [‡]Biology and [¶]Earth and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139; and [§]Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, MA 02115

Contributed by Jacqueline K. Barton, January 4, 2008 (received for review December 7, 2007)

Electrochemistry measurements on DNA-modified electrodes are used to probe the effects of binding to DNA on the redox potential of SoxR, a transcription factor that contains a [2Fe-2S] cluster and is activated through oxidation. A DNA-bound potential of +200 mV versus NHE (normal hydrogen electrode) is found for SoxR isolated from *Escherichia coli* and *Pseudomonas aeruginosa*. This potential value corresponds to a dramatic shift of +490 mV versus values found in the absence of DNA. Using Redmond red as a covalently bound redox reporter affixed above the SoxR binding site, we also see, associated with SoxR binding, an attenuation in the Redmond red signal compared with that for Redmond red attached below the SoxR binding site. This observation is consistent with a SoxR-binding-induced structural distortion in the DNA base stack that inhibits DNA-mediated charge transport to the Redmond red probe. The dramatic shift in potential for DNA-bound SoxR compared with the free form is thus reconciled based on a high-energy conformational change in the SoxR–DNA complex. The substantial positive shift in potential for DNA-bound SoxR furthermore indicates that, in the reducing intracellular environment, DNA-bound SoxR is primarily in the reduced form; the activation of DNA-bound SoxR would then be limited to strong oxidants, making SoxR an effective sensor for oxidative stress. These results more generally underscore the importance of using DNA electrochemistry to determine DNA-bound potentials for redox-sensitive transcription factors because such binding can dramatically affect this key protein property.