## Unexpected NO-dependent DNA Binding by the CooA Homolog from C. hydrogenoformans

Robert W. Clark<sup>1</sup>, Andrea J. Lee<sup>1</sup>, Robert L. Kerby<sup>2</sup>, Nicholas D. Lanz<sup>2</sup>, Hwan Youn<sup>2</sup>, Gary P. Roberts<sup>2</sup>, and Judith N. Burstyn<sup>1</sup>

CooA, the CO-sensing heme protein from *Rhodospirillum rubrum*, regulates the expression of genes that form a CO-oxidation system, allowing *R. rubrum* to utilize CO as a sole energy source. Recently, genomic searching has identified homologous CooA proteins in several other organisms, including the thermophile *Carboxydothermus hydrogenoformans*. To better understand the general gas-sensing regulation mechanism utilized by heme in these CooA proteins, we have spectroscopically and functionally characterized the CooA homolog from *C. hydrogenoformans* (Ch2340) to identify similarities and differences between this protein and CooA from *R. rubrum*. Surprisingly, and unlike *R. rubrum* CooA, we have discovered that Ch2340 CooA binds NO to form a 6-coordinate Fe(II)-NO heme that is active for DNA binding both *in vivo* and *in vitro*. Interestingly, the affinity of this NO-bound protein for DNA is remarkably similar to the Fe(II)-CO form. This is in striking contrast to the *R. rubrum* homolog, which is exquisitely specific for CO and forms a 5-coordinate Fe(II)-NO adduct that is inactive for DNA binding. The relevance of these results to the general activation mechanism of CooA and possible origins for the differential behavior exhibited by the two homologous CooA proteins will be presented.

<sup>&</sup>lt;sup>1</sup>Department of Chemistry, University of Wisconsin-Madison and <sup>2</sup>Department of Bacteriology, University of Wisconsin-Madison