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DISSERTATION

"REGULATION OF THE NO-SENSOR GUANYLYL CYCLASE ACTIVITY BY THIOL/DISULFIDE SWITCH"

by

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ABSTRACT

The nitric oxide (NO) sensor guanylyl cyclase (GC1) converts guanosine triphosphate (GTP) to cyclic-guanosine mono-phosphate (cGMP), an obligate heterodimer of α and β subunits. GC1 is stimulated by NO and the resulting production of cGMP is involved in physiological processes that include smooth muscle cell relaxation, neurotransmission, inhibition of platelet aggregation, and neuronal differentiation. Each subunit of GC1 has four domains: a heme-binding (HNOX), a Per-Arnt-Sim (PAS), a coiled-coil and catalytic domain. The enzyme is both a receptor and an effector as the NO signal is propagated from the HNOX domain of the β subunit to the catalytic domain, where cGMP is produced. However, the mechanism of NO activation remains poorly understood. GC1 is unusually cysteine (Cys)-rich and Cys are known to modulate the enzyme's activity through thiol-redox dependent modification. . Previous studies from our lab showed that GC1 can participate in a mixed-disulfide bond with protein disulfide isomerase and Thioredoxin 1. Others have shown that dithiol reagents that induce or reduce disulfide bonds modulate GC1 activity. Our hypothesis is that breakage and formation of disulfide(s) in GC1 contribute to the redox regulation of the enzyme.

Our result shows that dithiol reductants such as *tris(2-carboxyethyl)phosphine (TCEP)* reduce both NO potency and efficacy for GC1 activity. Mass spectrometry and molecular dynamics simulation indicated that β1Cys489 and β1Cys571 are potentially engaged in a thiol/disulfide switch. Mutational analysis of these Cys shows that they regulate GC1 activity by reducing its affinity to NO and its efficacy under reducing conditions. Cross-linking studies with dibromobimane (DiBrb), a fluorescent alkylating agent, show that GC1 not only contains vicinal thiols that may form disulfides but that disulfides differ under the basal and NO-stimulated states. These data supports the new idea that thiol/disulfide switch is involved in the mechanism of GC1 activation by NO. This study contributes to our understanding of GC1 signaling, its dependence on the cellular thiol redox environment and its potential disruption during development of oxidative cardiovascular diseases.