S3-3

Redox Regulation of OxyR Requires Specific Disulfide Bond Formation Involving a Rapid Kinetic Reaction Path

Cheolju Lee[1,*] & Seong Eon Ryu[2]

[1]*Biomedical Research Center, Korea Institute of Science and Technology and* [2]*Systemic Proteomics Research Center, Korea Research Institute of Bioscience and Biotechnology*

Cellular reactive oxygen species (ROS) are generated as byproducts of oxygen respiration and as second messengers in cellular signaling (1, 2). ROS are capable of oxidizing many cellular components including DNA, lipid membranes, and proteins, resulting in damage to cells. To avoid the harmful effects of ROS, cells have evolved to have delicate responses to the oxidative damage. The *Escherichia coli* OxyR transcription factor effectively senses low amounts of intracellular hydrogen peroxide (H_2O_2) and elicits the transcription of antioxidant genes (3). The *Escherichia coli* OxyR transcription factor is activated by cellular hydrogen peroxide through the oxidation of reactive cysteines. Although there has been substantial evidence for specific disulfide bond formation in the oxidative activation of OxyR (4), the presence of the disulfide bond has remained controversial (5, 6). By mass spectrometry analyses and *in vivo* labeling assays we demonstrate that oxidation of OxyR results in the formation of a specific disulfide bond between Cys199-Cys208 in the wild type protein (7). In addition, using various spectroscopic and time-resolved kinetic analyses, we show that OxyR activation occurs in a two step mechanism involving OxyR : hydrogen peroxide association forming a cysteine sulfenic acid at Cys199 and the subsequent conformation switch at about 10 s^{-1} accompanying specific disulfide bond between Cys199 and Cys208. The conformation switch results in a metastable form that is locally strained by about 3 kcal/mol, which serves as a driving force to restore the reduced conformation upon cleavage of the disulfide bond (8). Based on these observations we conclude that OxyR activation requires specific disulfide bond formation and that the rapid kinetic reaction path and conformation strain, respectively, drive the oxidation and reduction of OxyR (7; Figure 1).

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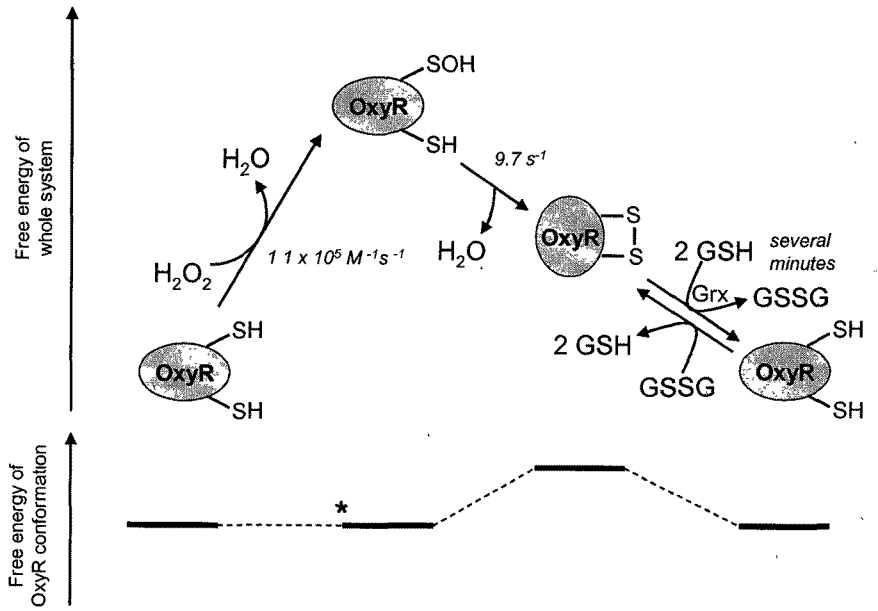

Figure 1. Redox cycle of OxyR