J2

Redox-Dependent Modulation of RsrA, an Anti-Sigma Factor Regulating Thioredoxin Operons in *Streptomyces coelicolor*

Jae-Bum Bae*, Joo-Hong Park and Jung-Hye Roe

School of Biological Sciences, College of Natural Science, Seoul National University

σ^R is a sigma factor responsible for inducing the thioredoxin system in response to oxidative stress in *Streptomyces coelicolor*. RsrA, an anti-sigma factor, specifically binds to σ^R and inhibits σ^R-directed transcription under reducing conditions. Exposure to H_2O_2 or thiol-specific oxidant diamide dissociates σ^R-RsrA complex. The redox-dependent regulation of σ^R-RsrA binding has been reported to involve thiol-disulfide exchange in RsrA, which contains 7 cysteines in 105 amino acid residues. Substitution mutagenesis of 4 cysteines at 11, 41, 44, and 62th position on RsrA abolished the activity of RsrA. Triple substitution mutant at 3, 31, 61th cysteines showed the same activity as wild type RsrA in σ^R binding, suggesting that 4 cysteines are sufficient for its activity. MALDI-tof mass-spectrometry peptide mapping showed that redox-dependent modulation of RsrA is accompanied by the formation of two intramolecular disulfide bonds within RsrA: one between cysteine 11 and cysteine 41/44 and the other one between cysteine 61/62 and cysteine 41/44, thus forming long-range disulfide bonds. Since RsrA has a conserved HX_3CX_2C motif, a putative metal binding site, the content of metals in wild type RsrA was investigated. Ca and Zn were detected in stoichiometric amounts. The content of Zn was systematically lower under oxidized conditions. About one mole of Zn per mole of RsrA was released upon oxidation, suggesting that Zn binds to RsrA depending on the thiol-disulfide status of the cysteines in RsrA being involved in redox-dependent modulation of RsrA. Experiments where Zn content of wild type and mutant RsrA was measured by PAR-PMPS assay, indicated that three cysteines at 3, 41, 44 coordinate a single Zn atom. The results imply cysteines at 41 and 44 coordinate Zn under reduced condition and form two long-range disulfide bonds upon oxidation, releasing Zn.