Regulation and Function of the Major Catalase (*katA*) Gene in *Pseudomonas aeruginosa* PA14

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Microbes have the capacity to rapidly adapt to peroxide stress. In particular, the peroxide-sensing transcriptional regulator OxyR plays a critical role in the regulatory networks governing such responses. The adaptive response to hydrogen peroxide (H_2O_2) in *Pseudomonas aeruginosa* involves OxyR and the major catalase, KatA, which is stably present in the extracellular milieu. Both proteins are required for peroxide resistance as well as acute virulence of this organism. However, neither the molecular basis nor the relationship between both proteins has yet been established. Here, we demonstrate that the transcriptional activation of the *katA* promoter (*katAp*) in response to H_2O_2 was abrogated in the *P. aeruginosa* PA14 *oxyR* null mutant. Promoter deletion analyses revealed that H_2O_2-mediated induction was dependent on a region of DNA -76 to -36 upstream of the H_2O_2-responsive transcriptional start site. This region harbored the potential operator sites (ORE, OxyR-responsive element) of the *Escherichia coli* OxyR binding consensus. Deletion of the entire ORE not only abolished H_2O_2-mediated induction, but also elevated the basal transcription, suggesting the involvement of OxyR and ORE in both transcriptional activation and repression. OxyR bound to the ORE both in vivo and in vitro, demonstrating that OxyR directly regulates the *katAp*. The predominant species of oxidized OxyR upon H_2O_2 exposure was sulfonic acid ($-SO_3H$) form at the conserved peroxidatic cysteine (C199) in *P. aeruginosa*. The uninduced transcription of *katAp* was also highly elevated in an *oxyR* mutant for C199 (C199S), but not in an *oxyR* mutant for C208 (C208S). In both mutants, however, *katAp* transcription was partially induced by H_2O_2 treatment, unlike in the *oxyR* null mutant; the H_2O_2-induction was delayed in the C199S mutant and lower in the C208S mutant. Taken together, our results suggest that *P. aeruginosa* OxyR is a bona fide transcriptional regulator of the *katA* gene, sensing H_2O_2 based on the conserved cysteines. Furthermore, the unmodified Cys 199 thiol is required for the repression of the *katA* transcription and the Cys 208 thiol oxidation is required for the transcriptional activation.