Regulation of the *katG-dpsA* operon and the importance of KatG in survival of *Burkholderia pseudomallei* exposed to oxidative stress

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Received 20 January 2003; revised 20 March 2003; accepted 28 March 2003

First published online 8 April 2003

Edited by Marc Van Montagu

Abstract Homologues of the catalase-peroxidase gene *katG* and the gene for the non-specific DNA binding protein *dpsA* were identified downstream of *oxyR* in *Burkholderia pseudomallei*. Northern experiments revealed that both *katG* and *dpsA* are co-transcribed during oxidative stress. Under conditions where the *katG* promoter is not highly induced, *dpsA* is transcribed from a second promoter located within the *katG-dpsA* intergenic region. A *katG* insertion mutant was found to be hypersensitive to various oxidants. Analysis of *katG* expression in the *oxyR* mutant indicates that OxyR is a dual function regulator that represses the expression of *katG* during normal growth and activates *katG* during exposure to oxidative stress. Both reduced and oxidized OxyR were shown to bind to the *katG* promoter. **© 2003 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.**

Key words: Melioidosis; Oxidative stress; Catalase-peroxidase; Non-specific DNA binding protein; OxyR; Gene regulation

1. Introduction

Burkholderia pseudomallei is a facultative bacterial intracellular pathogen that can cause melioidosis, a potentially fatal infection with septicemic, subacute, and chronic forms. *B. pseudomallei* can survive and multiply in human phagocytes in vitro, moreover, it can persist in a dormant stage in macrophages for months or years [1]. Currently, the mechanism by which this organism survives in human phagocytes is not known. Addition of superoxide dismutase and catalase enzymes significantly inhibits macrophage bactericidal activity against *B. pseudomallei* in vitro suggesting the importance of antioxidant enzymes for the intracellular survival of this pathogen [2]. KatG is a bifunctional enzyme having both catalase and peroxidase activities which protect *Mycobacterium tuberculosis* from killing by oxidative stress [3].

Melioidosis is a life-threatening disease but little is known about the genes involved in the oxidative stress response in its causative agent, *B. pseudomallei*. We began an investigation of the *B. pseudomallei oxyR* homologue and demonstrated that *oxyR* mutants are hypersensitive to oxidative stress, auto-aggregate, more readily form biofilms, and have decreased extracellular protease activity [4]. In this report, we cloned the catalase-peroxidase gene (*katG*) together with the non-specific DNA binding protein gene (*dpsA*). We monitored their expression and constructed a knockout mutant in order to determine the role *katG* plays in protecting *B. pseudomallei* against oxidative killing. Moreover, we demonstrated that OxyR can both repress and activate *katG* expression in vivo and bind to the *katG* promoter region in vitro.

2. Materials and methods

2.1. Media and growth conditions

B. pseudomallei P844 is a clinical isolate [5]. *B. pseudomallei* P844 and *Escherichia coli* were routinely maintained in Luria–Bertani (LB) medium [6]. *Pseudomonas* agar base supplemented with SR103E (cetrimide, fucidin, and cephaloridine) from Oxoid was used after conjugation as a selective medium to inhibit growth of *E. coli*. All cultures were grown at 37°C. Tetracycline (60 μg/ml), chloramphenicol (40 μg/ml), erythromycin (100 μg/ml), and trimethoprim (100 μg/ml) were added to media when required.

2.2. Cloning of full-length katG and dpsA

A 3.5-kb *Eco*RI-*Sal*I fragment of *B. pseudomallei* P844 chromosomal DNA, containing *recG*, *oxyR* and part of *katG*, was previously cloned to generate plasmid pR35 [4]. In order to clone the C-terminal part of *katG*, an *Xma*I genomic blot of *B. pseudomallei* P844 DNA was hybridized with a 700-bp *Xho*I probe from pR35, containing part of *katG*. A 6.0-kb hybridizing band was gel-purified and cloned into pBluescript KS. Restriction analysis and DNA sequencing verified that the newly cloned 6.0-kb *Xma*I fragment contained all of *katG*.

2.3. Northern analysis of katG and dpsA

Extraction of total RNA, using the modified hot acid phenol method, and Northern blot analysis were carried out as previously described [7]. Oxidants, *tert*-butylhydroperoxide (t-BOOH), H_2O_2 and menadione were added to mid-log-phase *B. pseudomallei* cultures to a final concentration of 0.5 mM. Induced and uninduced cultures were grown for 15 min before harvest for total RNA isolation.

2.4. Construction of chromosomal katG::lacZ transcriptional fusion strains

The 1064-bp *Eco*RV-*Bgl*II fragment containing the 5′ end of *katG* and upstream sequence was subcloned into pUC18 to generate pUC18*Sfi*I *lacZ*, subsequently the *Sfi*I fragment containing the *katG* promoter and *lacZ* reporter was excised and ligated into the modified minitransposon pUT-*dfr* [4] to create pUT-TnpG which was then conjugally transferred into *B. pseudomallei* P844 and stable trimethoprim-resistant transconjugants selected. Integration of the *katG::lacZ* transcriptional fusion into the chromosome of *B. pseudomallei* P844 and the *oxyR* knockout mutant R957 [4] created strains P844TnpG and R957TnpG, respectively.

2.5. Assays for the induction of the katG promoter by oxidative stress

Overnight cultures of *B. pseudomallei* P844TnpG and R957TnpG

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Abbreviations: t-BOOH, *tert*-butylhydroperoxide; NEM, *N*-ethylmaleimide; HOCl, hypochlorite

0014-5793/03/$22.00 © 2003 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(03)00328-4

were subcultured ($OD_{600} \approx 0.1$) in LB at 37°C. Mid-exponential-phase (~2 h) cells were induced with either 0.5 mM menadione, 1 mM t-BOOH, 2 mM H_2O_2 or 800 mM NaCl for 1 h before harvest. Cell lysates were prepared using bacterial protein extraction reagent (Pierce) and assayed for β-galactosidase activity using *o*-nitrophenyl-β-D-galactoside as the substrate as previously described [8].

2.6. *Construction of* katG *and* katG oxyR *mutants*

A *katG* knockout mutant was created by insertion of a tetracycline resistance plasmid into *katG*. Briefly, a 240-bp *Mlu*I-*Hin*cII fragment from pR35 was subcloned into pSPORT 1 (Life Technologies), excised with *Sca*I and *Eco*RI, then ligated into suicide vector pKNOCK-TC [9] to create pG221, which was mobilized from *E. coli* S17-1 λ*pir* into *B. pseudomallei* P844 by conjugation. The mutant designated G221 was confirmed as carrying a single insertion of pKNOCK-TC within *katG*. A Tc^r Cm^r *katG oxyR* double mutant, RG27, was created by chromosomal mobilization between G221 (*katG*) and R957 (*oxyR*). Both G221 and RG27 were shown by Southern analysis to have the desired gene disruptions.

2.7. *Complementation of* katG*,* oxyR*, and* katG oxyR *mutants by modified Tn5*

The 3.0-kb *Bam*HI-*Hin*dIII fragment, containing the *katG* upstream region along with *katG*, was cloned into pUT-*dfr* [4]. The minitransposon pUT-*dfr-katG* was then conjugally transferred into *B. pseudomallei* G221 and the stable trimethoprim- and tetracycline-resistant strain G221TnG was selected. Similarly, the 4.0-kb *Eco*RI fragment containing both *oxyR* and *katG* (pUT-*dfr-oxyR-katG*) was mobilized into RG27 by conjugation. The complemented strain, RG27TnRG, was selected on media containing trimethoprim, tetracycline and chloramphenicol. The *oxyR* mutant strain R957 was complemented by the minitransposon carrying *oxyR* and an erythromycin resistance gene (*ery*) from pIC20HEry [10].

2.8. *Purification of OxyR protein*

Primer R1 (5′-CGCCGGTCGACATTCACCGTCG-3′) and primer R2 (5′-CTTGCCATGGCGCTTACC-3′), which covered the 5′ and 3′ regions, respectively, were used to PCR amplify the *oxyR* gene from a plasmid pR35 template. The 700-bp PCR product was digested with *Sal*I and *Nco*I and ligated to pET2-blue (Novagen) digested with *Xho*I and *Nco*I to generate pET-*oxyR*. For purification of His-tagged OxyR, *E. coli* DE3 (Novagen) harboring pET-*oxyR* was grown in CG medium (Bio101) to log phase and induced with 1 mM IPTG for 1 h at 37°C. Cells were washed once with Tris buffer, pH 7.8 and resuspended in binding buffer (20 mM Tris, pH 8.0, 500 mM NaCl and 0.1 mM EDTA) plus 0.1% Triton X-100 before being sonicated on ice. A clear lysate was obtained after centrifugation at $10\,000 \times g$ for 10 min. The extract was loaded onto a Talon metal affinity column (Clontech). Elution of the bound OxyR by imidazole was carried out according to the manufacturer's procedure. The homogeneity of purified OxyR was determined by SDS–PAGE. Protein concentration was measured using the Bradford dye binding assay [11].

2.9. *Gel shift assay*

The assay was performed as previously described [12]. Briefly, the purified OxyR was dialyzed against TEG buffer. A 540-bp *Bam*HI-*Hin*cII fragment spanning the promoter region of *katG* was end-labeled using [α-^{32}P]dCTP and Klenow fragment. Gel shift reactions were performed by adding 3 fmol of labeled probe to TM buffer. To assay binding under reducing conditions, 200 mM dithiothreitol (DTT) was added to binding reactions.

2.10. *Oxidative stress sensitivity assays*

To test the susceptibility of *B. pseudomallei* strains to oxidative stress agents, disk inhibition assays were performed as previously described [4]. Thus, 6 μl of 0.5 M H_2O_2, 1 M menadione, 0.1 M *N*-ethylmaleimide (NEM), and 18 μl of 6% sodium hypochlorite (NaOCl) were used and the zones of growth inhibition around the disks were measured after 24 h of incubation.

2.11. *Nucleotide sequence accession number*

The GenBank accession number for *oxyR-katG-dpsA* genes is AY040244.

3. Results and discussion

3.1. *Cloning and sequence analysis of* katG *and* dpsA

We cloned a 6.0-kb *Xma*I fragment of *B. pseudomallei* P844 chromosomal DNA. DNA sequencing of the clone revealed that it overlapped the previous clone and contained two open reading frames oriented in the same direction. The first encoded the entire catalase-peroxidase enzyme (KatG) and the second encoded a protein of 162 amino acids with strong homology to the non-specific DNA binding protein (DpsA).

The deduced amino acid sequence of *B. pseudomallei* KatG (748 residues) shares very high levels of identity with eight other KatG sequences in the databases when compared and aligned using the CLUSTAL W program [13] (data not shown). The amino acid identities between *B. pseudomallei* KatG and its homologues in the databases are: 72% for *Mesorhizobium loti* (NP 107344), 68% for *Caulobacter virioides* (O31066), 66% for *Geobacillus stearothermophilus* (P14412), 64% for *Mycobacterium tuberculosis* (Q08129), 61% for *Legionella pneumophila* (Q9WXB9), *E. coli* (P13029) and *Vibrio cholerae* (AAF94714), and 58% for *Yersinia pestis* (NP 406785). The distal histidine refers to the residue forming the peroxide binding site on the distal side of the heme. The proximal residue is the histidine that binds to the proximal side of the heme. The distal and proximal histidine residues and sequences surrounding them are present in the *B. pseudomallei* clone and are highly homologous to those from other bacterial catalase-peroxidases [14]. Previously, the *B. pseudomallei* P844 catalase-peroxidase enzyme was purified, crystallized and analyzed by X-ray diffraction [15]. The enzyme is a homotetramer of 81.6-kDa subunits and contains heme *b*. The complete structure elucidation at high resolution of 1.7 Å of this enzyme is now in progress.

When the deduced amino acid sequence of DpsA (with a calculated molecular mass of 18 kDa) was analyzed and aligned with other Dps homologues, it revealed high homology (data not shown). The amino acid identities found are 74% for *Pseudomonas aeruginosa* (D83524), 58% for *Synechocystis* PCC6803 (S77503), 50% for *Xylella fastidiosa* (B82689), 47% for *Caulobacter crescentus* (AAK24837), and 30% for *Bacteroides fragilis* (AAG02618) and MrgA of *Bacillus subtilis* (G69660). DNA binding regions I and II, previously reported in Dps homologues from other bacteria [16], are conserved in *B. pseudomallei* DpsA.

3.2. *Regulation of* katG *and* dpsA *transcription*

To investigate the regulation of the expression of *katG* and *dpsA*, total RNA extracted from mid-exponential-phase cells, exposed to different oxidative stress conditions, was probed with radiolabeled *katG* and *dpsA* gene internal DNA fragments. The results are shown in Fig. 1A,B,D along with a map showing the genetic organization of both genes, including *oxyR*, together with the transcripts encoding each gene (Fig. 1C). Northern blot hybridization analysis revealed that expression of *katG* and *dpsA* mRNA was regulated at the transcriptional level. Under uninduced conditions, a transcript of approximately 2.9 kb was detected using a probe specific for *katG* (Fig. 1A), while a *dpsA*-specific probe detected a message of approximately 0.6 kb (Fig. 1B). By contrast, during growth under induced conditions in the presence of the oxidants t-BOOH, menadione, and H_2O_2, a common 3.5-kb transcript was detected encoding both *katG* and *dpsA* in addition to the

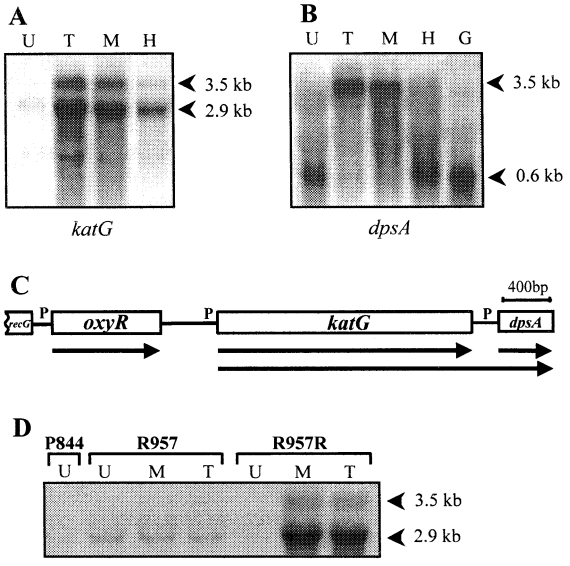

Fig. 1. Expression of *katG* and *dpsA* mRNA in response to oxidative stress and a diagram of their genetic organization. A: Northern analysis of *katG* mRNA from uninduced *B. pseudomallei* P844 cells (U), and from cells induced with t-BOOH (T), menadione (M), and H_2O_2 (H). B: Northern blot analysis of *dpsA* mRNA from *B. pseudomallei* P844 cells grown under the same conditions. Lane G contains RNA from the *katG* mutant, G221. Arrowheads indicate hybridizing mRNAs and their sizes (kb) are indicated. C: Diagram of the genetic organization of *oxyR*, *katG*, and *dpsA*. The arrows indicate the direction and extent of transcription. P indicates promoter regions. D: Northern analysis of *katG* mRNA from *B. pseudomallei* parent P844, *oxyR* mutant R957, and the complemented strain R957R. Total RNA was prepared from uninduced cells (U), and cells induced with menadione (M) and t-BOOH (T). Arrowheads indicate mRNA transcripts.

2.9-kb *katG* transcript (Fig. 1A,B, lanes T, M, H). Interestingly, under induced conditions, the 0.6-kb *dpsA* transcript was present in significant levels only during growth in the presence of H_2O_2 where it appeared to be induced only slightly (Fig. 1B, lanes T, M, H). H_2O_2 was also found to be a less effective inducer of *oxyR* expression in our previous study [4] suggesting that *B. pseudomallei* P844 may have high endogenous catalase activity that rapidly detoxifies H_2O_2. The fact that *dpsA* is transcribed on a 0.6-kb mRNA suggested that this message arose either from processing of the 3.5-kb *katG-dpsA* transcript or from initiation from a promoter situated in or near the *katG-dpsA* intergenic region. In order to differentiate between these two possibilities, Northern blot analysis was performed on total RNA extracted from the *katG* knockout mutant G221 grown in the presence of menadione. Only the 0.6-kb *dpsA* transcript was detected (Fig. 1B, lane G). Taken together, the Northern hybridization data indicate that the 3.5-kb *katG-dpsA* and 2.9-kb *katG* transcripts initiate from a promoter 5′ to *katG* while the 0.6-kb *dpsA* transcript is initiated from a separate promoter immediately upstream of *dpsA*.

Moreover, transcription initiation at the *dpsA* promoter appears to be inhibited by transcriptional read-through from the upstream *katG* promoter. This is suggested by the observation that the 0.6-kb *dpsA* transcript was absent when the upstream *katG* promoter was strongly induced (i.e. in the presence of t-BOOH or menadione, Fig. 1B, lanes T, M) and present only when the *katG* promoter was inactive (Fig. 1B, lanes U, G) or weakly induced by H_2O_2 (Fig. 1B, lane H). Co-transcription of *katG* and *dpsA* allows for a prompt response to oxidative stress. While KatG inactivates peroxides and hydroxyl radicals, Dps binds to the chromosome where it has been shown, in *E. coli*, to protect against the mutagenic effects of oxidative damage to the DNA [17]. KatG of *E. coli* is known to be regulated by OxyR during exponential-phase growth [18]. To test if *katG* of *B. pseudomallei* is regulated by OxyR, Northern blots of total RNA from the *oxyR* knockout mutant, R957 [4], was analyzed for *katG* mRNA during growth in the presence and absence of oxidants (Fig. 1D). The 3.5-kb mRNA (*katG-dpsA*) was not detected in R957, and the 2.9-kb mRNA (*katG*) was only very weakly expressed even in the presence of oxidants. The normal levels of the *katG* transcripts were restored and fully oxidant-inducible when a copy of *oxyR* was re-introduced into the mutant strain (Fig. 1D, R957R) indicating that the activation of *katG* transcription in response to oxidative stresses requires OxyR. The four putative OxyR binding tetranucleotide sequences (**ATTG**×7 **CCCG**×6 **ATTG**×7 **CAAG**×CTGCCA) and a putative −35 promoter element (underlined) were found 202 nucleotides upstream of the translation start site of *katG*. The residues in bold type within the putative OxyR binding site match the consensus sequence derived for *E. coli* OxyR-regulated promoters (ATAG×7 CTAT×7 ATAG×7 CTAT) [19]. Primer extension and DNase I footprinting experiments are needed to verify if these putative binding sequences are within the *katG* promoter.

3.3. katG *promoter responses to various stresses*

In order to further investigate the regulation of *katG* transcription response to both oxidative and osmotic stresses, β-galactosidase expression was monitored in strain P844TnpG, containing a chromosomal *katG-lacZ* transcriptional promoter fusion. The expression of *katG* in P844TnpG was induced eightfold, sixfold, and threefold after menadione, t-BOOH, and salt exposure, respectively (Fig. 2). The induction by oxidants was abolished in the *oxyR* knockout mutant R957TnpG. This is consistent with the Northern blot analysis result indicating that a functional OxyR is required to activate *katG* expression. It is worth noting that salt-induced *katG* promoter activity is OxyR-independent. In fact, *katG* expression is double in an *oxyR* mutant grown in the presence of high NaCl. In the case of *E. coli*, *katG*, in addition to being regulated by OxyR, is also a component of the *rpoS* regulon which has been shown to be activated by osmotic stress [20]. It is possible that *katG* in *B. pseudomallei* is also under control of both OxyR and RpoS thus explaining its activation by

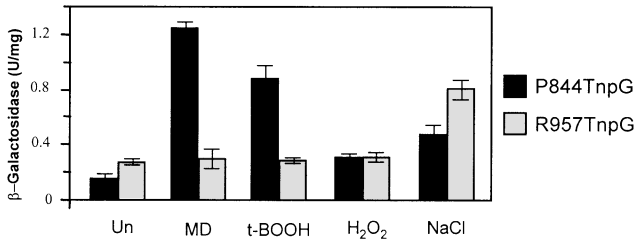

Fig. 2. Expression of the *katG* promoter in response to various stresses. β-Galactosidase activities in crude extracts of the *katG-lacZ* fusion parent (P844TnpG), and *oxyR* mutant (R957TnpG) prepared from uninduced cells (Un) and cells induced with menadione (MD), t-BOOH, H_2O_2, and NaCl. Each value shown is the mean of three separate experiments and error bars indicate S.E.M.

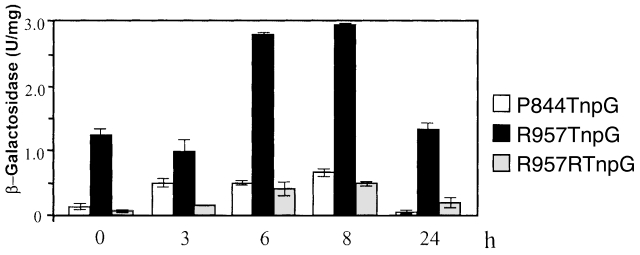

Fig. 3. Expression of *katG* promoter during growth. β-Galactosidase activities in crude extracts of a *katG-lacZ* fusion in parent (P844TnpG), *oxyR* mutant (R957TnpG), and the complemented strain (R957RTnpG). The enzyme activities were monitored and expressed as the mean of three separate experiments and error bars indicate S.E.M.

osmotic stress. Moreover, we observed that the *oxyR* mutant R957 exhibited a higher *katG* promoter activity during osmotic stress (this experiment, Fig. 2, Un) and higher basal *katG* mRNA expression during growth under uninduced conditions (Northern analysis, Fig. 1D) compared to the wild-type strain suggesting that OxyR represses *katG* transcription in the absence of oxidative stress.

3.4. katG *expression in wild-type and* oxyR *mutant during growth phase*

In order to determine whether OxyR functions as a repressor of *katG* transcription, β-galactosidase activities were monitored in wild-type (P844TnpG) and *oxyR* mutant (R957TnpG) backgrounds for 24 h (Fig. 3). Over the course of the experiment *katG* promoter activity was consistently higher in an *oxyR* mutant background (R957TnpG) compared to a wild-type background. Complementation of the *oxyR* mutant R957TnpG with a plasmid-borne *oxyR* in strain R957RTnpG reduced *katG* promoter activity to the level in the wild-type background. This finding suggests that OxyR, in addition to being an activator of *katG* when cells encounter oxidative threats, also acts as a repressor during the normal uninduced conditions. In *B. pseudomallei* P844, *katG* promoter activity reaches its highest level after 8 h of growth and then declines upon entry into stationary phase. This pattern is similar to that found in *E. coli* containing a *katG::lacZ* fusion. In *B. pseudomallei oxyR*, the expression of *katG* is higher than that of the wild-type in all phases of growth. This is in contrast to the observation in an *E. coli oxyR* mutant that *katG* expression is greatly reduced [21]. The results suggest that, unlike *E. coli* OxyR, *B. pseudomallei* OxyR represses *katG* expression during normal growth.

3.5. *Binding of both oxidized and reduced OxyR to* katG *promoter*

It has been shown in *E. coli* that during normal growth the bacterial cytosol is in a reduced state and that OxyR exists in a reduced form [22]. In the case of *Xanthomonas*, reduced OxyR was demonstrated to bind to and repress the *ahpC* promoter whereas oxidized OxyR activates *ahpC* expression [12]. We reported here that OxyR in untreated *B. pseudomallei* cells represses *katG* expression in vivo. This repression is reasonable and beneficial to keep the expression of *katG* low when cells are not exposed to an oxidative threat. We examined if purified OxyR protein can specifically bind to *katG* promoter. *B. pseudomallei* OxyR was purified to greater than 90% purity as judged by SDS–PAGE. OxyR was able to bind to the *katG* promoter under both oxidized and reduced conditions (Fig. 4). The differences in the pattern of binding of oxidized and reduced OxyR in the mobility shift may reflect dissimilar binding sites and affinities for the two forms. These data support our in vivo experiment that indicated that OxyR, in the absence of oxidative stress, represses *katG* expression. To our knowledge, repression of *katG* expression by OxyR has never been demonstrated in any bacterial system.

3.6. *Sensitivity of* katG *and* katG oxyR *mutants to various stresses*

The sensitivity of the *B. pseudomallei* P844 *katG* mutant G221 and the *katG oxyR* double mutant RG27 to several oxidants was measured. Both G221 and RG27 had increased sensitivity to H_2O_2, menadione, NEM, and hypochlorite (HOCl) killing compared to wild-type (P844) with RG27 having the highest sensitivity to these oxidants (Fig. 5). The altered sensitivities were restored to wild-type levels in the complemented strains G221TnG and RG27TnRG. KatG is a bifunctional enzyme having both catalase and peroxidase activities, therefore it was expected that the *katG* mutants would exhibit increased sensitivity to H_2O_2. Menadione, apart from being an intracellular superoxide generator, also causes a decreased total glutathione level, an increase in oxidized glutathione, and a decrease in the ratio of reduced to oxidized glutathione, thus shifting the cells to a more oxidized state and stimulating transcription of OxyR-inducible genes [23]. The thiol-alkylating agent NEM was reported to cause oxidative stress in human keratinocytes and, like menadione, rapidly depletes reduced glutathione [24]. Catalase protects *Xanthomonas* against electrophile toxicity of NEM [25]. HOCl exposure can generate hydroxyl radicals via a Fenton-type reaction and exponential-phase induction of the *oxyR* regulon protects *E. coli* cells against HOCl toxicity [26]. The fact that the lack of KatG expression causes *B. pseudomallei* to be more vulnerable to killing by different kinds of oxidants reflects the important roles *katG* plays in oxidative stress defense. Since catalase (KatA) of *Campylobacter jejuni* was found to be essential for its survival in macrophages [27], it would be interesting and of clinical importance to test if our mutants have an altered pattern of intramacrophage survival.

In conclusion, we described the genetic organization of *oxyR*, *katG*, and *dpsA* and their genetic regulation. Under uninduced conditions, both *katG* and *dpsA* are transcribed

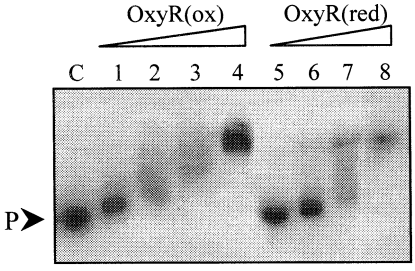

Fig. 4. Gel mobility shift assays of OxyR binding to the *katG* promoter region. To generate reduced conditions, 200 mM DTT was added to the binding reactions. ox and red indicate oxidized and reduced OxyR respectively. The following amounts of OxyR were added to the reactions: 75 ng (lanes 1 and 5), 150 ng (lanes 2 and 6), 250 ng (lanes 3 and 7) and 400 ng (lanes 4 and 8). P represents unbound probe. A labeled 540-bp DNA fragment containing the *katG* promoter was used as the probe.

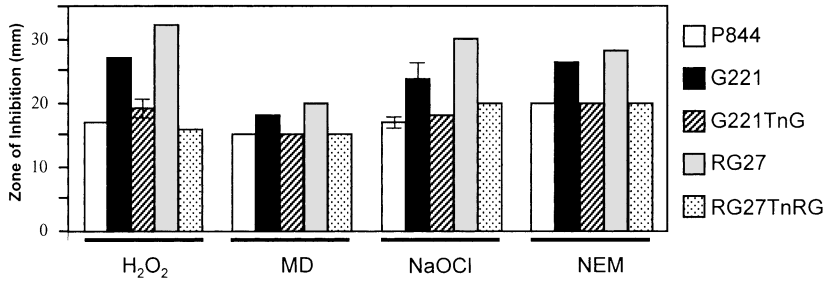

Fig. 5. Determination of levels of resistance to oxidative stress killing displayed by the *B. pseudomallei* parent strain P844, *katG* mutant G221, *katG oxyR* mutant RG27, and their complemented strains G221TnG and RG27TnRG. Determination of the levels of resistance to H_2O_2, menadione (MD), NEM, and NaOCl was performed as described in Section 2. Each value shown is the mean of three separate experiments and error bars indicate S.E.M.

monocistronically but when cells are exposed to oxidative stress both genes are activated and co-transcribed. OxyR is a dual function transcription regulator that represses *katG* expression under normal reduced conditions, but activates *katG* when cells encounter oxidative threats. KatG plays a significant protective role against various types of oxidants such as hydrogen peroxide, menadione, NaOCl, and NEM.

Acknowledgements: We thank M.F. Alexeyev and G. Shalom for providing pKNOCK and pGSTp, P. Munpiyamit for photograph preparation and J. Dubbs for critical reviewing the manuscript. This research was supported by grants from the Chulabhorn Research Institute and the senior research scholar RTA 4580010 grant from the Thailand Research Fund to S.M.

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