Response of *Mycobacterium tuberculosis* to Reactive Oxygen and Nitrogen Intermediates

Thomas R. Garbe, Nina S. Hibler, and V. Deretic

Department of Microbiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas, U.S.A.

ABSTRACT

Background: *Mycobacterium tuberculosis* is a significant human pathogen capable of replicating in mononuclear phagocytic cells. Exposure to reactive oxygen and nitrogen intermediates is likely to represent an important aspect of the life cycle of this organism. The response of *M. tuberculosis* to these agents may be of significance for its survival in the host.

Materials and Methods: Patterns of de novo proteins synthesized in *M. tuberculosis* H37Rv exposed to compounds that generate reactive oxygen and nitrogen intermediates were studied by metabolic labeling and two-dimensional electrophoresis.

Results: Menadione, a redox cycling compound which increases intracellular superoxide levels, caused enhanced synthesis of seven polypeptides, six of which appeared to be heat shock proteins. Chemical release of nitric oxide induced eight polypeptides of which only one could be identified as a heat shock protein. Nitric

oxide also exhibited a mild inhibitory action on general protein synthesis in the concentration range tested. Hydrogen peroxide did not cause differential gene expression and exerted a generalized inhibition in a dose-dependent manner. Cumene hydroperoxide caused mostly inhibition but induction of two heat shock proteins was detectable.

Conclusions: The presented findings indicate major differences between *M. tuberculosis* and the paradigms of oxidative stress response in enteric bacteria, and are consistent with the multiple lesions found in *oxyR* of this organism. The effect of hydrogen peroxide, which in *Escherichia coli* induces eight polypeptides known to be controlled by the central regulator *oxyR*, appears to be absent in *M. tuberculosis*. Superoxide and nitric oxide responses, which in *E. coli* overlap and are controlled by the same regulatory system *soxRS*, represent discrete and independent phenomena in *M. tuberculosis*.

INTRODUCTION

Although tuberculosis has never ceased to remain a global problem (1), it has been considered a conquered disease in developed countries. However, a recent resurgence of tuberculosis in the United States and other industrialized nations, which is linked to acquired immunodeficiency syndrome (AIDS) and socio-economic factors (2), is a reminder that more needs to be learned about its etiologic agent, *Mycobacterium tuberculosis*. This pathogen is readily taken up by mononuclear phagocytes where it is capable of multiplying. Stimulation with cytokines enables macrophages to reduce their mycobacterial burden (3–6), and based on experimental data it has been initially suggested that oxygen radicals are responsible for the intracellular killing of *M. tuberculosis* (7–9). More recent investigations however have shifted the emphasis from reactive oxygen species to reactive nitrogen intermediates (3–6), but perhaps both types of radicals are involved, as exemplified by hybrid products such as peroxynitrite (10).

In enteric bacteria, which currently serve as model systems for oxidative stress response (11,12), exposure to superoxide (O_2^-) generating compounds and hydrogen peroxide induces or enhances expression of over 90 genes and is controlled by several central regulators, two of which, OxyR and SoxRS, have been identified and thoroughly characterized (13–16) (Fig. 1A). The *oxyR* regulon is activated upon exposure to

Address correspondence and reprint requests to: Thomas R. Garbe, University of Texas Health Science Center at San Antonio, Department of Microbiology, 7703 Floyd Curl Drive, San Antonio, TX 78284-7739, U.S.A.
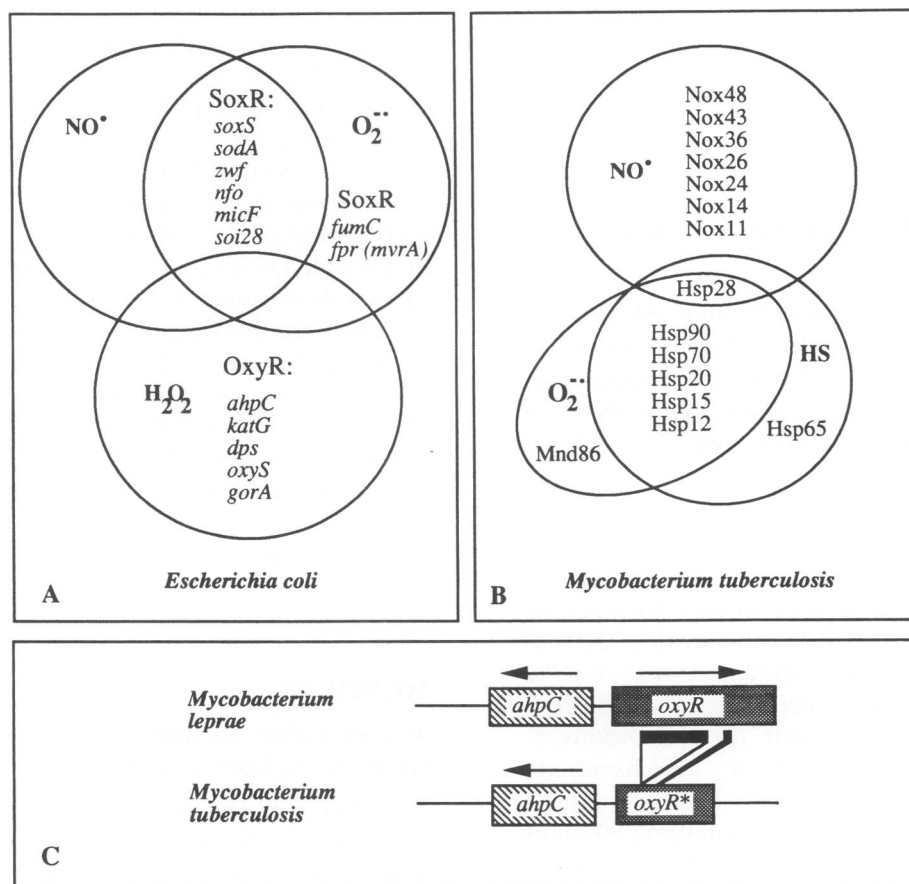
FIG. 1. Contrasting patterns of stress response in enteric bacteria and *M. tuberculosis*

The idiosyncrasies of stress response (circles) in enteric bacteria (A) and *M. tuberculosis* (B) can be summarized as follows: (i) No significant overlap between O_2^- and NO˙ regulon in *M. tuberculosis*. (ii) The majority of O_2^- induced polypeptides in *M. tuberculosis* are the previously characterized heat shock proteins (Hsp), with the exception of the newly identified O_2^- inducible polypeptide Mnd86. (iii) NO˙ induces a number of polypeptides (designated as Nox followed by apparent M_r) that appear not to be inducible by other stress factors tested. (iv) *M. tuberculosis* has multiple lesions in *oxyR* (24) as illustrated in Panel C (comparison with a functional *oxyR* gene from *M. leprae*; deletions are marked as filled rectangles). Consistent with these findings, there is no differential induction of gene expression in *M. tuberculosis* exposed to H_2O_2 except in the case of *katG*, which appears to be affected in combination with other cultural conditions (e.g., slightly elevated temperature) (24). HS, heat shock. Genes and protein names are explained in the text.

hydrogen peroxide and leads to increased synthesis of nine proteins, including catalase-peroxidase (KatG), glutathione reductase (GorA), alkyl hydroperoxide reductase (Ahp), and Dps, a nonspecific DNA-binding protein (17). The *soxRS* regulon in *Escherichia coli* is activated by superoxide generating compounds (15), nitric oxide (NO˙) (18,19), and by several oxidants even under anaerobic conditions (20,21). It has been suggested that the central regulator SoxR, which is an iron-sulfur protein (22), responds to the balance between reduced and oxidized flavodoxin and ferredoxin (21). This balance is disturbed by redox cycling compounds such as

menadione, which divert electrons from NADPH to the reduction of oxygen to generate O_2^- (21), resulting in the activation of at least 10 genes (15). These include the genes encoding Mn-containing superoxide dismutase (*sodA*), glucose 6-phosphate dehydrogenase (*zwf*), NADPH: ferredoxin oxidoreductase (*fpr*) (21), DNA repair endonuclease IV (*nfo*), and stable fumarase (*fumC*) (23).

We show here that the response of *M. tuberculosis* to oxidants differs from the response seen in *E. coli* and *Salmonella typhymurium* (Fig. 1B). These findings are consistent with the recent data indicating that the *M. tuberculosis* analog of

oxyR is inactivated by multiple mutations (24) (Fig. 1C), and suggest further differences between this organism and the currently known models of oxidative stress in enteric bacteria.

MATERIALS AND METHODS

Chemicals

S-nitroso-N-acetyl-D,L-penicillamine (SNAP) was from Alexis. Methyl-^{14}C–labeled molecular weight marker was from Amersham (Arlington Heights, IL, U.S.A.). All other chemicals were from Sigma Chemical Co. (St. Louis, MO, U.S.A.)

Cultures and Strains

M. tuberculosis H37Rv (ATCC 25618), was grown at 37°C and maintained at weekly passages on Middlebrook 7H10 (Difco Laboratories, Detroit, MI, U.S.A.) plates supplemented with 0.05% Tween 80 and OADC (oleic acid, albumin, dextrose, catalase) enrichment (Becton-Dickinson, BBL, Mountain View, CA, U.S.A.). Two hundred–milliliter polystyrene bottles (Corning Glassworks, Corning, NY, U.S.A.) containing 50 ml of Middlebrook 7H9 basal medium (Difco) without glycerol and detergent, supplemented with either ADC (albumin, dextrose, catalase) enrichment (Difco) or ADc (albumin-dextrose complex) enrichment, were inoculated with a loopful of bacterial material and incubated at 37°C on a continuous roller. ADc contained 5% bovine albumin fraction V (supplied by Boehringer Mannheim, Indianapolis, IN, U.S.A.), 2% glucose, and 0.85% sodium chloride and was filtrated through a 0.22-µm membrane.

### Metabolic Labeling and Two-Dimensional Gel Electrophoresis

M. tuberculosis cultures were incubated by continuous rolling for 3 days or as indicated and then used for labeling after pelleting by centrifugation at 3,000 × *g* for 5 min and 20°C and resuspending in ¹/₇ volume of the original growth medium. One-milliliter aliquots were exposed to tested compounds by adding 1 to 10 µl of solutions in water (hydrogen peroxide) or dimethyl sulfoxide (all other compounds) followed by addition of 10 µCi ^{35}S-methionine and ^{35}S-cysteine (NEN, protein labeling mix). After 4 hr of rolling incubation at 37°C, the samples were spun for 3 min in an Eppendorf microcentrifuge at maximum

speed. Approximately 50 µl of zirconium beads (0.1- to 0.15-mm diameter, Biospec Products, Inc., Bartlesville, OK, U.S.A.) and 100 µl of water were added to the pellets. Disruption of bacteria was achieved by vortexing in a mini-bead-beater-8 (Biospec Products) for 2 min at maximum speed. Equal volumes of the homogenates were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and by two-dimensional gel (2-D gel) electrophoresis as described previously (25,26). The proteins were transferred by electroblotting onto Immobilon-P membrane (Millipore, Marlborough, MA, U.S.A.) in 10 mM 3-[cyclohexylamino]-1-propanesulfonic acid buffer at pH 11 (NaOH) and 10% methanol at either 5 volts over night or 50 volts for 1 hr, and the filters were subjected to autoradiography.

RESULTS

M. tuberculosis Response to Superoxide Generating Compounds

The effects of O_2^- on protein synthesis in *M. tuberculosis* were examined by exposure to menadione, a redox cycling compound which increases intracellular levels of O_2^- (21,27,28). Samples from cultures treated with menadione concentrations that caused differential display of newly synthesized polypeptides by SDS-PAGE analysis were further examined by electrophoresis in 2-D gels. Several phenomena were observed: (i) The response to menadione was dependent on the growth stage. The two cultures shown in Figs. 2 and 3 were in different growth stages as noticeable by the differences in the patterns of de novo synthesized polypeptides seen in Panels A (Figs. 2 and 3), representing the two untreated control cultures. (ii) In the menadione treated samples, qualitative differences in the polypeptide induction and overall inhibition of protein synthesis were seen in the 3- and 7-day cultures. (iii) Most of the inducible proteins overlapped with several characterized heat shock proteins (26), namely Hsp15, Hsp28, Hsp70 (DnaK), and Hsp90 (Figs. 2 and 3). The up-regulated 20-kD polypeptide in Figs. 2B and 3B is most likely the previously identified Hsp20 (26). (iv) Occasionally, exposure to 300 µM menadione induced an 86-kD polypeptide (Mnd86) (data not shown). Since this polypeptide overlapped with the position in 2-D gels of a polypeptide reactive with polyclonal antibodies
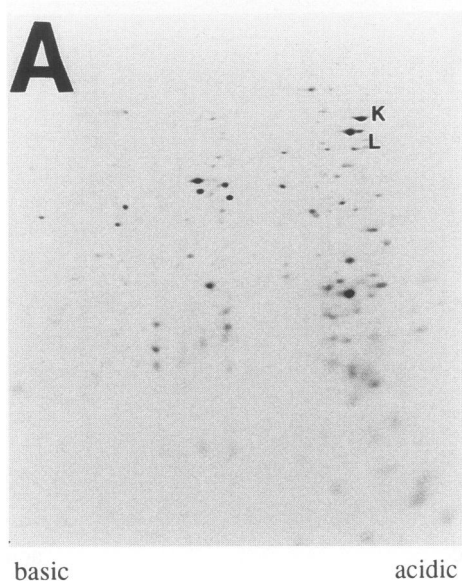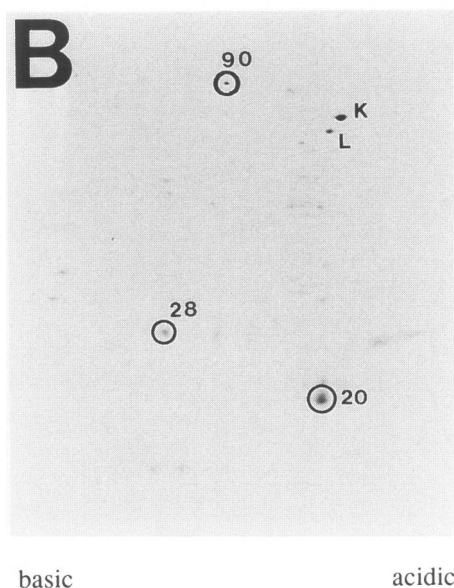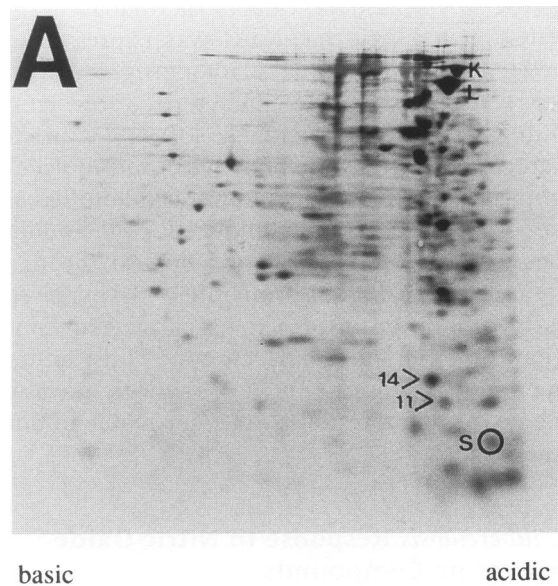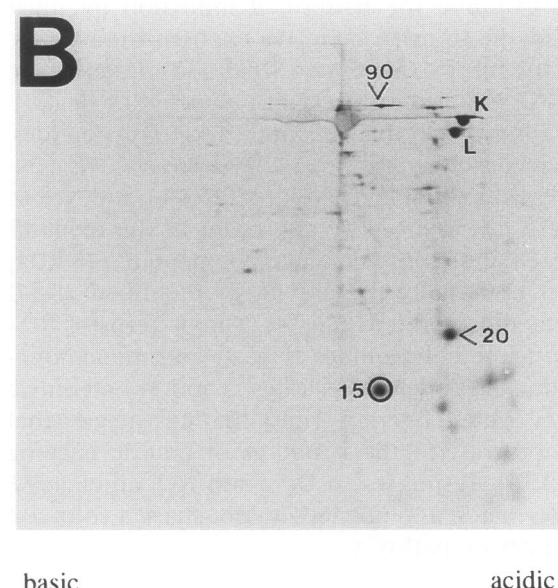
FIG. 2.   Early growth phase response in *M. tuberculosis* to menadione

Autoradiograms show 2-D gel profiles of metabolically labelled proteins in aliquots of a 3-day *M. tuberculosis* H37Rv culture in 7H9+ADc medium without any addition (A) and with 300 μM menadione, a superoxide generating redox cycling compound (B). Circles indicate induced polypeptides and numbers indicate their apparent molecular weights. K, DnaK (Hsp70) (26); L, GroEL (Hsp65) (26,35). DnaK is also up-regulated by menadione.

FIG. 3.   Late growth-phase response of *M. tuberculosis* to menadione

Autoradiographs from 2-D gels show profiles of metabolically labeled polypeptides of aliquots from a 6-day *M. tuberculosis* H37Rv culture in 7H9+ADc medium (A) and with 300 μM menadione (B). Several prominent polypeptides are highlighted and numbers indicate their apparent molecular weights. Polypeptides 14 and 11 kD in Panel A are identical with Nox14 and Nox11 of Fig. 4 and are repressed by menadione (B). The circled spot S overlaps with one of the major spots that occasionally show up by total protein stain (data not shown) and is possibly Hsp GroES, as judged by position and earlier published data (26). Other markings as in Fig. 2.

against *M. tuberculosis* catalase-peroxidase (29) (KatG), it is likely that KatG was expressed at elevated levels in some menadione-treated samples. (v) The previously characterized iron-containing superoxide dismutase (30,31) with its characteristic isoelectric point (26) and molecular mass of 23 kD (30), could not be identified as one of the differentially induced polypeptides (Figs. 2 and 3). Furthermore, superoxide dismutase activity did not comigrate with increased ^{35}S incorporation in proteins separated in nondenaturing gels (data not shown). Thus, either superoxide dismutase is not induced or any change may be too subtle to be detectable in our experiments.

M. tuberculosis Response to Nitric Oxide Generating Compounds

NO$^{.}$ activates the *soxRS* regulon in *E. coli* (18,19). To examine the pattern of induction by nitric oxide in *M. tuberculosis*, we exposed the bacteria to the nitric oxide donor SNAP (32). Exposure to SNAP induced a variety of polypeptides (Fig. 4). Among these, the 11- and 14-kD polypeptides, termed Nox11 and Nox14, respectively, were expressed at high levels (Fig. 4). Nox11 and Nox14 were not induced by any other of the oxidants tested. Only one inducible polypeptide (28 kDa) was potentially the previously identified 28-kD heat shock protein (Fig. 4). The patterns of NO$^{.}$-inducible polypeptides (Fig. 4) and menadione-inducible polypeptides (Figs. 2 and 3) had only a very limited overlap. These findings suggest that in contrast to the situation in enteric bacteria (18,19), responses to $O_2^{-.}$ and NO$^{.}$ differ in *M. tuberculosis*, and possibly do not share a common regulatory pathway.

M. tuberculosis Response to Hydrogen Peroxide and Organic Peroxides

Exposure of *M. tuberculosis* to a range of hydrogen peroxide concentrations did not display any differential gene expression as judged by SDS-PAGE (Fig. 5) and by patterns of de novo protein synthesis in 2-D gels (not shown). In contrast to *E. coli* and *S. typhimurium*, where hydrogen peroxide enhances synthesis of at least 30 polypeptides (16), the effect of hydrogen peroxide on *M. tuberculosis* was that of a generalized inhibition, and synthesis of most polypeptides ceased in a dose-dependent manner. Inhibition was clearly visible at concentrations as low as 100 μM (Fig. 5). Analysis by 2-D gel electrophoresis

basic   acidic

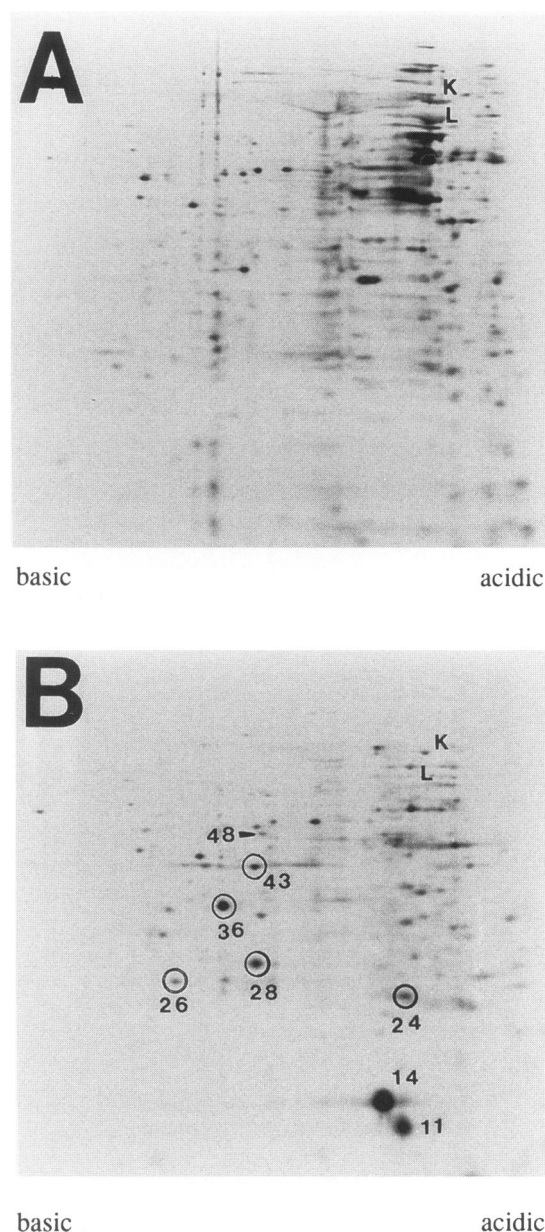basic   acidic

FIG. 4. *M. tuberculosis* response to nitric oxide generating compound SNAP

Autoradiograms of 2-D gels show newly synthesized proteins in aliquots of a 3-day *M. tuberculosis* H37Rv culture in 7H9+ADc medium. (A) Control (no additions). (B) 100 μM SNAP, an NO$^{.}$-generating compound. Circles or wedges highlight the most prominently induced polypeptides and numbers indicate their apparent molecular weights. Reference to these polypeptides is given in the text by the prefix Nox. K and L, as in Figs. 2 and 3.

showed no differential induction of polypeptides upon exposure to hydrogen peroxide with the exception of a variable presence of a polypeptide with an apparent M_r similar to the observed M_r of Mnd86 (data not shown). In our previous
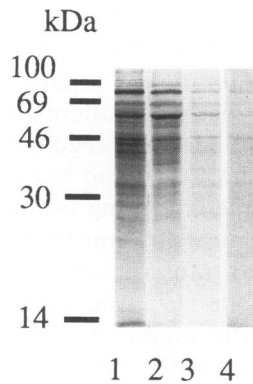
FIG. 5. Effects of hydrogen peroxide on protein synthesis in *M. tuberculosis*

SDS-PAGE autoradiogram shows metabolically labeled polypeptides in aliquots taken from a three day culture of *M. tuberculosis* H37Rv in 7H9+ADc exposed to increasing amounts of hydrogen peroxide. Lanes: 1, control; 2–4, 0.1 μM; 1 μM; and 10 μM hydrogen peroxide. Exposure of Lane 4 was twice as long as the other lanes.

report on *katG* studies (24), we could detect a 2-fold induction of the corresponding polypeptide only under slightly elevated growth temperatures. The overall lack of response to hydrogen peroxide is in sharp contrast to the reaction of enteric bacteria. These findings appear to be consistent with the recent observation that the putative *oxyR* gene of *M. tuberculosis* has an incomplete open reading frame and is most likely inactive (24).

To probe the response of the tubercle bacillus to organic peroxides, we employed cumene hydroperoxide. In repeated experiments, this compound proved to be more inhibitory than hydrogen peroxide. However, in contrast to hydrogen peroxide, a concentration of cumene hydroperoxide was found showing a pattern consistent with differential gene expression. The results of a titration experiment with cumene hydroperoxide, analyzed by SDS-PAGE, are shown in Fig. 6A. Induction of a 15- and a 28-kD polypeptide occurred at 200 μM cumene hydroperoxide and was further enhanced at 500 μM. This effect was not visible at 100 μM, a concentration that was nevertheless inhibitory for protein synthesis. Further analysis of the inducible polypeptides by 2-D gel electrophoresis yielded a distinct pattern depicted in Fig. 6B. The cumene hydroperoxide-inducible 15- and 28-kD polypeptides overlap with the positions of the previously characterized heat shock proteins Hsp15 and Hsp28 (26). Figure 6B also shows 45- and 53-kD polypeptides (Chp45 and Chp53), not discernible on single dimension SDS-PAGE autoradiograms (Fig. 6A).
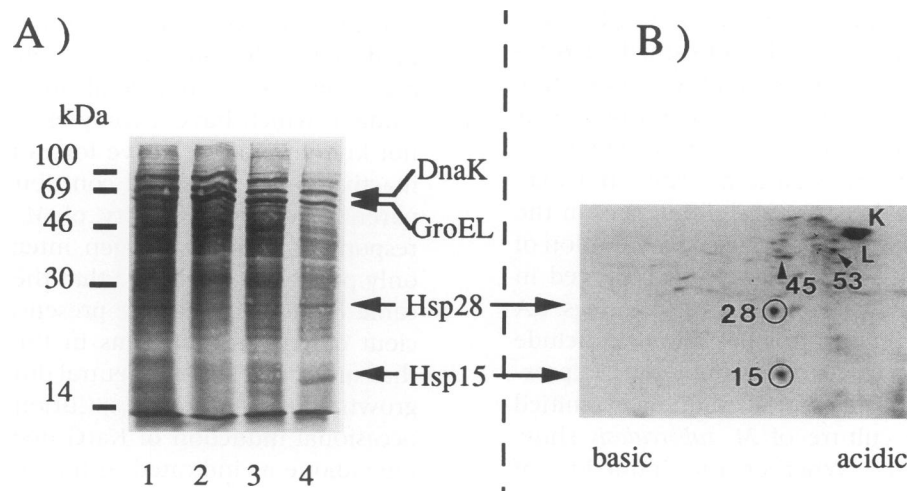

FIG. 6. *M. tuberculosis* response to cumene hydroperoxide

(A) SDS-PAGE analysis of metabolically labelled polypeptides in aliquots from culture grown in 7H9+ADC medium exposed to increasing amounts of cumene hydroperoxide. Lanes: 1, 37°C control; 2–4, 100 μM; 200 μM; and 500 μM cumene hydroperoxide. (B) Analysis by 2-D gel electrophoresis of the 500 μM cumene hydroperoxide treated sample (Panel A, Lane 4). Two major induced polypeptides are labeled as heat shock proteins 15 and 28 on the basis of their positions and earlier published data (26). The 45- and 53-kD polypeptides are referred to in the text as Chp45 and Chp53. DnaK and GroEL (Hsp70 and Hsp65) are indicated as markers to facilitate comparisons.

DISCUSSION

In this work, the patterns of gene expression in *M. tuberculosis* in response to challenges with reactive oxygen and nitrogen intermediates have been examined and compared. Several conclusions can be drawn: (i) *M. tuberculosis* has a very limited response to hydrogen peroxide which is in agreement with the recently reported naturally occurring lesions in the putative *oxyR* gene of *M. tuberculosis* (24) (Fig. 1C). (ii) Exposure to redox cycling agents, which increase endogenously generated O_2^-, causes induction of a number of gene products. However, the majority of these polypeptides appear to overlap with the heat shock proteins (Fig. 1B). (iii) A vigorous and complex display of differential gene expression is observed upon exposure to NO˙ generating compounds. (iv) There is very little overlap between O_2^--induced response and heat shock proteins on one side and NO˙-induced polypeptides on the other (Fig. 1B), suggesting that they may be controlled by independent regulatory mechanisms.

Perhaps the most intriguing observation presented here is the differential display of gene expression in response to nitric oxide. Preliminary studies with NO˙-generating compounds other than SNAP (e.g., nitroso glutathione, diethylenetriamine nitric oxide adduct) support the data shown in this work. NO˙ gas was not used in the present study, since a recent report (19) indicates that a slow but continued release of NO˙ has a more profound effect than a bolus of NO˙, which may be quickly consumed in reactions with oxygen. Using several NO˙ generating reagents we have observed induction of similar polypeptides but also noticed some variability in 2-D gel electrophoresis patterns. The latter may be partly due to the structural differences in the tested chemicals but is also caused by variation of metabolic activity in *M. tuberculosis* observed in parallel untreated control cultures (e.g., Figs. 2A and 4A). Nevertheless, it is possible to conclude that nitric oxide elicits differential gene expression in this organism since all aliquots examined from the same culture of *M. tuberculosis* show consistent results. Whether the induction of Nox11, Nox14, and additional polypeptides (Fig. 1B) is related to the pathogenesis and virulence of *M. tuberculosis* is currently under investigation. The most prominent polypeptides induced by all NO˙ generating compounds tested are being further characterized.

Another observation of potential relevance for the idiosyncrasies of oxidative stress response in *M. tuberculosis* is the difference in patterns of O_2^-- and NO˙-induced polypeptides. In contrast to this observation, recent studies in *E. coli* indicate that in this bacterium there are major overlaps between O_2^- and NO˙ regulons (Fig. 1). The central regulator of O_2^- and NO˙ response in *E. coli* is SoxR, a redox sensing iron-sulfur protein (22) which, upon activation by a redox signal, causes increased transcription of 10 genes via a regulatory cascade (19). The analyses presented here provide little support for possible overlaps between O_2^-- and NO˙-inducible systems in *M. tuberculosis*. It is also worth noting the conspicuous absence of detectable induction of superoxide dismutase upon exposure to menadione. Furthermore, six of the seven polypeptides consistently inducible with menadione appear to be heat shock proteins. One possible explanation for this phenomenon is that the effects of O_2^- on gene expression may be indirect, i.e., via protein denaturation (33), a process suggested to induce heat shock response (34).

The observations reported here and elsewhere (24) suggest that *M. tuberculosis* and other members of the *M. tuberculosis* complex have undergone a partial loss of response to oxidative stress in the course of their evolution. Some vestiges of the tubercle bacillus ancestor's capacity to adjust to the stress imposed by reactive oxygen intermediates can be seen by the presence of an inactive *oxyR* gene upstream of *ahpC* (24). The lesions in *oxyR* have been observed in all strains and members of *M. tuberculosis* complex examined to date, in contrast to the situation in *Mycobacterium leprae* and several other mycobacteria studied, which have a complete *oxyR* gene. It is not known what selective forces resulted in the inactivation of *oxyR* and contributed to the apparently reduced capacity of *M. tuberculosis* to respond to reactive oxygen intermediates. It is only possible to speculate that these systems became obsolete due to the presence of more efficient defense mechanisms in this organism, or that such processes are neutral during the in vivo growth of *M. tuberculosis*. Curiously, we found occasional induction of KatG upon exposure to menadione as indicated in this work and also a slight change with hydrogen peroxide, most prominently observed under growth at elevated temperatures (24). However, the detected increases in newly synthesized KatG appear to be sensitive to small variations in culture conditions, and any conclusion regarding potential regulation must await further genetic investigations.

In contrast to the apparent lack of specific response to reactive oxygen intermediates is the synthesis of at least seven novel proteins in response to NO˙. While the role of this radical remains controversial for the infections in humans, members of the *M. tuberculosis* complex infect other host species that have a demonstrable inducible NO˙ synthase in macrophages. Furthermore, several studies (3–6) suggest that NO˙-mediated killing of intracellular *M. tuberculosis* takes place in macrophages from such sources. Whether the members of the *M. tuberculosis* complex have evolved specific regulatory and defense mechanisms to cope with exposure to NO˙ is currently under investigation.

ACKNOWLEDGMENTS

REFERENCES

1. Kochi A. (1991) The global tuberculosis situation and the new control strategy of the World Health Organization. *Tubercle* **72:** 1–6.
2. Murray JF, Mills J. (1990) Pulmonary infectious complications of human immunodeficiency virus infection. *Am. Rev. Respir. Dis.* **141:** 1356–1372.
3. Flesch I, Kaufmann SHE. (1991) Mechanisms involved in mycobacterial growth inhibition by gamma interferon–activated bone marrow macrophages: Role of reactive nitrogen intermediates. *Infect. Immun.* **59:** 3213–3218.
4. Denis M. (1991) Interferon-gamma–treated murine macrophages inhibit growth of tubercle bacilli via the generation of reactive nitrogen intermediates. *Cell. Immunol.* **132:** 150–157.
5. Chan J, Xing J, Magliozzo RS, Bloom BR. (1992) Killing of virulent *Mycobacterium tuberculosis* by reactive nitrogen intermediates produced by activated murine macrophages. *J. Exp. Med.* **175:** 1111–1122.
6. Chan J, Tanaka K, Carroll D, Flynn J, Bloom BR. (1995) Effects of nitric oxide synthase inhibitors on murine infection with *Mycobacterium tuberculosis. Infect. Immun.* **63:** 736–740.
7. Jackett PS, Aber VR, Mitchison DA, Lowry DB. (1981) The contribution of hydrogen peroxide resistance to virulence of *Mycobacterium tuberculosis* during the first six days after intravenous infection of normal and BCG-vaccinated guinea pigs. *Br. J. Exp. Pathol.* **62:** 34–40.
8. Jackett PS, Andrew PW, Aber VR, Lowrie DB. (1983) Guinea pig alveolar macrophages probably kill *M. tuberculosis* H37Rv and H37Ra in vivo by producing hydrogen peroxide. *Adv. Exp. Med. Biol.* **162:** 99–104.
9. Walker L, Lowrie DB. (1981) Killing of *Mycobacterium microti* by immunologically activated macrophages. *Nature (Lond.)* **293:** 69–70.
10. Zhu L, Gunn C, Beckman JS. (1992) Bactericidal activity of peroxynitrite. *Arch. Biochem. Biophys.* **298:** 452–457.
11. Farr SB, Kogoma T. (1991) Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium. Microbiol. Rev.* **55:** 561–585.
12. Demple B, Amábile-Cuevas CF. (1991) Redox redux: The control of oxidative stress responses. *Cell* **67:** 837–839.
13. Christman MF, Morgan RW, Jacobson FS, Ames BN. (1985) Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium. Cell* **41:** 753–762.
14. Tsaneva IR, Weiss B. (1990) *soxR*, a locus governing a superoxide response regulon in *Escherichia coli* K-12. *J. Bacteriol.* **172:** 4197–4205.
15. Amábile-Cuevas CF, Demple B. (1991) Molecular characterization of the *soxRS* genes of *Escherichia coli*: two genes control a superoxide stress regulon. *Nucleic Acids Res.* **19:** 4479–4484.
16. Storz G, Altuvia S. (1994) OxyR regulon. *Methods Enzymol.* **234:** 217–223.
17. Altuvia S, Almiron M, Huisman G, Kolter R, Storz G. (1994) The *dps* promoter is activated by OxyR during growth and by IHF and s^s in stationary phase. *Mol. Microbiol.* **13:** 265–272.
18. Nunoshiba T, DeRojas-Walker T, Wishnok JS, Tannenbaum SR, Demple B. (1993) Activation by nitric oxide of an oxidative-stress response that defends *Escherichia coli* against activated macrophages. *Proc. Natl. Acad. Sci. U.S.A.* **90:** 9993–9997.
19. Nunoshiba T, DeRojas-Walker T, Tannenbaum RS, Demple B. (1995) Roles of nitric oxide in inducible resistance of *Escherichia coli* to activated murine macrophages. *Infect. Immun.* **63:** 794–798.

20. Privalle CT, Fridovich I. (1990) Anaerobic biosynthesis of the manganese-containing superoxide dismutase in *Escherichia coli*. Effects of diazenedicarboxylic acid bis(N,N'-dimethylamide) (diamide). *J. Biol. Chem.* **265:** 21966–21970.

21. Liochev SI, Hausladen A, Beyer Jr WF, Fridovich I. (1994) NADPH:ferredoxin oxidoreductase acts as a paraquat diaphorase and is a member of the *soxRS* regulon. *Proc. Natl. Acad. Sci. U.S.A.* **91:** 1328–1331.

22. Hidalgo E, Demple B. (1994) An iron-sulfur center essential for transcriptional activation by the redox-sensing SoxR protein. *EMBO J.* **13:** 138–146.

23. Liochev SI, Fridovich I. (1992) Fumarase C, the stable fumarase of *Escherichia coli*, is controlled by the *soxRS* regulon. *Proc. Natl. Acad. Sci. U.S.A.* **89:** 5892–5896.

24. Deretic V, Philipp W, Dhandayuthapani S, et al. (1995) *Mycobacterium tuberculosis* is a natural mutant with inactivated oxidative stress regulatory gene: Implications for sensitivity to isoniazid. *Mol. Microbiol.* **17:** 889–900.

25. O'Farrell PZ, Goodman HM, O'Farrell PH. (1977) High resolution two-dimensional electrophoresis of basic as well as acidic proteins. *Cell* **12:** 1133–1142.

26. Young DB, Garbe TR. (1991) Heat shock proteins and antigens of *Mycobacterium tuberculosis*. *Infect. Immun.* **59:** 3086–3093.

27. Walkup L, Kogoma T. (1989) *Escherichia coli* proteins inducible by oxidative stress mediated by superoxide radical. *J. Bacteriol.* **171:** 1476–1484.

28. Greenberg JT, Demple B. (1989) A global response induced in *Escherichia coli* by redox-cycling agents overlaps with that induced by peroxide stress. *J. Bacteriol.* **171:** 3933–3939.

29. Heym B, Zhang Y, Poulet S, Young D, Cole ST. (1993) Characterization of the *katG* gene encoding a catalase-peroxidase required for isoniazid susceptibility of *Mycobacterium tuberculosis*. *J. Bacteriol.* **175:** 4255–4259.

30. Zhang Y, Lathigra R, Garbe T, Catty D, Young D. (1991) Genetic analysis of superoxide dismutase, the 23 kilodalton antigen of *Mycobacterium tuberculosis*. *Mol. Microbiol.* **5:** 381–391.

31. Cooper JB, McIntyre K, Badasso MO, et al. (1995) X-ray structure analysis of the iron-dependent superoxide dismutase from *Mycobacterium tuberculosis* at 2.0 Ångstroms resolution reveals novel dimer-dimer interactions. *J. Mol. Biol.* **246:** 531–544.

32. Gibson A, Babbedge R, Brave SR, et al. (1992) An investigation of some S-nitroso-thiols, and of hydroxy-arginine, on the mouse anococcygeus. *Br. J. Pharmacol.* **107:** 715–721.

33. Weiner L, Kreimer D, Roth E, Silman I. (1994) Oxidative stress transforms acetylcholinesterase to a molten-globule-like state. *Biochem. Biophys. Res. Commun.* **198:** 915–922.

34. Bukau B. (1993) Regulation of the *Escherichia coli* heat shock response. *Mol. Microbiol.* **9:** 671–680.

35. Kong TH, Coates ARM, Butcher PD, Hickman CJ, Shinnik TM. (1993) *Mycobacterium tuberculosis* expresses two chaperonin-60 homologs. *Proc. Natl. Acad. Sci. U.S.A.* **90:** 2608–2612.