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Role of *Salmonella* Flavohemoglobin in Nitric Oxide and Redox Homeostasis

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Salmonella enterica is a Gram-negative enteric bacterium that is pathogenic for humans and other animal hosts. Host phagocytic cells use inducible nitric-oxide (iNOS) to generate the antimicrobial radical, nitric oxide (NO·) (1). Intracellular pathogens such as *Salmonella* must resist the antimicrobial actions of NO· and NO· mediated reactive nitrogen species (RNS) produced by host cells. To this end pathogens possess several NO· metabolizing enzymes. To examine the importance of RNS metabolism to *Salmonella* virulence, the virulence of isogenic mutant strains lacking flavohemoglobin Hmp, GSNO reductase, flavorubredoxin, and cytochrome *c* nitrite reductase were compared. The *hmp* mutant strain exhibited almost complete loss of virulence, whereas the other mutants possessed virulence similar to that of wild type *Salmonella*. Abrogation of murine-inducible NO· synthase restored virulence to *hmp* mutant *Salmonella*, indicating that the Hmp flavohemoglobin promotes *Salmonella* virulence by detoxifying NO· generated by host cells. The *hmp* mutant *Salmonella* were also more susceptible to chemically-generated NO· and to iNOS-expressing macrophages.

The biochemistry of flavohemoglobin have been characterized heme and flavoreductase domains. NO· binds Hmp at its heme ligand (2). Electrons are transferred from NAD(P)H to the ferric heme ligand via FAD, resulting in reduction of NO· to form a heme-bound nitroxyl anion equivalent (NO⁻)(3). NO⁻/HNO is further converted to NO_3^- or N_2O in the presence or absence of oxygen, respectively. Site-directed mutagenesis of Hmp employed in this study showed that mutation of either heme or flavoreductase domain of Hmp abrogates *Salmonella* resistance to nitrosative stress, consistently indicating the electron transfer from NAD(P)H to FAD to heme for NO· metabolism in *Salmonella* Hmp. However, under aerobic conditions in the absence of NO·, elevated Hmp expression increased susceptibility to hydrogen peroxide. The flavoreductase domain but not the heme domain was required for Hmp-mediated susceptibility to oxidative stress in an iron-dependent manner. This provides a rationale for the negative regulation of *hmp* expression under iron-rich conditions. A ferric uptake regulator Fur has been known as the ideal regulator for *hmp* transcription in *Salmonella* (4). But a screen
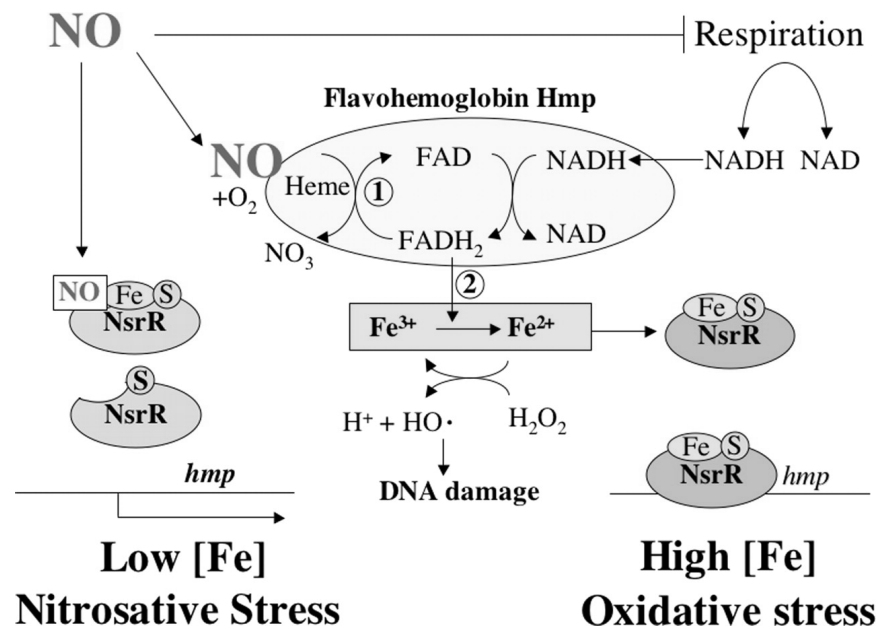
Fig. Model of NO · and redox homeostasis maintenance by flavohemnoglobin Hmp.

for the regulator of *hmp* transcription identified the transcriptional regulator NsrR but not Fur. The *nsrR* mutation derepressed *hmp* transcription in iron-rich media, comparable with mRNA levels observed in NO·-treated or iron-chelator-treated wild type cells. NsrR is a homolog of Fe-S containing IscR transcriptional regulator, which controls expression of the *isc/suf* regulons that encode enzymes required for Fe-S cluster biosynthesis (5).

The following figure summarizes this study. In the presence of NO·, inhibition of bacterial respiration can increase intracellular pools of NAD(P)H (6, 7), which might enhance oxidative damage unless electrons are directed to ferric-nitrosyl Hmp to promote reaction of heme-bound NO· with O_2. In this way Hmp can actually ameliorate oxidative stress (pathway 1). However, bacterial respiration can also be inhibited in the absence of NO·, e.g. by antimicrobial peptides produced by host cells. Under such circumstances, elevated levels of NAD(P)H can lead to reduction of Hmp-bound FAD. Intermolecular transfer of these reducing equivalents could promote oxidant damage. Consequently, expression of Hmp in the absence of NO· may increase FAD- and iron-dependent damage by hydrogen peroxide (pathway 2).

In conclusion, this study shows that the flavohemoglobin Hmp is the principal enzyme responsible for aerobic NO· metabolism by *Salmonella* but requires precise regulation to avoid the exacerbation of oxidative stress that can result if electrons are shuttled to extraneous iron.

Key references

1. Fang, F. C. (2004) Nat. Rev. Microbiol. 2, 820-832.

2. Hernandez-Urzua, E. *et al.* (2003) J. Biol. Chem. 278, 34975-34982.

3. Hausladen, A., Gow, A., and Stamler, J. S. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 10108-10112.

4. Crawford, M. J., and Goldberg, D. E. (1998) J. Biol. Chem. 273, 34028-34032.

5. Kiley PJ and Beinert H. (2003) Curr Opin Microbiol. 6, 181-185.

6. Stevanin, T. M. *et al.* (2000) J. Biol. Chem. 275, 35868-35875.

7. Woodmansee, A. N., and Imlay, J. A. (2002) J. Biol. Chem. 277, 34055-34066.