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MICROBIOLOGY

S-Nitrosylation Signaling in *Escherichia coli*

Ivan Gusarov and Evgeny Nudler*

Most bacteria generate nitric oxide (NO) either aerobically by NO synthases or anaerobically from nitrite. Far from being a mere by-product of nitrate respiration, bacterial NO has diverse physiological roles. Many proteins undergo NO-mediated posttranslational modification (S-nitrosylation) in anaerobically grown *Escherichia coli.* The regulation of one such protein, OxyR, represents a redox signaling paradigm in which the same transcription factor controls different protective genes depending on its S-nitrosylation versus S-oxidation status. We discuss a structural model that may explain the remarkable stability and specificity of OxyR S-nitrosylation.

The response of *Escherichia coli* and other bacteria to nitric oxide (NO) has been studied extensively because of its clinical relevance (*1*–*3*). Activated macrophages generate large amounts of NO as a defense against pathogens (*4*–*6*). Thus, interfering with mechanisms that protect bacteria against NO may be an effective strategy to mitigate infection. However, bacteria were exposed to NO in the environment well before the evolutionary appearance of immune cells. For eons, NO was the only accessible form of nitrogen for ancient organisms (*7*), forcing bacteria to develop multiple mechanisms to safely use it. With the decrease of NO in Earth's changing atmosphere, microorganisms developed pathways for the anaerobic synthesis of NO by nitrite reductase and aerobic synthesis of NO by nitric oxide synthases (*8*). These pathways ultimately appear in eukaryotes (*9*).

The proteins that initially sensed NO eventually evolved to monitor O_2. For example, hemoglobin is thought to have developed originally to bind NO, and bacterial single-subunit hemoglobins are still the first line of defense against nitrosative stress (*10*). Only later in evolution did hemoglobins acquire a high affinity for O_2 to serve as its carriers. Just as bacteria evolved proteins to carry and make use of NO and O_2, they needed to also evolve transcription factors to sense these gases and respond by adjusting bacterial gene expression to changing redox environments. Seth *et al*. investigated the complex nature of one such transcription factor, OxyR (*11*).

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Originally denoted as an oxidative stress sensor, reduced OxyR binds promoter DNA. Upon oxidation, the Cys^{199} residue of OxyR forms an intramolecular disulfide bond with its Cys^{208} residue to promote cooperative binding to RNA polymerase and to activate numerous oxidative stress defense genes (*12*). It has been proposed that OxyR could also be S-nitrosylated to activate transcription (*13*), but subsequent genome-wide screens for nitrosative stress response genes failed to demonstrate a major role for OxyR in this implementation (*1*–*3*).

When comparing the results of screens for the nitrosative stress response, it is important to consider the specific experimental conditions. Mukhopadhyay *et al*. and Flatley *et al*. treated *E. coli* with exogenous *S*-nitrosoglutathione (GSNO) or nitrite in rich media under aerobic or chemostat (physiological steady-state) conditions, respectively (*1*, *3*). Both GSNO and nitrite primarily donate the nitrosonium ion (NO^+) under these experimental conditions. Unlike NO, NO^+ cannot diffuse freely through membranes. In contrast, Seth *et al*. (*11*) examined physiologically produced, endogenous NO. They compared anaerobic gene expression in bacteria respiring on either nitrate (to promote NO production) or fumarate (to inhibit NO production) in defined media. During anaerobic respiration, nitrate as a terminal electron acceptor is reduced to nitrite and further to NO or ammonia. Under these conditions, NO was continuously produced, and if not controlled, it would have accumulated to potentially toxic concentrations. The results of Seth *et al*. suggested that OxyR monitors the amount of intracellular S-nitrosylation and regulates the expression of genes dedicated to prevent it.

Seth *et al*. demonstrated that Cys^{199} of OxyR, which is responsible for the oxidative stress response, was nitrosylated in vivo in response to endogenous nitrosative stress (*11*). Only Cys^{199}, one of six Cys residues in OxyR, was S-nitrosylated, indicating that OxyR S-nitrosylation is highly specific. Two complementary mechanisms of S-nitrosylation have been described. According to the autocatalytic mechanism of protein nitrosylation, the hydrophobic core of a protein accumulates NO and accelerates the otherwise slow trimolecular reaction of NO oxidation through micellar catalysis (*14*). Alternatively, NO can be oxidized to NO^+ by reaction with a transition metal (*15, 16*). The latter reaction can occur in both aerobic and anaerobic conditions. NO^+ can interact with solvent-exposed thiols in the vicinity of amino acid side chains that promote and stabilize S-nitrosylated (SNO) adducts (*17*). Seth *et al*. demonstrate that in the case of anaerobic respiration on nitrate, divalent cations are necessary for S-nitrosylation. However, because nitrate reductase (which converts nitrate to nitrite) was essential for SNO formation, whereas nitrite reductase (which converts nitrite to NO) was not, the molecular mechanism of the transition of NO_2^- to SNO awaits clarification.

Seth *et al*. also reported that OxyR S-nitrosylation activated transcription of a strikingly different set of genes than did OxyR oxidation. Although the results of Seth *et al*. suggested that the products of OxyR-SNO–activated genes help to manage endogenous NO activity, most of these proteins have no known function in nitrosative stress response. One of the few genes induced by both exogenous and endogenous nitrosative stresses, *hcp*, is particularly notable (*1*, *11*). Despite possessing hydroxylamine reductase activity, *hcp* is not induced by hydroxylamine, nor does it protect *E. coli* against hydroxylamine (*18*). Hcp protein is implicated in defending *Salmonella enterica* against nitrosative stress (*19*). Seth *et al*. demonstrated the importance of Hcp in defending *E. coli* against macrophage-derived NO (*11*). The regulation of *hcp* transcription is complex. At least three transcription factors—FNR, NarL, and OxyR—bind to the *hcp* promoter, and deletions of either *fnr* or *oxyR* abolish *hcp* transcription (*11, 18*). Determination of the molecular function of Hcp may help to explain the complexity of its regulation.

The authors' findings regarding S-nitrosylation in bacteria and the cellular response to it are provocative, challenge earlier models (*3*), and should spark further investigation. From the clinical perspec-

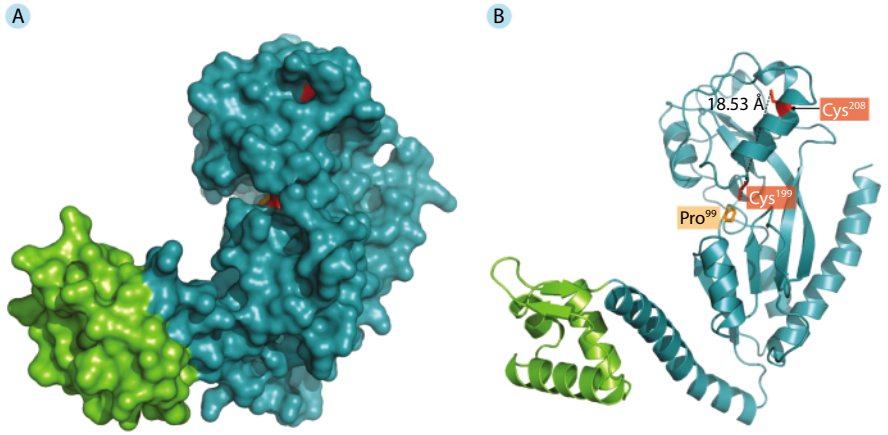

Fig. 1. Structural model of full-length OxyR as predicted by I-TASSER. Molecular surface (**A**) and cartoon (**B**) representations were generated in Pymol. Catalytically active cysteine residues (Cys^{199} and Cys^{208}) are shown in red, the proline (Pro^{99}) in the ligand-binding pocket in orange; a dotted line indicates the distance between Cys^{199} and Cys^{208}. The DNA binding domain is light green. The model has a C score of 0.86 and TM score of 0.83 ± 0.08.

tive, it would be important to catalog and compare S-nitrosylated proteins between nonpathogenic and pathogenic *E. coli* and its disease-causing relatives, such as *Salmonella*. Another exciting issue is whether endogenous S-nitrosylation occurs and has a role in signaling in Gram-positive bacteria. Some Gram-positive bacterial species, including the notorious pathogens *Staphylococcus aureus* and *Bacillus anthracis*, have NO synthases and generate NO aerobically to defend against various types of chemical stress (*20*). From the mechanistic point of view, it would be interesting to determine the structural changes induced by S-nitrosylation in a protein and to explain how these changes alter its specific activity.

Only a partial crystal structure of OxyR is available (*21*). Using I-TASSER (*22*), we built a three-dimensional model of full-length OxyR (Fig. 1), which resembles LysR-type transcriptional regulators with most similarity to CbnR (*23*). Thus, we predict that OxyR also binds DNA as a pair of dimers, causing the DNA to bend (*23*). OxyR and other LysR-type structural homologs share a common ligand-binding site predicted to be between Cys^{199} and Pro^{99} (Fig. 1). The surface representation (Fig. 1A) suggests that Cys^{199} in this ligand-binding pocket would be easily accessible to small molecules, such as peroxide or NO, but not to reducing molecules, such as glutathione, which may explain the high stability of OxyR-SNO and other redox modifications of this protein (*13*). The I-TASSER model has the position of Cys^{208}, which is supposed to form disulfide with Cys^{199} upon oxidation, not only far from Cys^{199} (distance 18.53 Å, Fig. 1B), but also separated from it by a rigid α helix (Fig. 1A). Thus, according to this structural model, OxyR has to undergo a dramatic conformational change to form such a disulfide bond. It would be interesting to compare the structural changes of OxyR caused by either oxidation or S-nitrosylation.

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