Point of VIEW

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A Twist on Heme Signaling

Thomas Spiro*

Department of Chemistry, University of Washington, Seattle, Washington 98195

ABSTRACT Proteins in the H-NOX family act as sensors of NO or O_2. This family includes soluble guanylate cyclase (sGC), the NO sensor that is responsible for vasodilation and neurotransmission in mammals. The crystal structures of bacterial H-NOX domains have revealed a highly distorted heme cofactor. This distortion has now been shown to be associated with a concerted displacement of the entire N-terminal half of the protein. This displacement likely provides the mechanism for transducing the ligand binding event into signaling.

How do biological systems sense the presence of the gaseous diatomic molecules CO, NO, and O_2? They are nonpolar, or nearly so, and have no handles for charge or H-bond interactions that proteins typically use for small-molecule binding. Nature's answer is the heme prosthetic group, to which these molecules bind strongly, through back-donation of electrons from the heme Fe. The heme group has been recruited to proteins that transduce the gas binding event into an enzymatic reaction or into gene activation or repression, thereby acting as sensors. How does this transduction work? On page 703 of this issue, Olea *et al.* (*1*) provide an exciting clue. For at least one important family of heme sensor proteins, the heme seems to signal by doing the twist.

This family has been dubbed H-NOX (heme nitric oxide/oxygen binding) (*2*). In eukaryotes, the principal H-NOX protein is soluble guanylate cyclase (sGC), an enzyme of enormous interest because it is the receptor for NO. sGC is responsible for regulation of vasodilation and neurotransmission through production of the secondary messenger, cyclic GMP, when NO binds to its heme (*3*). H-NOX domains are also found in prokaryotes and likely serve in sensory systems for NO or O_2 (*2*). They are homologous with the heme-binding domain of sGC. Crystal structures are available for H-NOX domains from the bacteria *Thermoanaerobacter tengcongensis* (*Tt* H-NOX) (*4, 5*) and *Nostoc* (*Ns* H-NOX) (*6*). A striking feature of these structures is that the hemes are highly distorted from their normal planar geometry. To varying degrees, the four heme pyrrole rings swivel and twist out of the plane.

Is the heme distortion part of the sensing mechanism? To test this idea, Olea *et al.* (*1*) mutated a proline residue in *Tt*H-NOX to alanine. P115 butts up against pyrrole D (Figure 1), which experiences the greatest out-of-plane displacement. Olea *et al.* reasoned that the less sterically encumbered alanine might allow the heme to relax. Indeed they found a significantly flatter heme in the P115A variant (Figure 2). Strikingly, the heme flattening is associated with significant displacement (3.8 Å rms deviation) of the entire N-terminal half of the molecule, composed of helices A, B, and C, and the connecting loops (Figure 2). This displacement is connected to a number of nonbonded interactions, which are seen to be relaxed when the heme flattens in the P115A variant. In particular, the A helix residues Met1 and Ile5 are in contact with the methyl and propionate substituents of pyrrole A and shift away from the heme as pyrrole A moves into the heme plane.

The concerted N-terminal displacement suggests an obvious pathway for connecting the ligand binding event to the activation of the sensory apparatus, in which the H-NOX domain is presumably imbedded. *Tt* H-NOX is a member of the Tar4 family of receptors and is fused to a predicted methyl-accepting chemotaxis protein (MCP) (*2*). There are two predicted membrane-spanning regions between the H-NOX and MCP domains, which would then be on the same side of the membrane. Ligation-induced displacement at the interdomain

*Corresponding author, spiro@chem.washington.edu.

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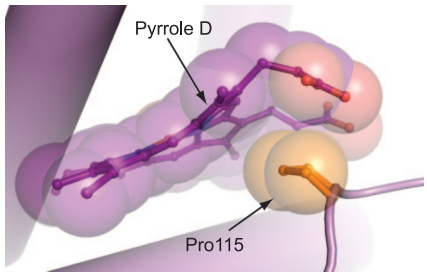

Figure 1. Heme prosthetic group and a view of its distortion in *Tt* H-NOX, showing the nonbonded contact with Pro115. Adapted from Olea, C., Boon, E., Pellicena, P., Kuriyan, J., and Marletta, M. (2008) Probing the function of heme distortion in the H-NOX family, *ACS Chem. Biol.* 3, 703–710. Copyright 2008 American Chemical Society.

contact is therefore a plausible mechanism for signal transduction.

Olea *et al.* (*1*) also detected functional consequences at the heme. The Fe(III/II) reduction potential decreased significantly in the P115A variant, by 170 mV. This effect is opposite to what might have been expected (*7*) from studies of model compounds, which suggest that heme flattening favors Fe(II) over Fe(III). Thus other factors may override the heme distortion effect on redox potential.

In addition, the O_2 affinity increased in P115A, mainly because of a 5-fold reduction in the rate of O_2 dissociation. No significant changes in bond length or H-bond distances (the bound O_2 is H-bonded by a distal tyrosine residue) could be seen between native and P115A adducts. However, the authors noted a tilt in the bond connecting the heme Fe with the proximal histidine ligand (78° bond angle) in the native protein, whereas this bond is perpendicular to the heme plane in the P115A variant. They suggested that the perpendicular orientation improves the imidazole-Fe bond strength, thereby stabilizing the Fe–O_2 complex.

These intriguing findings raise many questions. Is the heme distortion directly responsible for signaling, or are other interactions involved? One structural feature to consider is the tight network of H-bonds connecting the heme propionate substituents on pyrroles A and D (*4*), the rings that figure prominently in the heme distortion. As these pyrroles swivel in and out of the plane, the propionate interactions must be affected. The H-bonds are donated by a triad of residues, Tyr-Ser-Arg, which is common to all H-NOX sequences. Computational modeling (*8*) has suggested that the heme distortion itself requires little energy, but distortion-coupled changes in propionate H-bonds could involve significant energy. It has been suggested that these H-bonds can account for anomalous vibrational spectroscopic signatures of H-NOX CO adducts.

Another issue is the relationship of ligand affinity to the heme distortion. To function in sensing, the heme distortion must be caused by ligand binding. Indeed, high-resolution crystal structures of the NO-binding protein nitrophorin (*9*), which also features significantly distorted heme, have revealed that the distortion increases significantly when NO replaces water as a heme ligand. However, if ligand binding favors heme distortion, then conversely heme distortion must favor ligand binding. Yet Olea *et al.* found *stronger* O_2 binding for the flatter P115A variant. Clearly, other factors than heme flattening must be involved (the Fe-histidine tilt noted by the authors may be one of them), but the apparent paradox does warn of complexities that vitiate a simple correlation of ligation and heme distortion.

Finally, there is the question of how these new results can illuminate the mechanism of sGC activation. Although sGC is not membrane-bound, it also is constructed of a heme domain at one end and a catalytic (cyclase) domain at the other, with a connecting domain (PAS-like domain) in between (*6*). If ligand binding induces concerted displacements in sGC, as in *Tt* H-NOX, then coupling at the interface between heme and cyclase domains may be the pathway for activation. However, binding of NO to sGC induces release of the proximal histidine from the heme, after transient formation of a six-coordinate adduct. The histidine release is synchronous with cyclase activation (*10*) and has been inferred to be a central event in signaling. Is this release connected to heme distortion, or does it involve a separate mechanism? A linkage between the two is suggested by the finding

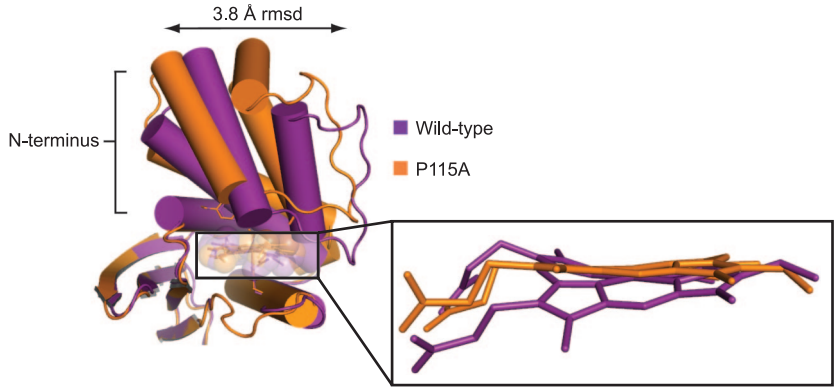

Figure 2. Overlay of wild-type (purple) and P115A (gold) variants of *Tt* H-NOX molecules, showing the heme flattening and the concerted movement of the N-terminal half of the structure, associated with the residue substitution. Adapted from Olea, C., Boon, E., Pellicena, P., Kuriyan, J., and Marletta, M. (2008) Probing the function of heme distortion in the H-NOX family, *ACS Chem. Biol.* 3, 703–710. Copyright 2008 American Chemical Society.

that heme out-of-plane vibrational modes are induced in the resonance Raman spectrum of the sGC-CO adduct upon addition of synthetic molecule activators YC-1 (*11*) or BAY-41-2272 (*12*). The normally low activity of the CO adduct is boosted by these activators to levels comparable with that induced by NO. These out-of-plane modes are indicators of heme distortion. In addition, the Raman spectra reveal that the activators induce release of the proximal histidine in a fraction of the sGC-CO molecules. Thus, these activators connect heme distortion with a tendency for histidine release. These observations reinforce the relevance of the new H-NOX structures for our understanding of sGC.

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