

HEME DEFORMATION AND A NOVEL HEME-ASSISTED NITROSILATION OF A THIOLATE LIGAND IN NITROPHORINS FROM BLOOD-SUCKING INSECTS

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Nitrophorins, heme proteins from blood-sucking insects, serve to transport nitric oxide (NO) from the insect salivary gland to the tissue of a victim, where vasodilation caused by NO release leads to enhanced feeding for the insect. For an efficient transport and release to occur, nitrophorins use a ferric heme center as an NO carrier. Stabilization of the ferric state against NO-dependent autoreduction, which would result in a ferrous heme and nearly irreversibly bound NO, is a key feature of these proteins. Nitrophorins from *R. prolixus* (*rNP*) are the first example of heme proteins with heme group contained in an exclusively β -sheet environment. Binding of NO to *rNP* is pH sensitive and is associated with a large conformational change in the protein [1]. Based on our ultra-high resolution structures of numerous *rNP* complexes, we hypothesize that steric interactions between the heme and the lipocalin type β -barrel of the protein result in a severe deformation of the heme, and as a consequence, stabilization of the ferric state.

A different way to ensure the reversibility of NO binding has been uncovered in nitrophorin from *C. lectularius* (*cNP*). *cNP*, completely unrelated to *rNP*, is a thiolate-bound heme protein with some structural elements resembling proteins from the cytochrome P450 family. Similarly to *rNP*, NO binding to *cNP* is pH sensitive and the heme group is stabilized in the ferric state. However, our structural, spectroscopic and kinetic data reveal that NO binding can occur at both the heme and the proximal cysteine, suggesting a unique, built-in mechanism for NO binding and release that involves reversible heme-assisted nitrosilation of the thiolate ligand. This novel mechanism may have implications for studies of other heme-thiolate proteins like NO synthase or cytochromes P450.

REFERENCE

1. Weichsel, A., Andersen, J.F., Roberts, S.A. and Montfort, W.R. Nitric oxide binding to nitrophorin 4 induces complete distal pocket burial. **Nature Struct. Biol.** 7 (2000) 551-554.