Nitric Oxide: A Biological Messenger

Caroline M. Pavot Literature Seminar November 23, 1999

Nitroglycerin has been used as a chest pain reliever for over a century, but it was not until twenty years ago that its effect was associated to its ability to release nitric oxide[1]. This led to the discovery that nitric oxide (NO) is produced in many cells as a signal molecule from the aerobic decomposition of L-arginine into L-citrulline, catalyzed by the enzyme nitric oxide synthase. Its physiological effects include the regulation of vasodilatation[2], gene expression[3], muscle contraction[4], and calcium and potassium transport[5]. NO can also induce cell-damage (Parkinson's Disease, HIV dementia) mainly by reacting with superoxides to form peroxynitrites[6]. Owing to its multiple roles, NO has encouraged a lot of research in view of possible medical applications.

The message carried by NO is expressed by the interaction of NO with a receptor. This usually happens at the metal center of a metalloenzyme but also occurs via nitrosation of thiols. In blood flow regulation, the binding of NO to heme centers is a crucial step in the signaling pathway.

The first discovered and best-understood effect of NO is its activation of the protein soluble guanylate cyclase (sGC) by interaction with a heme iron in the regulatory domain of the enzyme[2]. Studies of the heme coordination support a mechanism of activation in which the binding of NO induces a change in the protein structure, increasing its ability to produce cyclic 3',5'-guanosine monophosphate (cGMP). cGMP is a secondary signal molecule that is able to activate potassium channels, which are involved in blood vessel relaxation.

As there is no crystal structure available for sGC, the information concerning this enzyme has been obtained mainly by biochemical assays and spectroscopic studies. It has been shown that NO activates sGC *in vivo* by 100 to 400-fold, whereas CO has no effect. EPR[7], Resonance Raman[8], and UV-vis spectroscopy[9] (Fig. 1) on the native-enzyme as well as on manganese and cobalt substituted enzymes[10] show that prior to activation, the heme is 5-coordinated with a histidine (probably His 105, as indicated by mutagenesis[11]) as the proximal ligand. The activated conformation of the enzyme has been found to contain a 5-coordinate heme complex, obtained by breaking the proximal Fe-His bond upon NO addition (fig. 2). CO, on the contrary, increases the strength of the proximal ligand bond, forming a non-activated 6-coordinate species.

Resonance Raman indicates a negatively charged distal pocket, which could be responsible for the low binding affinity of O_2. In addition, this could cause rapid dissociation of NO to deactivate sGC[8].

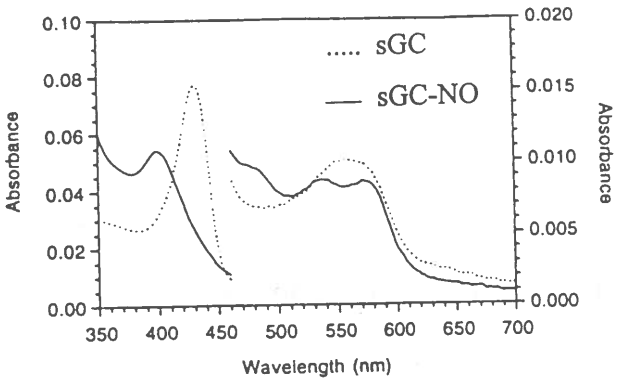

Figure 1

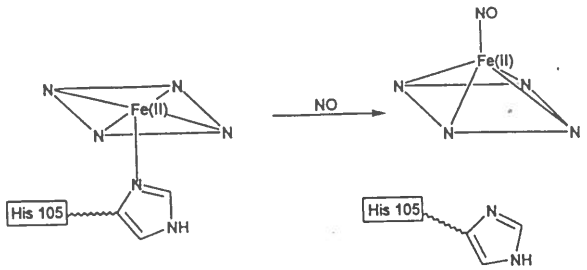

Figure 2

NO has also been shown to interact with hemoglobin. At first it was considered to be a NO scavenger pathway to eliminate NO as NO_3^-. This interaction has been shown recently to be involved in the regulation of blood vessel contraction[12]. NO first binds to the heme center of the deoxygenated hemoglobin[13], and is then transferred to a cysteine residue on the protein[14], to form an *S*-nitrosothiol. This species could then generate NO by decomposition, or transfer NO to another thiol. *S*-nitrosothiols have been observed *in vivo* in different situations. Their formation could be catalyzed by metalloproteins[15]. *S*-nitrosothiols have often been regarded as NO carriers, delivering the molecule at greater distances than NO alone, thus avoiding its degradation. In this case, the *S*-nitrosothiol appears solvent exposed (that is, liable to NO release) only in the T (deoxygenated) state of hemoglobin. NO would therefore play a role in oxygen sensing, increasing the blood flow when oxygen is scarce[12].

Although toxic, NO has been shown to play many important roles in *in vivo* signaling. The crystal structure of sGC, and mechanism of NO dissociation from this enzyme, as well as more information about the role of *S*-nitrosothiol *in vivo*, would greatly enhance our knowledge of blood-flow regulation, and would certainly help in the study of other NO regulation pathways. This should have significant importance for medical applications, particularly for cardiovascular and neuralgic diseases.

References

1. Murad, F. "Nitric Oxide Signaling: Would You Believe That a Simple Free Radical Could Be a Second Messenger, Autacoid, Paracrine Substance, Neurotransmitter, and Hormone?," *Recent Prog. Horm. Res.* **1998**, *53*, 43-60.

2. Ignarro, L. J.; Ballot, B.; Wood, K. S. "Regulation Of Soluble Guanylate Cyclase Activity By Porphyrins and Metalloporphyrins," *J. Biol. Chem.* **1984**, *259*, 6201-7.

3. Foster, M. W.; Cowan, J. A. "Chemistry Of Nitric Oxide With Protein-Bound Iron Sulfur Centers. Insights On Physiological Reactivity," *J. Am. Chem. Soc.* **1999**, *121*, 4093-4100.

4. Abraham, R. Z.; Kobzik, L.; Moody, M. R.; Reid, M. B.; Stamler, J. S. "Cyclic GMP Is a Second Messenger By Which Nitric Oxide Inhibits Diaphragm Contraction," *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.* **1998**, *119A*, 177-183.

5. Bolotina, V. M.; Najibi, S.; Palacino, J. J.; Pagano, P. J.; Cohen, R. A. "Nitric Oxide Directly Activates Calcium-dependent Potassium Channels In Vascular Smooth Muscle," *Nature (London)* **1994**, *368*, 850-3.

6. Dawson, V. L.; Dawson, T. M. "Nitric Oxide In Neurodegeneration," *Prog. Brain Res.* **1998**, *118*, 215-229.

7. Stone, J. R.; Sands, R. H.; Dunham, R.; Marletta, M. A. "Electron Paramagnetic Resonance Spectral Evidence For the Formation Of a Pentacoordinate Nitrosyl-heme Complex On Soluble Guanylate Cyclase," *Biochem. Biophys. Res. Commun.* **1995**, *207*, 572-7.

8. Deinum, G.; Stone, J. R.; Babcock, G. T.; Marletta, M. A. "Binding Of Nitric Oxide and Carbon Monoxide To Soluble Guanylate Cyclase As Observed With Resonance Raman Spectroscopy," *Biochemistry* **1996**, *35*, 1540-7.

9. Stone, J. R.; Marletta, M. A. "Spectral and Kinetic Studies On the Activation Of Soluble Guanylate Cyclase By Nitric Oxide," *Biochemistry,* **1996**, *35*, 1093-9.

10. Dierks, E. A.; Hu, S.; Vogel, K. M.; Yu, A. E.; Spiro, T. G.; Burstyn, J. N. "Demonstration Of the Role Of Scission Of the Proximal Histidine-iron Bond In the Activation Of Soluble Guanylyl Cyclase Through Metalloporphyrin Substitution Studies," *J. Am. Chem. Soc.* **1997**, *119*, 7316-7323.

11. Zhao, Y.; Schelvis, J. P. M.; Babcock, G. T.; Marletta, M. A. "Identification Of Histidine 105 In the b1 Subunit Of Soluble Guanylate Cyclase As the Heme Proximal Ligand," *Biochemistry* **1998**, *37*, 4502-4509.

12. Gow, A. J.; Luchsinger, B. P.; Pawloski, J. R.; Singel, D. J.; Stamler, J. S. "The Oxyhemoglobin Reaction Of Nitric Oxide," *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 9027-9032.

13. Gow, A. J.; Stamler, J. S. "Reactions Between Nitric Oxide and Hemoglobin Under Physiological Conditions," *Nature (London)* **1998**, *391*, 169-173.

14. Chan, N.-L.; Rogers, P. H.; Arnone, A. "Crystal Structure Of the S-Nitroso Form Of Liganded Human Hemoglobin," *Biochemistry* **1998**, *37*, 16459-16464.

15. Inoue, K.; Akaike, T.; Miyamoto, Y.; Okamoto, T.; Sawa, T.; Otagiri, M.; Suzuki, S.; Yoshimura, T.; Maeda, H. "Nitrosothiol Formation Catalyzed By Ceruloplasmin. Implication For Cytoprotective Mechanism In Vivo," *J. Biol. Chem.* **1999**, *274*, 27069-27075.