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Why NO?†

Teddy G. Traylor* and Vijay S. Sharma

Departments of Chemistry, 0506, and Medicine, 0652, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0506

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Four recent discoveries have greatly increased interest in nitric oxide. (1) It has been shown that the presence of NO is involved in several important biological events including vascular smooth muscle relaxation (Ignarro, 1989), platelet deaggregation (Azuma et al., 1986; Furlong et al., 1987; Rodomski et al., 1987), neuronal communication (Garthwaite, 1991), and possibly photoreceptor signaling (Stryer, 1986; Horio & Murad, 1991). (2) This involvement of NO has been found to occur through activation of guanylate cyclase, a heme-containing enzyme which catalyzes the reaction GTP → cGMP (Ignarro et al., 1981; Murad, 1986). (3) Nitric oxide released by murine macrophages and other cells after immunological activation acts as a cytotoxic molecule for invading intracellular microorganisms and tumor cells (Marletta et al., 1988; Hibbs et al., 1988; Stuehr et al., 1989; Moncada et al., 1991a). All of the enzymes affected by cytotoxic NO including ribonucleotide reductase (the rate-limiting enzyme in DNA replication) contain catalytically active non-heme iron coordinated to sulfur atoms (Hibbs et al., 1990). In all cases the inhibition of enzyme activity was accompanied by the loss of intracellular iron from the target cells (Hibbs et al., 1984). (4) An enzyme, nitric oxide synthase, converts L-arginine to NO (Bredt & Snyder, 1990; Moncada et al., 1991b; Mayer et al., 1989, 1990; Knowles et al., 1989). The endogenously produced NO is known as endothelium-derived relaxation factor (EDRF) because of its role in the relaxation of vascular smooth muscles. These and related observations have raised the title question, "why NO?" What makes NO so special that it, rather than CO, O_2, or other ligands, is used as a trigger for these important processes.

A possible answer is simply that NO is an extraordinarily different ligand with regard to its reaction with hemes and non-heme iron proteins. First, and possibly irrelevant, is the fact that NO binds to both iron(II) porphyrins and iron(III) porphyrins, which sets it apart from CO and O_2 (Klein & Hartree, 1973; Sharma et al., 1983, 1987; Sharma & Ranney, 1978). But the key to the choice of NO to trigger some new reaction is probably in its unusual binding affinities to various forms of iron(II) porphyrins. We compare the two reactions (eqs 1 and 2), where Hm represents an iron(II) porphyrin

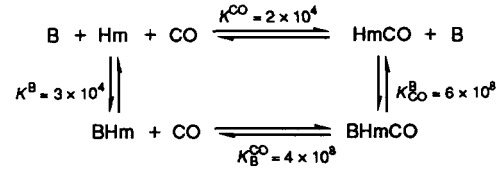
$$\mathrm{Hm} + \mathrm{L} \rightleftharpoons \mathrm{HmL} \qquad (1)$$

$$\mathrm{BHm} + \mathrm{L} \rightleftharpoons \mathrm{BHmL} \qquad (2)$$

without ligands (4-coordinated heme) and B represents an alkylimidazole.

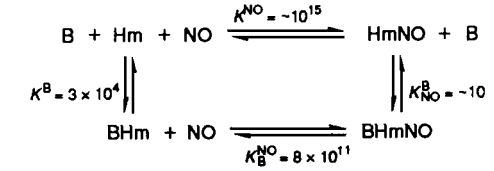
With CO, isocyanides, pyridines, and many other ligands $K_2 > K_1$; that is, B and L are synergistic, each increasing the binding affinity of the other (Rougee & Brault, 1975; White et al., 1979; Momenteau, 1976). Not so with NO! The ex-

Scheme I: Carbon Monoxide Binding[a]



[a] The data used here are from Rougee and Brault (1973, 1975), White et al. (1979), Traylor et al. (1979), and Geibel et al. (1978).

Scheme II: Nitric Oxide Binding[a]



[a] A different set of titrations results in an estimate of $K^{NO} \cong 10^{14}$ M^{-1}. The additional data are from Shimazu et al. (1984), Rose and Hoffman (1983), Scheidt and Frisse (1975), and Romberg and Kassner (1979).

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* Address correspondence to this author at the Department of Chemistry.

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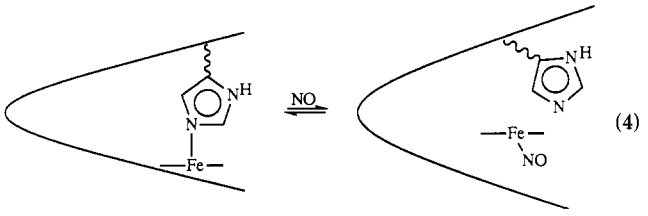
traordinary difference between NO and other ligands such as CO is illustrated in Schemes I and II, one for CO and the other for NO. Some of the equilibrium constants are not accurately known, not having been measured directly. But an error of 10-fold would not affect the conclusions. Although Schemes I and II are qualitatively similar, their quantitative differences are so large as to have major qualitative differences in the chemical consequences outlined below. The first striking thing about these schemes is the incredibly large value of K^{NO}, the equilibrium constant for binding NO to 4-coordinate heme. But the important point for the special nature of NO as a trigger is that it binds better *without proximal base*, i.e., $K^{NO} \gg K_B^{NO}$. Correspondingly, the binding constant for the proximal imidazole B is *decreased* by about 10^3-fold upon addition of NO. This gives a great driving force for releasing imidazole.

$$\text{HmIm} + \text{NO} \rightarrow \text{HmNO} + \text{Im} \qquad (3)$$

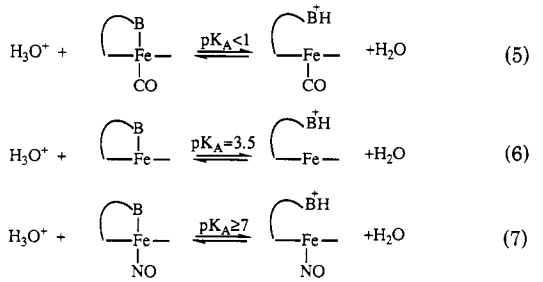
While it is too early to speculate about the *exact* nature of the mechanism of activation of guanylate cyclase by NO, the following scenario, adopted from suggestions which have been published, seems appealing.



(4)

The release of imidazole could then act as a general base or simply serve to release the protein for a change in conformation. The published visible spectra and EPR data on model compounds and guanylate cyclase are consistent with reaction 4 (Ignarro et al., 1986; Rose & Hoffman, 1983; Scheidt & Frisse, 1975; Ignarro et al., 1984; Rubanyi et al., 1990). Similarly, the cytotoxic effect of NO and loss of intracellular iron from the target cells are preceded by the replacement of the diamagnetic EPR signal by the paramagnetic signal (Pellat et al., 1990; Vanin, 1967).

These considerations suggest that the role of nitric oxide as an activator of soluble guanylate cyclase as a signal-transducing enzyme and as an effector molecule in cytotoxicity requires breaking of the proximal base to iron bond (Ignarro et al., 1989). The calculations given above, although approximate, indicate that nitric oxide is able to achieve this by its repulsive trans effect. Neither O_2 nor CO or, for that matter, ionic ligands of ferric heme are capable of this effect. The special effect of NO is illustrated by the following equations for chelated protoheme:



In the presence of CO base elimination takes place at very low pH values; in the absence of ligands (CO or NO) base elimination takes place below pH = 3.5 while in the presence of NO base elimination is complete at all pH values. The pK_A of chelated nitrosylprotoheme is simply the pK_A of the free imidazole (Geibel et al., 1975).

We have recently demonstrated this effect with myoglobin–NO which completely loses its proximal base and becomes free protoheme–NO inside the pocket at pH = 4. Since it is already known that proximal strain (T-state effect) releases proximal base when (and only when) NO is bound (in both hemoglobin and model compounds), this means that a delicate balance of proximal strain and pH can control the release of proximal base. Three important effects accrue from this base release upon binding NO. The base becomes free for catalytic action; the protein becomes free for conformational change; the heme–NO species becomes free to diffuse out of the protein. Any or all of these three effects (all peculiar to NO) could play important roles in guanylate cyclase.

An additional property of HmNO might also be important. It can be estimated from Schemes I and II, knowing that the additions of CO or NO to 4-coordinated heme are diffusion controlled (Traylor et al., 1979), that the 5-coordinated species heme–CO and heme–NO dissociate with rate constants of 10^4 s^{-1} and $\sim 10^{-6}$ s^{-1}, respectively. Therefore, HmNO is stable and can diffuse freely through the protein or solution. It has essentially no affinity for the bases in the protein whereas HmCO has a very high affinity for bases and is at the same time unstable toward dissociation.

Finally NO is easily destroyed by oxidation or reduction, making control of its concentration easy to accomplish. The affinity of NO for iron(III) porphyrins is low, and the dissociation rates are fast. Therefore, iron(III) porphyrins are not likely to play any special role in the triggering effects.

Several vasodilators carry out their pharmacological function by releasing nitric oxide. The best known of these is nitroglycerin which has been in use since the 19th century. Alfred Nobel, whose fortune was based on the discovery of the use of stabilized nitroglycerin as dynamite, wrote to a friend in 1885, "It is the irony of fate that I should be ordered by my doctors to take nitroglycerin internally" (Vane et al., 1990).

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