ORGANIC LETTERS

2006
Vol. 8, No. 14
3065−3067

Mechanism of NO Transfer from NO-Donors (SNAP and G-MNBS) to Ferrous Tetraphenylporphyrin in CH_3OH

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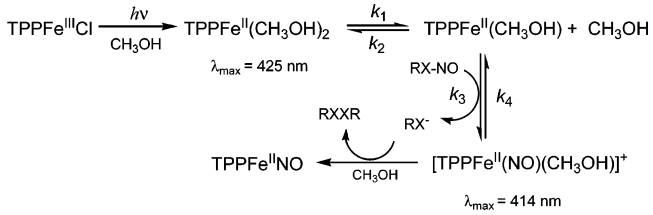
Received April 27, 2006

ABSTRACT



The mechanism of NO transfer from NO-donors (SNAP and G-MNBS) to ferrous tetraphenylporphyrin (TPPFe^{II}) in CH_3OH is discovered for the first time by using a laser flash technique. The results show that the NO transfer is completed by NO^+ transfer followed by electron transfer rather than direct NO transfer in one step.

Nitric oxide (NO), which is among the simplest of molecules, has been found to have many important functions including blood pressure control, neurotransmission, and immune response in mammalian biology.[1] It is now believed that NO can activate the hemeproteins such as soluble-guanylyl cyclase (sGC) by binding its heme moiety.[2] Therefore, it is very important to understand the interaction mechanism of iron center proteins with NO. Much attention has been given to this aspect in the past two decades, and the iron complex, especially the iron porphyrin as a good model, has been widely used in the mechanistic studies.[1−10] In fact, the concentration of free NO in mammalian tissue is very low (∼4 nM). The species most related to NO found in mammalian tissue are nitrate, nitrite, and NO-donors.[5,11,12] Since the NO-donor compounds, such as *S*-nitrosothioles, *N*-nitrosamines, etc., have shown many of the same biological properties as does NO alone and have been regarded as the best candidates for the endogenous storages and transports of NO,[12−14] it is certain that the interactions of hemeproteins

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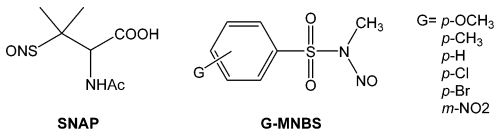
10.1021/ol061021m CCC: $33.50 © 2006 American Chemical Society
Published on Web 06/07/2006

or iron porphyrin with widely existing NO-donors should be more common and important than that with free NO in vivo.[15a] However, the investigation of this aspect is relatively rare.[15b] One of the main reasons is that the ferrous porphyrin is extremely sensitive to oxygen and the pure compound is very difficult to obtain, which strictly limits experimental progress. But, in recent papers Hoshino et al.[16,17] reported that the ferrous porphyrin can be quantitatively generated from $TPPFe^{III}Cl$ in the degassed methanol solution by laser irradiation, which encouraged us to examine the reaction of NO-donors (G-MNBS and SNAP) with ferrous porphyrin



in the degassed methanol solution by using a laser flash technique. Herein, we wish to report the detailed experimental results for the first time.

$TPPFe^{III}Cl$ in the degassed methanol solution was treated by using a laser beam to quantitatively yield $TPPFe^{II}(CH_3OH)_2$ ($\lambda_{max} = 425$ nm), which was confirmed by the previous work.[16,17] The generated $TPPFe^{II}(CH_3OH)_2$ is relatively stable without any decay over milliseconds after the laser pulse. However, when the NO-donor MNTS (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) was added to the degassed methanol solution before the laser pulse, the formed absorption peak at 425 nm due to $TPPFe^{II}(CH_3OH)_2$ decayed instantly, and at the same time, a new absorption peak at 414 nm appeared with an isoabsorptive point at 417 nm (Figure 1). The decay

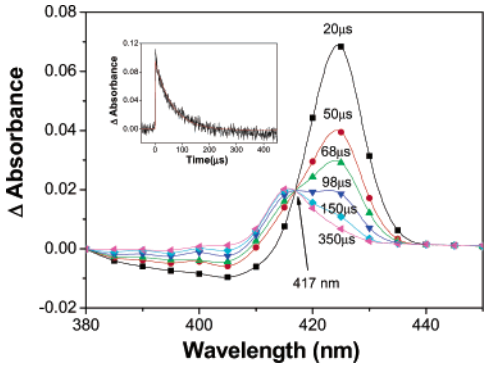


Figure 1. Transient absorption spectra for the $TPPFe^{III}Cl$ (1.0×10^{-5} M) in the presence of MNTS (2.0×10^{-4} M) in degassed methanol solution at 298 K. Inset: The decay curve of the transient monitored at 425 nm.

rate of $TPPFe^{II}(CH_3OH)_2$ was increased with increasing concentration of the added NO-donor, and finally approached the upper limit (Figure 2).

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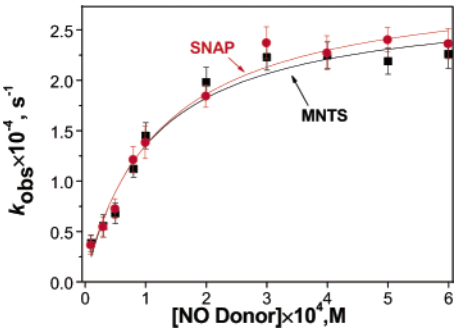


Figure 2. The plots of decay rate of $TPPFe^{II}(CH_3OH)_2$ versus the concentration of NO-donors [SNAP (red circle) and MNTS (black square)].

From Figure 1, it is clear that the formed $TPPFe^{II}(CH_3OH)_2$ from $TPPFe^{III}Cl$ is unstable in the presence of MNTS in Ar-saturated methanol and only remains several hundred microseconds after the laser pulse. Since the decay rate of $TPPFe^{II}(CH_3OH)_2$ is obviously dependent on the concentration of the NO-donor, it is conceivable that $TPPFe^{II}(CH_3OH)_2$ decay is due to the reaction of $TPPFe^{II}(CH_3OH)_2$ with the NO-donor.[18]

According to the previous study,[19] the new absorption peak at 414 nm resulting directly from the decay of $TPPFe^{II}(CH_3OH)_2$ is due to $[TPPFe^{II}(NO)(CH_3OH)]^+$ ($\lambda_{max} = 414$ nm),[19] which suggests that the decay of $TPPFe^{II}(CH_3OH)_2$ in the presence of MNTS in Ar-saturated methanol could be directly caused by NO^+ transfer from MNTS to $TPPFe^{II}(CH_3OH)_2$. To further support this suggestion, the substituted *N*-methyl-*N*-nitrosobenzenesulfonamides (G-MNBS) were used to replace MNTS to examine the Hammett substituent effect on the decay rate of $TPPFe^{II}(CH_3OH)_2$. The results are shown in Figure 3. From Figure 3, it is clear that the decay rate of

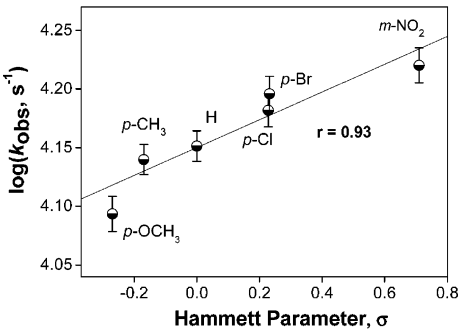


Figure 3. Correlation of log k_{obs} for the G-MNBS/TPPFeCl system ($T = 298$ K) versus the Hammett parameter σ.

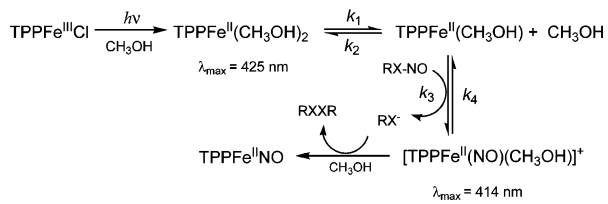
$TPPFe^{II}(CH_3OH)_2$ is obviously increased as the substituent of G-MNBS goes from an electron-donating group (EDG) to an electron-withdrawing group (EWG) and the Hammett reaction constant ρ (the line slope) is positive, which indicates

that the negative charge on the G-MNBS was inceased during the reaction of G-MNBS with $TPPFe^{II}(CH_3OH)_2$, meaning that the break of the N−NO bond in G-MNBS during the reaction took place by heterolytic dissociation (NO^+ transfer) rather than homolytic dissociation (neutral NO transfer). Evidently, this result strongly supports the suggestion above that the formation of $[TPPFe^{II}(NO)(CH_3OH)]^+$ was derived from NO^+ transfer from G-MNTS to $TPPFe^{II}(CH_3OH)_2$. But it is worth mentioning here that the NO^+ transfer was not performed by the initial N−NO bond heterolytic fission of MNTS to release NO^+ and the following combination of the released NO^+ with $TPPFe^{II}(CH_3OH)_2$ to form $[TPPFe^{II}(NO)(CH_3OH)]^+$. The reason is that the N−NO bond heterolytic dissociation energies of G-MNBS (range from 49.5 kcal/mol for *p*-OCH_3 to 44.3 kcal/mol for *m*-NO_2)[20] are quite large. The practical pathway of NO^+ transfer from (G-MNBS) to $TPPFe^{II}(CH_3OH)_2$ could be the concerted process of the heterolytic fission of the N−NO bond and the formation of the Fe−NO^+ bond.

When we prolonged the observation on the formed absorption peak at 414 nm, it is clear that after about 400 μs from the beginning of the laser irradiation, the absorbance of $[TPPFe^{II}(NO)(CH_3OH)]^+$ at 414 nm began to decrease (Figure S2, Supporting Information). At the same time, the absorbance at 401 nm, owing to $TPPFe^{II}NO$,[21] was increased (Figure S4, Supporting Information), meaning that the formed $[TPPFe^{II}(NO)(CH_3OH)]^+$ finally became the more stable product $TPPFe^{II}NO$[22] by deriving an electron from the nitranion G-$MBSN^-$ formed in the former reaction. In fact, according to the reduction potential of $TPPFe^{II}NO$ (E_{red} = 0.43 V vs $Fc^{+/0}$)[23] and the oxidation potential of the nitranions G-$MBSN^-$ (E_{ox} = 0.201 V vs $Fc^{+/0}$ for *p*-H),[20] the electron transfer from G-$MBSN^-$ to $[TPPFe^{II}(NO)(CH_3OH)]^+$ to give $TPPFe^{II}NO$ and the neutral radical G-$MBSN^\bullet$ is thermodynamically favorable ($\Delta G = -5.3$ kcal/mol). The formed radical G-$MBSN^\bullet$ was easy to dimerize to release energy, which can further promote the former reaction. According to the statements above, the mechanism of the NO transfer from MNBS to iron porphyrin TPPFeCl initiated by laser irradiation may be proposed as shown in Scheme 1. Since similar phenomena were observed when SNAP, another very important NO-donor,[12] was used to replace G-MNBS to react with $TPPFe^{II}NO$ (Figure 2, as well as Figures S1, S3, and S5 in the Supporting Information), the two reaction systems should have a similar mechanism.

Scheme 1 shows that an equilibrium exists between $TPPFe^{II}(CH_3OH)_2$ and $TPPFe^{II}(CH_3OH)$ in methanol solution.[24] The decay rate (k_{obs}) of $TPPFe^{II}(CH_3OH)_2$ can be expressed by eq 1 according to the steady-state approximation of $TPPFe^{II}(CH_3OH)$. The equilibrium constants K ($K = k_1k_3/k_2k_4$) for the formation of $[TPPFe^{II}(NO)(CH_3OH)]^+$ were obtained from the plots of k_{obs} versus the concentration of NO-donor (Figure 2) with the results of $1.0 \pm 0.3 \times 10^5$ M^{-1} and $1.2 \pm 0.3 \times 10^5$ M^{-1} for SNAP and MNTS, respectively. The large K values indicate that the NO^+ transfer from the NO-donors to $TPPFe^{II}(CH_3OH)$ is thermodynamically favorable.

Scheme 1



$$k_{obs} = \frac{k_1k_3[\text{NO-donor}] + k_2k_4}{k_2 + k_3[\text{NO-donor}]} \quad (1)$$

In summary, from the present investigation it is clear that ferrous porphyrin is a good NO acceptor, which can easily accept an NO from NO-donors (G-MNBS and SNAP) to become nitrosyl iron porphyrin $TPPFe^{II}NO$. The practical pathway for the NO transfer contains two separate steps: initial NO^+ transfer followed by electron transfer. The thermodynamic analysis shows that both reaction steps are thermodynamically favorable. Considering that the hemeproteins and NO-donors widely exist in vivo,[5−15] the NO transfer from NO-donors via the hemprotein to the target molecules should be both very common and important. The present study could give important insight into a greater understanding of the interaction of NO-donors with hemeprotein and the possible mechanism of NO transfer in vivo.

Acknowledgment. Financial support from MSTC (2004CB719905), NSFC (20125206, 20332020, 20472038, 20421202), MEC (20020055004), and NSFT (033804311) is gratefully acknowledged.

Note Added after ASAP Publication. The reference citation at the end of the first page was incorrectly shown as "22−14" in the version published ASAP June 7, 2006; the corrected version was published ASAP June 12, 2006.

Supporting Information Available: Experimental details including transient absorption spectra of the $TPPFe^{II}$ decay in the presence of SNAP as well as the kinetic trace. This material is available free of charge via the Internet at http://pubs.acs.org.

OL061021M

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