Controlled NO Transfer

Light-Activated Transfer of Nitric Oxide from a Porous Material**

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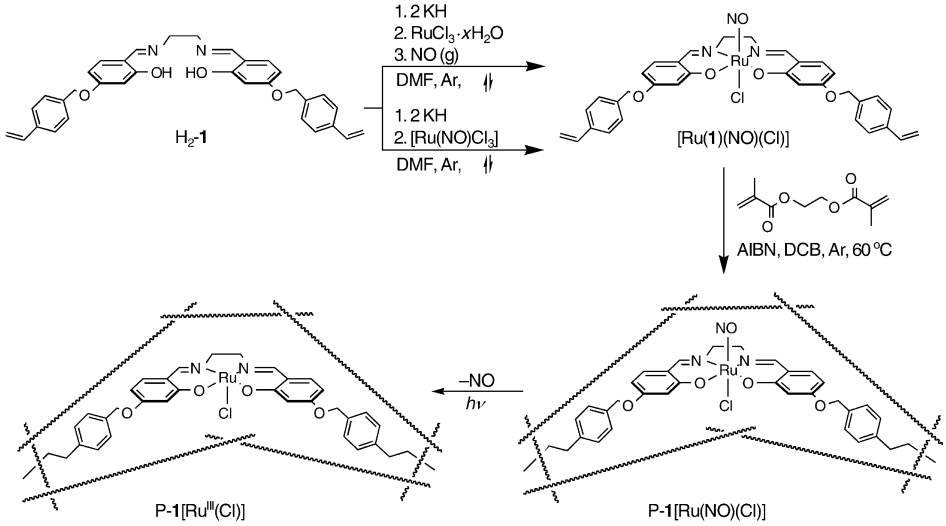
Nitric oxide (NO) is an important biomolecule in mammalian biology, with a range of effects that include regulatory functions in the cardiovascular, respiratory, and nervous systems.[1] The linkage of abnormal NO levels to numerous diseases[1,2] and the potential use of NO as an anti-biofouling agent for in vivo medical devices[3] further underscores the need for new storage–release systems. There is ample evidence that a diverse group of systems will be necessary to meet therapeutic demands.[4] One continuing challenge is the preparation of materials that controllably release NO without leaching toxic by-products.[3,5] A promising approach for such materials utilizes covalently immobilized metal nitrosyl (M–NO) complexes within porous polymeric hosts.[6] These materials combine the properties of the M–NO units, such as reversible NO binding, with those of the porous hosts. We report herein the synthesis and properties of a highly cross-linked, network polymer containing immobilized Ru–NO sites. Light triggers the controlled release of NO, as demonstrated by the transfer of NO in solution to a metalloporphyrin and myoglobin.

We have previously described a material containing covalently immobilized cobalt(II) bis[2-hydroxo-4-(4-vinylbenzyloxy)benzaldehyde]ethylenediiminate complexes ([Co^{II}(**1**)], see Scheme 1 for H_2-**1**) within porous hosts for the storage and release of NO.[6] This material, P-**1**[Co^{II}], bound NO rapidly and selectively with slow release under ambient conditions (about 40% removal of NO over 14 days). It was prepared by a template copolymerization method, similar to that used to make molecularly imprinted polymers.[7] Molecular precursors are used in polymerization, since this allows for structural manipulation of the immobilized sites. In our design,[6,8] substitutionally inert metal complexes containing polymerizable groups are used as templates to form the immobilized sites: Multiple covalent connections of the metal–NO template complex to the polymeric hosts minimizes leaching. Monomeric Ru–NO complexes with ligands related to $\mathbf{1}^{2-}$ photolytically transfer NO in solution.[9] We reasoned that the Ru–NO complex with $\mathbf{1}^{2-}$ could be prepared and utilized as a template to make a new material containing NO. Therefore, the template complex [Ru(**1**)(NO)(Cl)] was synthesized and used to produce the corresponding porous methacrylate polymer P-**1**[Ru(NO)(Cl)], a material with which controllable transfer of NO is achieved with light.

The two methods for preparing [Ru(**1**)(NO)(Cl)] are outlined in Scheme 1. The use of NO and $RuCl_3$ follows literature methods and gives yields of about 45%. A second method uses [$Ru(NO)Cl_3$] and produces [Ru(**1**)(NO)(Cl)] in a slightly higher yield of 54%. This latter method is advantageous because it does not require the use of NO gas during synthesis. Data from NMR, electronic absorbance, and FT-IR spectroscopy as well as mass spectrometry, are consistent with the formulation of this monomer as [Ru(**1**)(NO)(Cl)] (see the Supporting Information).



Scheme 1. Preparative routes for [Ru(**1**)(NO)(Cl)], P-**1**[Ru(NO)(Cl)], and P-**1**[Ru(Cl)]. AIBN = azobisisobutyronitrile, DCB = 1,2-dichlorobenzene.

[Ru(**1**)(NO)(Cl)] contains polymerizable styryloxy groups for covalent immobilization within a porous network polymer. The copolymerization procedure for preparing P-**1**[Ru(NO)(Cl)] employs [Ru(**1**)(NO)(Cl)], ethylene glycol dimethacrylate (EGDMA) as the cross-linking agent, the

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radical initiator AIBN, and 1,2-dichlorobenzene as the porogen (Scheme 1). The monomer [Ru(**1**)(NO)(Cl)] is stable towards NO release under the reaction conditions, as long as light is absent during polymerization. A monolithic solid was isolated, which was washed continuously with CH_2Cl_2, pulverized, and sieved to an appropriate particle size. Particles between 125 and 75 μm have an average pore diameter of 60 Å, a pore volume of 0.63 mL per gram of polymer, and a surface area of 400 m^2 per gram of polymer.

The spectroscopic analysis of P-**1**[Ru(NO)(Cl)] indicates that the Ru–NO complex remains intact during polymerization. The FT-IR spectrum of P-**1**[Ru(NO)(Cl)] shows a band at 1824 cm^{-1}, which is assigned to the NO stretch,[10] and the electronic absorption spectrum of the polymer suspended in toluene has an absorbance band at $\lambda_{max} = 373$ nm. Furthermore, P-**1**[Ru(NO)(Cl)] is EPR silent at 77 and 4 K, consistent with antiferromagnetic coupling between the unpaired electron of NO and the low-spin d^5 Ru^{III} center. The spectroscopic features found for P-**1**[Ru(NO)(Cl)] are nearly identical to those for the monomeric precursor [Ru(**1**)(NO)(Cl)] (see the Supporting Information).

Photolytic release of NO is observed for P-**1**[Ru(NO)(Cl)], and broad-band irradiation with a Hg arc lamp converts P-**1**[Ru(NO)(Cl)] into P-**1**[Ru(Cl)]. This process was monitored with EPR spectroscopy (Figure 1).

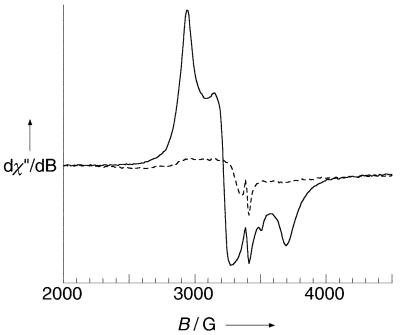

Figure 1. The X-band EPR spectra (4 K) for P-**1**[Ru(NO)(Cl)] (----) and after broad-band irradiation for 2 h to form P-**1**[Ru^{III}(Cl)] (—).

The X-band EPR spectrum of photolyzed P-**1**[Ru(NO)(Cl)] contains a rhombic signal with *g* values at 2.3, 2.1, and 1.8, which is indicative of immobilized ruthenium(III) complexes with $S = 1/2$ ground states (i.e., Ru^{III} complexes that have lost NO). This spectrum is identical to that of P-**1**[Ru(Cl)] prepared independently (see the Supporting Information).

Nitric oxide is also photoreleased from suspensions of P-**1**[Ru(NO)(Cl)] in various solvents. For instance, irradiation of P-**1**[Ru(NO)(Cl)] suspended in toluene with 370 nm light results in the formation of P-**1**[Ru(Cl)], which has characteristic absorbance bands at $\lambda_{max} = 400$ nm (shoulder) and 660 nm. The photolysis of NO appears to be dependent on the solvent. Nitric oxide loss from P-**1**[Ru(NO)(Cl)] is slower from suspensions in noncoordinating solvents, including CH_2Cl_2, toluene, and cyclohexane, than in coordinating solvents, such as acetonitrile and water. A likely explanation for this observation is that coordinating solvents bind to the site on the ruthenium center left vacant after photolysis, which prevents NO rebinding, thus hastening release. Similar observations were found for solution studies of related monomeric Ru–NO complexes.[9]

Irradiation of a suspension of P-**1**[Ru(NO)(Cl)] at 370 nm causes the heterogeneous transfer of NO to cobalt(II) tetraphenylporphyrin ([Co^{II}(TPP)]) dissolved in CH_2Cl_2. The photolytic transfer was monitored with electronic absorbance spectroscopy (Figure 2) and shows clean conversion of [Co^{II}(TPP)] into [Co^{II}(TPP)(NO)] with sharp isosbestic points at $\lambda_{max} = 397$, 420, and 425 nm. The NO transfer to [Co^{II}(TPP)] is completed in less than 20 min and gives an apparent first-order rate constant k_1 of 0.0030 s^{-1} (inset of Figure 2). This rate is slower than that found for homogeneous NO transfer from the monomer [Ru(**1**)(NO)(Cl)] ($k_1 \approx 0.01$ s^{-1}, see the Supporting Information).

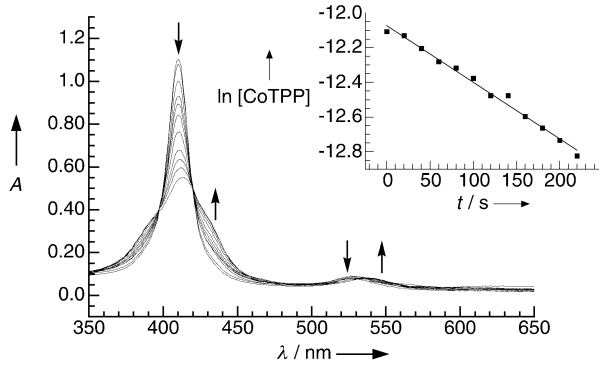

Figure 2. Overlaid electronic absorption spectra demonstrating the photolytic transfer of NO from P-**1**[Ru(NO)(Cl)] to 5.5×10^{-6} M [Co^{II}(TPP)] in CH_2Cl_2. Each spectrum was collected after 20 s of irradiation at $\lambda = 370$ nm. Inset: Plot used to determine a first-order rate constant of $k_1 = 0.0030$ s^{-1}. *A* = absorbance.

Figure 3 shows an NO-release profile for aqueous suspensions of particulate P-**1**[Ru(NO)(Cl)]. Continuous irradiation for 50 min at pH 7.2 for particles smaller than 75 μm produces an increase in the amount of NO released to a maximum of 15.5(4) μmol of NO per gram of polymer (19% yield);[11] no additional release was observed at longer irradiation times (Figure 3a). P-**1**[Ru^{III}(Cl)] was also produced from this photolysis and gave the expected EPR signal, whose spin concentration is 14(1) μmol of Ru^{III} sites per gram of polymer. Figure 3b illustrates that larger particles (0.5 to 1.0 mm) produce a maximum of 2.0(4) μmol of NO per gram of polymer after 80 min of irradiation. To demonstrate that release is only caused during irradiation, light-dark-light sequences were examined (Figure 3b). The amount of NO released correlated with the time period of irradiation, and no release was observed during the dark phase. The amount of NO released in these studies was monitored indirectly by determining the nitrite concentration with the Griess spectrophotometric method,[12] and shows that photolysis of P-**1**[Ru(NO)(Cl)] gives controllable NO production in an aqueous medium at physiological pH.

Equine skeletal muscle myoglobin (Mb) was used to establish that P-**1**[Ru(NO)(Cl)] can transfer NO to proteins. Many heme proteins, such as Mb, bind NO.[13] Moreover, Mb has been implicated as an in vivo NO scavenger[14] because of

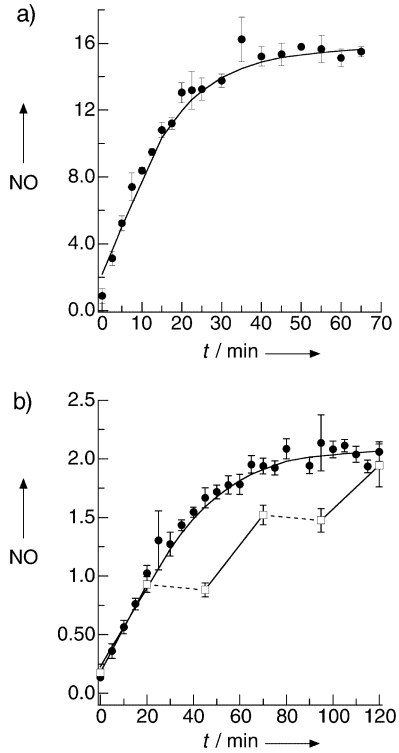

Figure 3. Plots of the release of NO (in μmol NO per gram of polymer) as a function of time for particles a) smaller than 75 μm and b) between 0.5 and 1 mm. (●) Continuous irradiation, (□) light-dark-light sequences, (—) irradiation phases, (-----) dark phases.

its high concentrations in cardiac cells and striated muscles, both of which use NO as an intercellular messenger.[15] Irradiation of P-**1**[Ru(NO)(Cl)] with 370 nm light at pH 7.2 converted 81 % of reduced Mb into Mb(NO) within 20 min. The NO transfer is illustrated in Figure 4 by the shift in the Mb Soret band from $\lambda_{max} = 435$ to 420 nm. Nearly identical shifts were reported for Mb and Mb(NO);[16] furthermore, control spectra of equine skeletal muscle Mb and Mb(NO) display identical properties. The spectral data corresponded to a first-order rate constant of $k_1 = 5.0 \times 10^{-4}\ s^{-1}$ (inset of Figure 4).

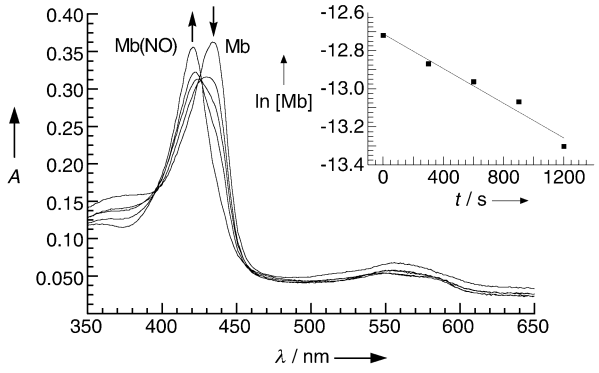

Figure 4. Overlaid electronic absorption spectra demonstrating the photolytic transfer of NO from P-**1**[Ru(NO)(Cl)] to 3.0×10^{-6} M Mb in 50 mM phosphate buffer, pH 7.2. Each spectrum was collected after 5 min of irradiation at $\lambda = 370$ nm. Inset: Plot used to determine a first-order rate constant of $k_1 = 5.0 \times 10^{-4}\ s^{-1}$. *A* = absorbance.

Our findings with P-**1**[Ru(NO)(Cl)] for the release and transfer of NO illustrate that photolytic processes can be incorporated into biomaterials to controllably dispense this biologically important compound. While photolytic release of NO has been reported from compounds[9,17,18] and a self-assembled monolayer,[19] our findings represent the first example, to our knowledge, in which light triggers NO release from a material. A photorelease mechanism as shown for P-**1**[Ru(NO)(Cl)] complements those reported for other materials, in particular materials containing diazeniumdiolates, which control NO release by chemical manipulations. Materials such as P-**1**[Ru(NO)(Cl)] can potentially be used to accurately distribute NO to specific targets for in vitro and in vivo studies.

Experimental Section

P-**1**[Ru(NO)(Cl)]: Under an argon atmosphere, [Ru(**1**)(NO)(Cl)] (0.0745 g, 0.107 mmol, 5 mol %), EGDMA (0.40 g, 2.0 mmol, 94 mol %), and AIBN (0.0040 g, 0.021 mmol, 1 mol %) were combined in a thick-wall polymerization tube (Ace Glass). The porogen DCB (1.5 g) was added to the mixture, and the tube was sealed, covered with aluminum foil, and heated to 60 °C. After 24 h a brown monolithic polymer was obtained and continuously extracted with CH_2Cl_2 overnight. The resulting polymer was pulverized in a mortar and pestle, sieved to a particle size between 0.5 and 1.0 mm, and dried under reduced pressure to yield 0.482 g of P-**1**[Ru(NO)(Cl)].

NO release studies: Suspensions of P-**1**[Ru(NO)(Cl)] particles were prepared by adding 2 mL aliquots of phosphate buffer solution (50 mM, pH 7.2) to P-**1**[Ru(NO)(Cl)] (20 mg) in Pyrex test tubes, which were sealed and stored in the dark. Samples were shaken and exposed to light at $\lambda = 350$ nm (≈ 24 W) from a Rayonet photochemical reactor for defined time periods, after which a sample (50 μL) was transferred to a well of a 96-well microtiter plate. Each sample well was treated with components of the Griess reagent system (Promega). The amount of nitrite was determined spectrophotometrically with a SpectraMax 190-plate reader (Molecular Devices) monitoring absorbance at 520 nm.

NO transfer studies: In a typical experiment, a solution of the NO acceptor with known concentration (≈ 10 mg for [Co(TPP)], ≈ 100 mg for Mb) and P-**1**[Ru(NO)(Cl)] particles (0.5–1 mm) were placed in a 1.00-cm Suprasil quartz cuvette and sealed under an inert atmosphere with a rubber septum. The sample was irradiated with a broad emission Hg arc lamp (Oriel) equipped with a 370 nm wavelength band pass filter (Omega Optical). Spectra were collected in defined intervals and recorded on a Varian Cary 50 spectrophotometer at room temperature. Reduced equine skeletal muscle Mb was prepared from metMb (Sigma) using dithionite (≈ 1.2 equiv).

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