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Release of Nitric Oxide from a Sol−Gel Hybrid Material Containing a Photoactive Manganese Nitrosyl upon Illumination with Visible Light

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Nitric oxide (NO) is an endogenously produced reactive gaseous molecule with regulatory, protective, and deleterious effects, depending on its local concentration.[1] A large number of NO donors have been synthesized for use in studying numerous biological processes as well as for therapeutic applications.[2] For the latter, release of NO to specific locales is often required. The vast majority of NO-releasing materials have been developed as coatings for improved biocompatibility of blood-contacting medical devices.[3] These types of materials utilize diazeniumdiolates embedded within or covalently linked to polymers with spontaneous release of NO in biological solutions over long periods of time. Reports on materials that release NO only when activated by an external light trigger (either UV or visible) have been quite limited.[4−7] Also, such NO donors yield small quantities of NO upon illumination with visible light. Here we report the synthesis and properties of a sol−gel-derived hybrid material (**1·HM**) encapsulating the photoactive manganese nitrosyl $[Mn(PaPy_3)(NO)]ClO_4$ (**1**, $PaPy_3H$ = *N*,*N*-bis-(2-pyridylmethyl)amine-*N*-ethyl-2-pyridine-2-carboxamide).[8] This material releases NO with high quantum efficiency when activated by visible light. The release of NO is accompanied by a dramatic color change (green to orange, Figure 1). Such a material could be used for rapid generation of NO at a specific site and transfer of NO to biological targets.

As part of our ongoing research to develop photoactive metal nitrosyls as convenient NO donors,[9,10] we have recently reported the water-soluble complex $[Mn(PaPy_3)(NO)]ClO_4$ (**1**) which rapidly releases NO upon activation with visible light of low intensity (25−50 W tungsten lamp).[8] Complex **1** is stable in biological buffers and has been used to transfer NO to cytochrome *c* oxidase and papain.[11,12] In the present work, we have synthesized a hybrid material by incorporating **1** into a sol−gel polymer (**1·SG**) and then coating the polymer with polyurethane for enhanced stability (**1·HM**). Polyurethanes are widely used in medical devices due to their excellent biocompatibility.[13] The polyurethane coating effectively prevents leakage of **1** from **1·HM** without significantly suppressing NO flux.[14] Containment of **1** in the material also lowers the cytotoxicity of the nitrosyl in biological systems. In contrast to the diazeniumdiolates, the stability of **1** in aqueous solutions (in the dark) allows controlled delivery of NO with visible light as the external on/off switch.[8,11,12]

1·SG was prepared from 1 mL of tetraethoxysilane (TEOS), 1.5 mL of ethanol (EtOH), 0.8 mL of water (pH 5, made acidic with aqueous $HClO_4$), and 10 mg of **1**. The deep green mixture was homogenized by stirring and allowed to gel for 4 days in the dark. The dark green gel sample thus obtained was soaked in triisopropylsilane for 48 h. The gel was finally rinsed with EtOH and water. This sample of **1·SG** (shown in Figure 1) was soft and could be cut and shaped with a razor blade. To add more structural strength, **1·SG** was then coated with four layers of Tecoflex SG-80A polyurethane (gift from Noveon, Wilmington, MA) dissolved in THF and dried in the dark under ambient conditions for 5 h to afford **1·HM**, a rigid and optically transparent material.

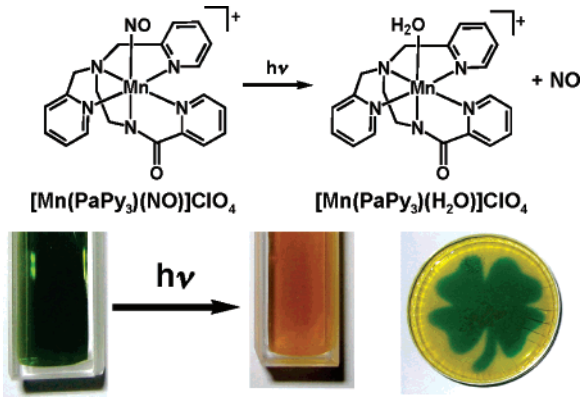

Figure 1. Photodissociation of NO from **1** (top) and **1·SG** (bottom left). Selective irradiation of a circular slice of **1·SG** with a black plastic shamrock on top; nonirradiated area is green, while the photolyzed area is yellow (bottom right). Light source: 25 W tungsten bulb.

Incorporation of **1** into the **1·SG** did not change the ability of the complex to release NO upon irradiation with visible light (either 25 W tungsten bulb or 10 mW 532 nm laser light). Exposure of green **1·SG** (λ_{max} = 635 nm) to light caused the color of the gel to bleach to orange−yellow. This process is easily followed visually (Figure 1) and spectrophotometrically. As shown in Figure 1 (bottom right), spatial differentiation between the site of illumination and nonillumination is quite remarkable. It is therefore possible to deliver NO to a specific locale by **1·HM** by either constricting the light beam or by properly covering the surrounding areas.

Immobilization of **1** into the sol−gel matrix did not alter the photoproperties of the nitrosyl to a great extent. For example, much like **1**, the light-induced NO loss from **1·SG** followed a pseudo-first-order behavior. The value of the NO off rate constant (K_{NO}) of **1·SG** under illumination with a 100 W tungsten lamp (distance between the lamp and the gel = 8 cm) was found to be 2.5×10^{-3} s^{-1}, while that of **1** dissolved in water under the same lamp was 8.7×10^{-3} s^{-1}.[8]

To determine the efficiency of the loss of NO from **1** and **1·SG** with visible light, quantum yield measurements were performed with the actinometer Actinochrome N (475/610), using 10 mW 532 nm light (photolysis source: DCR-11 Nd:YAG laser).[15] The quantum yield of **1** dissolved in water was determined to be 0.55, while that of **1·SG** was 0.25. One must note the high quantum yield of **1** compared to that of other NO donors that have later been used for incorporation in NO-releasing materials. For example, the quantum yields of (*S*)-nitroso-*N*-acetylpenicillamine (SNAP, in phosphate buffer, 550 nm light), [Ru(salen)(Cl)(NO)] (in MeCN, 365 nm light), and $[Ru(salen)(H_2O)(NO)]Cl$) (in water, 365 nm light) are 0.04,[16] 0.13, and 0.05, respectively.[17] Clearly, **1** is the most efficient photoactive NO donor that can be activated with

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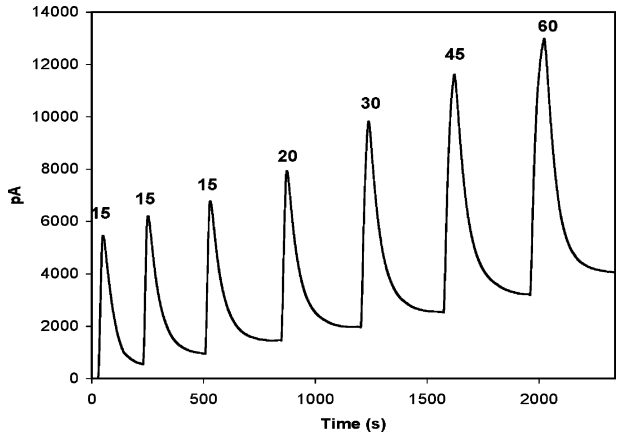

Figure 2. Amperogram of NO generated from a pellet of **1·HM** in water, monitored with an inNO-T NO-electrode (Harvard Apparatus). The numbers on the top of the peaks denote the number of seconds of illumination with a 50 W tungsten lamp.

visible light. Although SNAP and the Ru nitrosyls have been incorporated in NO-releasing materials,[4,5,7] their quantum yield values have not been determined. The high quantum yield of **1·SG** indicates that the NO-releasing power of **1** is not dramatically curtailed upon immobilization in a sol−gel matrix.

Although **1** is stable within the sol−gel matrix (in the dark), it is not covalently attached. As a result, when **1·SG** is immersed in aqueous solution, **1** slowly leaks out of the sol−gel matrix (the aqueous solution turns light green within hours). To prevent this leaching, **1·SG** was coated with polyurethane to make **1·HM**. This polyurethane coat completely sealed **1** inside the hybrid material (no leakage within 24 h) while still retaining the NO photoactivity. When a pellet of **1·HM** was immersed in water and exposed to flashes of visible light (50 W tungsten lamp), NO was detected by an NO electrode (inNO-T NO-measuring system, Harvard Apparatus). As shown in Figure 2, the amount of NO (as indicated by the current) was proportional to the exposure time, a fact that clearly demonstrated that the hybrid material **1·HM** photoreleased NO in a very linear fashion. We have also incorporated **1** in polyurethane film. Such films, however, rapidly lose **1** in solution. The sol−gel matrix and the polyurethane coat are therefore necessary for successful immobilization of **1** in **1·HM**.

1·HM is a convenient NO donor for biological targets. For example, when a pellet of **1·HM** was immersed into a solution of horse heart Mb (phosphate buffer, pH 7) in the dark, no change in the Soret band (λ_{max} = 410 nm) was observed. Upon addition of dithionite, the only change observed was the expected reduction of Mb (λ_{max} = 432 nm). However, when the solution was exposed to visible light, the Mb−NO adduct (λ_{max} = 420 nm) was formed rapidly (Figure 3). The reaction was clean and complete within 5 min. The rapid transfer of NO from **1·HM** to reduced Mb *under visible light* is especially notable. The [Ru(salen)(NO)Cl]-derived porous material reported by Borovik and co-workers took 20 min for complete transfer of NO to reduced Mb under UV light (λ = 370 nm).[5]

In summary, we have shown that the manganese nitrosyl [Mn(PaPy$_3$)(NO)]ClO$_4$ (**1**) can be incorporated into a sol−gel matrix

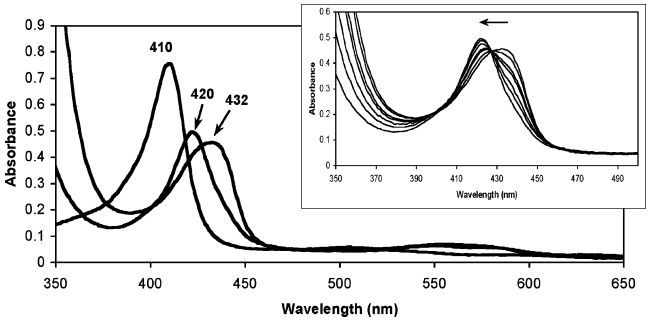

Figure 3. Transfer of NO from **1·HM** to reduced Mb in pH 7 phosphate buffer with a 50 W tungsten lamp. Reduced Mb was prepared from met Mb (Sigma) using dithionite (∼1.2 equiv). Inset: Spectra collected at 45 s intervals of illumination.

(**1·SG**), and it still rapidly releases substantial amounts of NO upon irradiation with visible light. Application of a polyurethane coat to **1·SG** affords the hybrid material **1·HM**, a convenient NO donor that delivers NO to selected locales upon exposure to light in a controlled manner. Since **1·HM** requires low-intensity visible light to trigger the release of NO (instead of UV light), it is more suitable for biological targets. In addition, the polyurethane coating makes **1·HM** very biocompatible.[14] It is therefore possible to use **1·HM** as an NO donor to initiate NO-induced cell death at a specific location (such as in photodynamic therapy of cancer).[18] Such experiments are in progress in this laboratory.

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