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Isolation of a *Se*-Nitrososelenol: A New Class of Reactive Nitrogen Species Relevant to Protein *Se*-Nitrosation

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Nitric oxide (NO) is a messenger molecule implicated in a number of physiological processes.[1,2] One of the mechanisms by which NO affects cellular processes is through direct interaction with cellular proteins by nitrosylation and nitrosation reactions. *S*-Nitrosation of cysteine residues to produce *S*-nitrosothiols (RSNOs) is one of the most important NO-mediated modifications of proteins.[3,4] Recently, there have been several reports suggesting that the interactions of NO (or NO-derived species) with the SeH groups of selenoproteins are also involved in NO-mediated cellular functions.[5−11] For example, glutathione peroxidase (GPx), an essential selenium-containing antioxidant enzyme, is inactivated by treatment with RSNO as well as by endogenous NO,[6−11] presumably through selenocysteine *Se*-nitrosation.[7,9] In contrast to the extensive studies undertaken on *S*-nitrosothiols, however, no chemical information about their selenium analogues has been available to date, despite their potential physiological importance.[12] To elucidate the mechanism of NO-mediated modification of selenoproteins, reference data on *Se*-nitrosated species are indispensable. Here we report the synthesis of a stable *Se*-nitrosated derivative of an organoselenol, a *Se*-nitrososelenol (RSeNO), and its crystal structure and spectral properties. This *Se*-nitrososelenol can be formed by direct transnitrosation from RSNO to a selenol, while it is reduced to the selenol by treatment with dithiothreitol, as proposed in the hypothetical pathway for the NO-mediated inactivation of GPx.[7,9]

Because the selenium−nitrogen bond of the Se−NO group is considered to be weaker than the sulfur−nitrogen bond of the S−NO group, it is likely that *Se*-nitrososelenols are more labile than *S*-nitrosothiols, which are already notorious for their very facile bimolecular decomposition to give disulfides and NO. Previously we reported that *S*-nitrosothiols can have a long lifetime if their bimolecular decomposition is sterically suppressed by the dendrimer-type substituents,[13,14] including a Bpq group[14,15] shown in Figure 1. This methodology is also expected to be effective for the stabilization of *Se*-nitrososelenols. *Se*-Nitrososelenol **2** was prepared by nitrosation of selenol **1** bearing a Bpq group.[16] Treatment of **1** with an excess of ethyl nitrite in degassed $CDCl_3$ led to the quantitative formation of *Se*-nitrososelenol **2**, which was isolated as reddish purple crystals with a melting point of 130.0−132.5 °C (decomp) by recrystallization from toluene−hexane in 89% yield (Figure 1).[16] Formation of **2** was also observed in the reaction of **1** with *S*-nitrosoglutathione (GSNO) (vide infra). *Se*-Nitrososelenol **2** is sensitive to atmospheric oxygen but stable toward water. In $CDCl_3$, no decomposition of **2** was observed after 1 week at room temperature. The structure of **2** was determined by ^{1}H, ^{13}C, and ^{77}Se NMR spectroscopy, UV−vis and IR spectroscopy, and X-ray crystallographic analysis.[16]

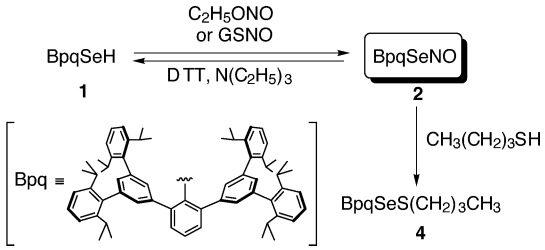

Figure 1. Synthesis and reactions of *Se*-nitrososelenol **2**.

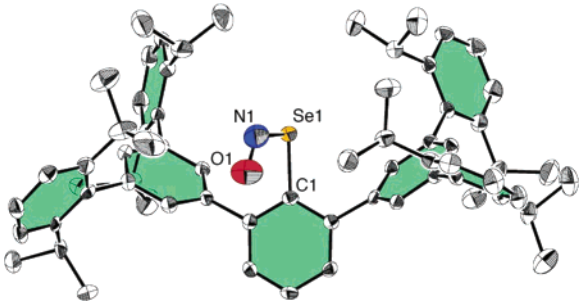

Figure 2. ORTEP drawing of *Se*-nitrososelenol **2** (30% probability). Selected bond lengths (Å), bond angles (deg), and a torsion angle (deg): O(1)−N(1), 1.162(6); N(1)−Se(1), 2.107(6); Se(1)−C(1), 1.915(3); O(1)−N(1)−Se(1), 116.2(3); N(1)−Se(1)−C(1), 94.88(16); O(1)−N(1)−Se(1)−C(1), −2.2(5). In the crystalline state, there is a rotational disorder of the N−O moiety around the C−Se bond in the ratio of 0.81:0.19, and only the major component is shown. One molecule of benzene is included in the asymmetric unit, which is omitted for clarity.

Figure 2 shows the crystal structure of **2** with selected bond lengths, bond angles, and a torsion angle. The Se−N bond length (2.107(6) Å) and the N−O bond length (1.162(6) Å) are consistent with a selenium−nitrogen single bond and a nitrogen−oxygen double bond. The observed bond lengths and angles are in good agreement with the calculated values for a model *Se*-nitrososelenol, C_6H_5SeNO (Se−N, 2.097 Å; N−O, 1.172 Å; O−N−Se, 115.4°), obtained by density functional theory (DFT) calculation at the B3LYP/6-31G(d) level,[17] indicating that the structure around the SeNO functionality of **2** is not affected by the two bulky substituents at the ortho positions. The C−Se−N−O linkage adopts only the syn conformation, which is similar to the aromatic *S*-nitrosothiols structurally characterized so far.[14,18]

The ^{77}Se NMR spectrum ($CDCl_3$) of **2** shows a signal at δ 2229 ppm, about 2100 ppm lower field than that of selenol **1** (δ 135), suggesting the strong magnetic deshielding effect of the NO moiety. Such an extreme low-field shift was also found in theoretical calculation. By the gauge-including atomic orbital (GIAO) calculation at the B3LYP/6-311G(3d)[Se]:6-311G(d)[C,O,N,H]//B3LYP/6-31G(d) level, the chemical shift of **2** was estimated to be δ 2449

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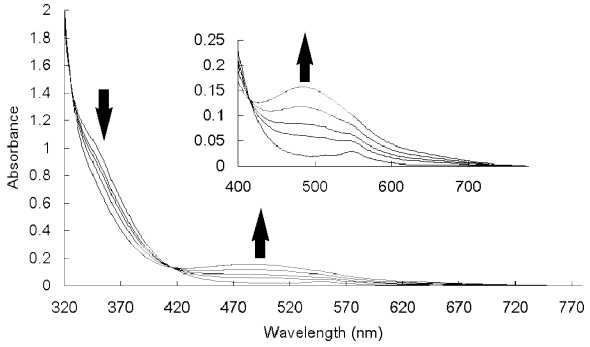

Figure 3. UV−vis spectral change during the reaction of selenol **1** (1.6 M) with GSNO (1.8 M) in THF−water (4 mL, THF:water = 3:1 v/v). Inset: magnified view for the range of 400−780 nm.

ppm. The UV−vis spectrum of **2** shows the absorption maximum at 485 nm (ϵ 150) in chloroform. The time-dependent DFT calculation for C_6H_5SeNO at the B3LYP/6-311+G(2d)//B3LYP/6-31G(d) level shows two characteristic bands at 495 nm (n−π* transition) and 711 nm (π−π* transition), although the intensity of the latter is nearly zero. The observed 485 nm band can be reasonably assigned to the n−π* transition, which shows a bathochromic shift of about 140 nm compared to that of the corresponding *S*-nitrosothiol, BpqSNO (**3**) (345 nm).[14] The π−π* transition band of **2** is considered to be so weak that it could possibly be hidden underneath the broad absorption feature of the n−π* transition. In the IR spectrum of **2**, the N−O stretching band was observed at 1563 cm^{-1}, which is slightly higher than that of BpqSNO (**3**) (1548 cm^{-1}).[14]

GPx contains an essential selenocysteine residue at its active site and catalyzes the reduction of hydroperoxides by glutathione. Recently it has been reported that GPx also acts as an intracellular NO scavenger and is inactivated by exogenously administered and endogenously produced NO.[6−11] Such dysfunction results in an increase in cellular peroxide levels, which induces adaptive defense responses or otherwise apoptotic cell death.[8−11] The inactivation of GPx induced by NO donors has been investigated in vitro and in cell culture. It has been proposed that bovine GPx is specifically inactivated by *S*-nitroso-*N*-acetylpenicillamine (SNAP) in vitro by a two-step reaction.[7,9] The first step is *Se*-nitrosation of a selenocysteine moiety, which can be reversed by addition of a reducing agent such as dithiothreitol (DTT). The second reaction involves the formation of a sulfur−seleno bridge between Cys^{91} and Sec^{45} in bovine GPx and cannot be reversed by a reducing agent.[19] As a model for this mechanism, the formation of *Se*-nitrososelenol **2** by transnitrosation from an *S*-nitrosothiol to selenol **1** was examined, and it was revealed that *S*-nitrosoglutathione (GSNO), one of the important NO carriers in the biological system, undergoes NO transfer to selenol **1** (Figure 1). When the reaction of selenol **1** with GSNO in degassed THF−water (THF:water = 3:1 v/v) at room temperature in the dark was monitored by UV−vis spectroscopy, a decrease in the absorbance of GSNO at 334 nm and a corresponding increase in a maximum at 483 nm of *Se*-nitrososelenol **2** were observed, with clear isobestic points at 328 and 415 nm (Figure 3). This indicates that the direct transnitrosation occurs from GSNO to the SeH group of **1** to produce *Se*-nitrososelenol **2**. Treatment with an excess of DTT in the presence of triethylamine quantitatively reduced *Se*-nitrososelenol **2** to the parent selenol **1** (Figure 1). The reaction of **2** with an excess of 1-butanethiol led to the quantitative formation of selenenyl sulfide **4** (Figure 1).[20] These results coincide with the proposed mechanism for the NO-mediated GPx inactivation described above, and strongly suggest the possible involvement of *Se*-nitrosation of selenoproteins by NO-derived species in redox regulation of cellular functions.

Modification of antioxidant enzymes such as GPx by reactive nitrogen species has been suggested as playing a pivotal role in NO-related cellular signaling cascades. The stable *Se*-nitrososelenol obtained in this study is expected to serve as a reference compound for identification of yet unconfirmed *Se*-nitrosated species in proteins and understanding of their physiological functions.

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Supporting Information Available: Experimental procedures and characterization data for new compounds (PDF); X-ray crystallographic data of **2** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Selenenyl sulfide **4** is not reduced by DTT under the conditions where *Se*-nitrososelenol **2** is reduced to selenol **1**.

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