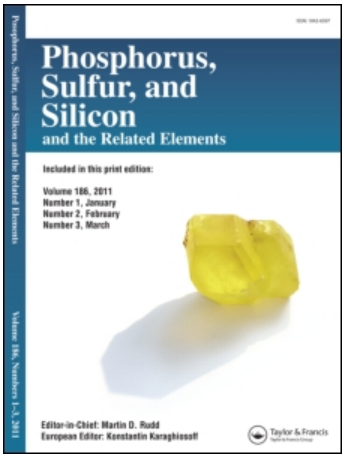


Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Modeling of Selenoprotein Nitrozation: Synthetic Approaches

Cathleen Wismach[a]; Wolf-Walther Mont du[a]; Delia Bugnariu[a]; Thorsten Gust[a]
[a] Institut fuer Anorganische und Analytische Chemie, Technische Universitaet Braunschweig, Braunschweig, Germany

To cite this Article Wismach, Cathleen , Mont du, Wolf-Walther , Bugnariu, Delia and Gust, Thorsten(2005) 'Modeling of Selenoprotein Nitrozation: Synthetic Approaches', Phosphorus, Sulfur, and Silicon and the Related Elements, 180: 3, 713 — 720

To link to this Article: DOI: 10.1080/15389580590903186

URL: http://dx.doi.org/10.1080/15389580590903186

Phosphorus, Sulfur, and Silicon, 180:713–720, 2005

ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426500590907435

Taylor & Francis
Taylor & Francis Group

Modeling of Selenoprotein Nitrozation: Synthetic Approaches

Cathleen Wismach
Wolf-Walther du Mont
Delia Bugnariu
Thorsten Gust
Institut fuer Anorganische und Analytische Chemie, Technische Universitaet Braunschweig, Braunschweig, Germany

Model reactions have been carried out to evaluate the question of what kind of links may exist between the biological cycles of nitrogen oxide (NO) and those of selenoproteins, especially the amino acid selenocysteine (Sec). To collect information about the properties of the as-yet unknown selenonitrites (RSeNO) in comparison with the well-known thionitrites, (RSNO), the interaction of nitrosating reagents with a choice of molecular thiols and related selenoles as model compounds have been studied. Selenol nitrosation is clearly preferred in vitro *to thiol nitrosation, but selenonitrites are thermally significantly less stable than the related thionitrites, suggesting that selenonitrites may be important, but yet-undetected intermediates in selenoprotein chemistry. Chemical trapping of RSeNO was achieved for the first time by its 1,4-addition to dimethylbutadiene leading to a stable unsaturated oxime.*

Keywords Nitrogen oxide; nitrosation; oxime; selenol; selenonitrite; thionitrite

STATE OF RESEARCH: THIONITRITES

In connection with the recent intense research on all aspects of the biochemistry of NO, the presence of *S*-nitrosothiols (thionitrites, RSNO) in physiological systems was recognized.[1–5] Thionitrites have been

Received January 21, 2004; accepted August 25, 2004.

This work was supported by the *Deutsche Forschungsgemeinschaft* (DFG). We also thank C. Vinas and F. Teixidor (Barcelona, Spain) for samples of the carboranylthiol, G. Mugesh (Bangalore, India) for oxazolinyl derivatives, W. Kaim (Stuttgart, Germany) for EPR measurements, P. G. Jones and L. Ernst (Braunschweig, Germany) for support with X-ray crystallography and two-dimensional NMR spectroscopy, respectively, and U. Jahn (Braunschweig, Germany) for helpful discussions on radical reactions.

Address correspondence to Wolf-Walther du Mont, Institut fuer Anorganische und Analytische Chemie, Technische Universitaet Braunschweig Hagenring 30, 38106 Braunschweig, Germany. E-mail: w.du-mont@tu-bs.de

detected *in vivo*; they appear to contribute to the anti-platelet aggregation effects of nitroprusside and their concentration in blood plasma can be up to 1 μM and they are of potential medical use as NO donors. *S*-nitrosohaemoglobin is apparently involved in vascular control.[6] In physiological systems, RSNO can be generated from thiols and NO in the presence of oxidizing agents.[7] Their chemical synthesis was long known before their physiological role was recognized: thionitrites are formed by acid-catalyzed reactions of nitrite with thiols in aqueous systems, or by the reaction of organic nitrites with thiols in organic solvents. Until 1999, *S*-nitroso-acetylpenicillamine (SNAP) was the only structurally charcterized RSNO compound.[8,9] Recently, several research groups have been contributing to structural studies on aliphatic and aromatic RSNO. Protecting groups apparently help to stabilize RSNO against decomposition reactions; among the more stable compounds are SNAP and *S*-nitrosoglutathion (GSNO), whereas (free) *S*-nitrosocystein (CysNO) is a very labile compound. The typical decomposition pathway of thionitrites is homolytic S-N cleavage leading to disulfides (by thiyl radical dimerisation) and molecular NO.[5,10] Photolysis of GSNO confirmed this radical pathway, but under aerobic conditions O_2 participates in the reaction sequence, i.e., radical trapping by O_2 is accompanied by NO oxidation to NO_2.

An important finding in studies on thionitrites was the inhibition of RSNO homolysis by ethylenediamine tetracicetic acid (EDTA). This result revealed that previous studies had neglected the role of trace metal catalysis in RSNO decomposition. RSNO cleavage by monovalent copper shows analogies induced by electrochemical reduction.

THIONITRITES, PEROXYNITRITES, AND SELENOENZYMES

Biochemical studies revealed that GSNO also is enzymatically cleaved by the thioredoxin system.[11] The active centers of mammalian thioredoxine reductases (TrxR) contain a selenocystein moiety; any particular role of this selenolate function in course of GSNO cleavage by TrxR is not known.

There are some hints in the medical literature that mammalian glutathione peroxidase (GPx), another selenium-containing antioxidant enzyme, potentiates the inhibition of platelet function by catalyzing RSNO cleavage.[12] Experimentally, it was confirmed that selenocystine as well as GPx, with RSH as cofactor catalyse, the dismutation of GSNO and of SNAP to the corresponding disulfides and free NO.[5,13] The cofactor leads to the formation of selenols that attack RSNO. The particular

$$\mathrm{RSeSeR} + \mathrm{R'SH} \rightarrow \mathrm{RSeSR'} + \mathrm{RSeH}$$

$$\mathrm{RSeSR'} + \mathrm{RS'H} \rightarrow \mathrm{R'SSR'} + \mathrm{RSeH}$$

$$2\ \mathrm{GSNO} \xrightarrow[+\,\mathrm{R'SH}]{\text{catalyst}} \mathrm{GSSG} + 2\ \mathrm{NO}$$

SCHEME 1 Diselenide-supported GSNO decomposition.[13]

selenol-RSNO reaction step was, to our knowledge, never verified experimentally (Scheme 1).

The oxidation of selenocysteine (Sec) is involved in the interaction of GPx with NO-donating SNAP.[14] Intermediates with Se–S and with Se–OH functions were proposed. The particular mechanistic role of selenium in these reactions, however, has not been experimentally clarified.

The ability of selenoproteins to effect peroxynitrite reduction to nitrite has been recognised by Sies et al. (Scheme 2);[15–18] a possible subsequent nitrosating reaction of nitrite with selenol functions, however, has not yet been detected.

$$\mathrm{RSeH} + \mathrm{O{=}NOO^{\ominus}} \rightarrow \mathrm{RSeOH} + \mathrm{ONO^{\ominus}}$$

$$\mathrm{RSeOH} + 2\ \mathrm{GSH} \rightarrow \mathrm{RSeH} + \mathrm{GSSG} + \mathrm{H_2O}$$

SCHEME 2 Selenol-catalysed peroxynitrite reduction.[15–18]

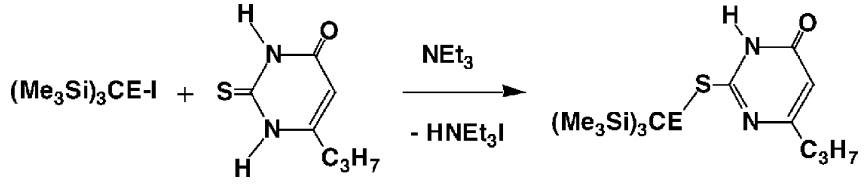
In summary, reactions of selenocysteine precursors and of selenocysteine-containing proteins with RSNO and with other sources of NO or NO^+ have been reported in the recent literature, but the role of selenium in these reaction has not yet been subjected to experimental evidence. Especially, the results of Hou, Guo and Wang[13] would propose that in course of the GPx- or selenocystin-catalysed decomposition of RSNO, selenocysteine moieties and RSNO might generate shortlived *selenonitrite* species (RSeNO) that would decompose into R_2Se_2 and NO.

In biological systems, selenol nitrosation (that has yet been neglected in research at this point) may be competitive to thiol nitrozation (which is experimentally well established). To gain a deeper insight into the role of selenols in the biochemistry of NO, we found it necessary to evaluate the basic properties of the as yet unknown RSeNO compounds. This includes finding concepts to stabilize selenonitrites as far as possible.

CHOICE OF SUBSTITUENTS FOR THIOL AND SELENOL NITROZATION EXPERIMENTS

To test our concept to synthesise selenonitrites that should be as long-lived as possible, we studied substituent effects on the stability of related thionitrites. Concerning nitrosyl halides X–N=O (X=F, Cl, Br, I), the tendency to homolytic dissociation of the N–X bond decreases with increasing electronegativity of the halogen. This prompted us to study the nitrozation behavior of thiols with electron-withdrawing substituents. On the other hand, sterically protecting triarylmethyl groups and bulky ortho-substituted aryl groups apparently enhance the thermal stability of thionitrites. This may be due to disfavoring bimolecular decomposition pathways and disfavoring disulfide formation.[19–25]

Disulfide and diselenide formation is particularly suppressed when sterically demanding trisyl [trisyl = Tsi = $(Me_3Si)_3C$] substituents enforce electronically unfavourable *trans*-conformation. Trisyliodosulfane (TsiSI)[22] and trisyliodoselane (TsiSeI)[19] are uniquely thermally stable but quite reactive sulfenyl and selenenyl halides with 2-center-2-electron S–I or Se–I bonds. Both compounds react in a straightforward fashion with antithyroid drugs like PTU (propylthiouracil, Scheme 3), modeling the proposed deiodinase enzyme inhibition.[22–27]



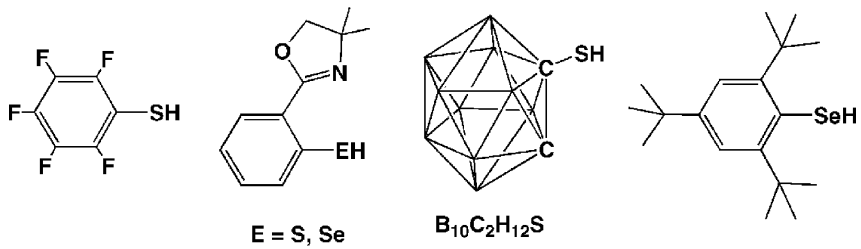
SCHEME 3 Sulfenyl and selenenyl iodide (E = S, Se) reactions with PTU.[22–27]

An alternative approach to stable selenenyl iodides is the use of intramolecular coordination which stabilizes Se-I bonds as part of 3-center-4-electron systems.[28–31]. Such selenenyl iodides show little tendency to undergo transformation into diselenide and molecular iodine.

NITROZATION EXPERIMENTS

As a thiol with a strongly electron-withdrawing substituent, we chose the pentafluorophenyl derivative. This thiol reacts with nitrosating agents (*t*-butylnitrite or *i*-amylnitrite) at −50°C leading to red solutions. The desired thionitrite, however, cannot be isolated due to complete loss of NO under disulfide formation (Scheme 4).

As a thiol with a sterically demanding but strongly electron-withdrawing substituent we chose *o*-carboranylthiol. With nitrosating



SCHEME 4 Thiols and selenole that give unstable nitrosation products.[22,23]

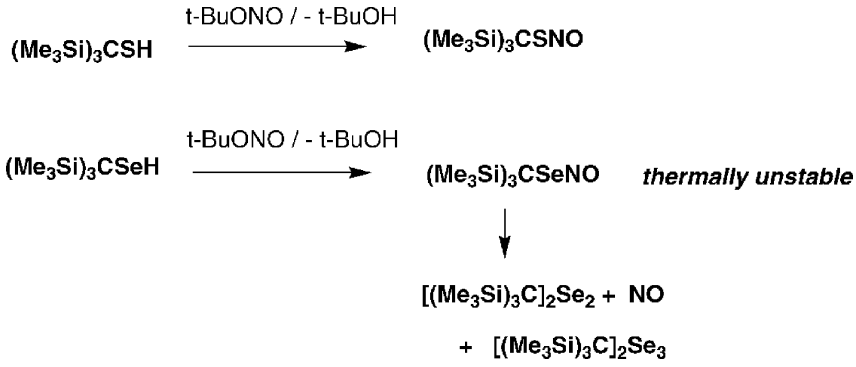
agents, green solutions containing the thionitrite are formed; attempted isolation of the green compound, however, was unsuccessful due to disulfide formation.

As a thiol with the abiltiy for intramolecular coordination that would allow formation of a Se-N bond as part of a 3-center-4-electron system, we chose 2-(4,4-dimethyl-2-oxazolinyl)phenylthiol. This thiol reacts with nitrosating agents (*t*-butylnitrite or *i*-amylnitrite) at −20°C forming red solutions that allow NMR-detection of the desired thionitrite which, decomposes during the work-up procedure and furnishes only the known disulfide as an isolated product. The intramolecularly coordinated thionitrite is apparently only a transient compound at room temperature.

2-(4,4-dimethyl-2-oxazolinyl)phenylselenol nitrozation occurs even at −78°C, instantaneously forming deep-red solutions that turn yellow-brown (due to diselenide formation), even at −78°C within 1–2 h. The selenol is more reactive towards *t*-butylnitrite than the related thiol, but the selenonitrite also is much less stable than the thionitrite.[32]

With trisylthiol, nitrozation does not occur at −78°C. At room temperature, however, nitrozation is complete within about 30 min. The new thionitrite can be isolated as a solid compound in a pure state. This encouraging result led us to persue this steric approach for selenonitrite stabilization. Trisylselenol indeed instantaneously reacts with the nitrosating agent at −78°C to form deep-red solutions that contain TsiSeNO (Scheme 5). These solutions, as well as the residue left after the evaporation of all volatiles, stay deep-red for a long time when kept at −78°C. ^{77}Se-NMR spectra show that the signal of trisylselenol disappears after addition of the nitrozating agent, but no other ^{77}Se-NMR signal can be resolved.[32]

Thermal decomposition of the red material leads to loss of gaseous NO and formation of the diselenide that can be identified as main product by its ^{77}Se-NMR signal, accompanied by smaller signals of the related triselenide. The absence of ^{77}Se-NMR absorptions in the red



SCHEME 5 Nitrozation of trisylthiol and trisylselenol.[22,23,32]

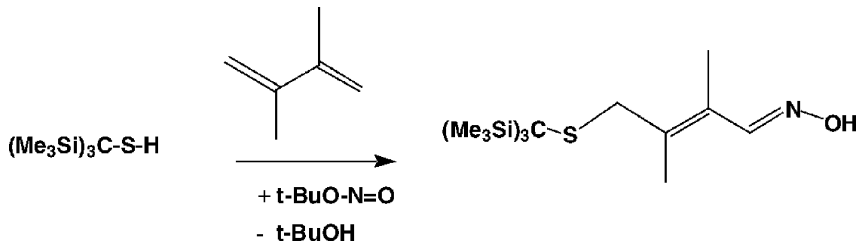
product coincides with the appearence of an EPR quintet signal that can be modeled by coupling with two equivalent ^{14}N nuclei ($a_N = 7{,}6$ G), as in a radical species of the composition $TsiSe(NO)_2^{\cdot}$. After the decomposition of this species, another radical with the coupling pattern of a nitroxide radical $(RCH_2)_2NO$ (involving one ^{14}N and four equivalent ^1H nuclei) is observed. Using *i*-amylnitrite in place of *t*-butylnitrite, a slightly different secondary radical $(RCH_2)_2NO$ is detected. Related radicals were not observed in solutions of decomposing TsiSNO.

Since TsiSeNO could not be crystallized as a metastable compound, it was desirable to develop a typical trapping reaction that could be used for both thionitrites and shortlived selenonitrites.

TRAPPING EXPERIMENTS

Encouraged by the report on a cycloadditon reaction of the NO group of a shortlived nitrosophosphate compound with butadiene derivatives,[33] we studied model reactions of the stable thionitrite TsiSNO with butadiene derivatives. With 2,3-dimethylbutadiene a stable 1:1 adduct was isolated which turned out not to be a heterocyclic adduct, but an α,β-unsaturated oxime derivative from the 1,4-addition of TsiSNO to the diene system (unambiguous assignment by two-dimensional NMR spectroscopy).[32] Independent from our study, Cavero et al.[34] detected this type of addition using tritylthionitrite (Ph_3CSNO). In that case, a mixture of (*E*)- and (*Z*)-stereoisomers was detected by NMR. Starting with TsiSNO, only one isomer was observed (Scheme 6). An X-ray crystal structure determination confirmed that the isolated compound is the (*E*)-configurated isomer, forming dimers through intermolecular H bridges involving the =N–OH groups.[32]

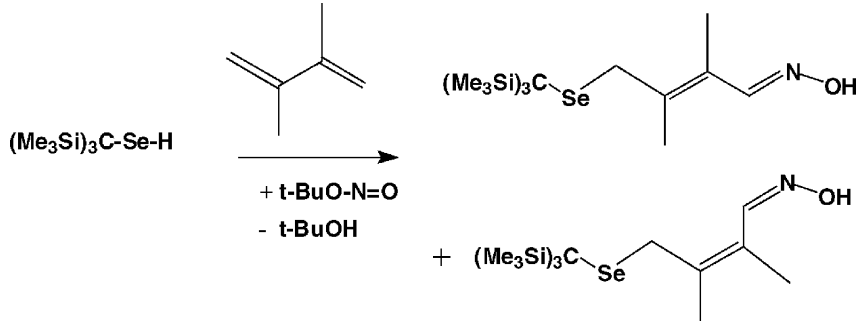
The thionitrite trapping reaction with 2,3-dimethylbutadiene (Scheme 6) is also a very good probe for shortlived thio- and



SCHEME 6 Trapping of thionitrite as a α,β-unsaturated oxime.[32]

selenonitrites, since the very characteristic ^{13}C NMR resonance from the C=NOH function (δ ^{13}C = 149 ppm) should allow to detect derivatives of this oxime, even in complex reaction mixures.

Carrying out trisylselenol nitrozation reactions in the presence of excess 2,3-dimethylbutadiene as a trapping reagent allowed detection of very weak oxime ^{13}C-NMR signals. The purified material (obtained in a very small yield) exhibits, in fact, two ^{13}C-NMR signals close to 149 ppm, and also two complete $^{1}H/^{13}C$- and ^{77}Se-NMR sets from two isomers, apparently (*E*)- and (*Z*)-isomers of the trapping product (Scheme 7).



SCHEME 7 Trapping of selenonitrite as α, β-unsaturated oximes.

Synthetic experiments concerning the approach to modified functional thio- and selenonitrites are under way.

CONCLUSION

Selenol nitrozation is clearly preferred *in vitro* to thiol nitrozation, but selenonitrites (RSeNO) are thermally significantly less stable than the related thionitrites, which suggests that selenonitrites may be important, however yet undetected intermediates in selenoprotein chemistry. Chemical trapping of RSeNO was achieved for the first time by its 1,4-addition to dimethylbutadiene leading to a stable, unsaturated δ-seleno oxime.

REFERENCES

[1] L. J. Ignarro, *Angew. Chem.*, **111,** 2003 (1999).
[2] J. L. Ignarro, *Angew. Chem. Int. Ed. Engl.*, **38,** 1882 (1999).
[3] F. Murad, *Angew. Chem.*, **111,** 1976 (1999).
[4] F. Murad, *Angew. Chem. Int. Ed. Engl.*, **38,** 1856 (1999).
[5] K. Wang, W. Zhang, M. Xian, Y.-C. Hou, X.-C. Chen, J.-P. Cheng, and P. G. Wang, *Curr. Med. Chem.*, **7,** 821 (2000).
[6] L. Jia, C. Bonaventura, J. Bonaventura, and J. S. Stamler, *Nature*, **380,** 221 (1996).
[7] S. Goldstein and G. Czapski, *J. Am. Chem. Soc.* **118,** 3419 (1996).
[8] D. L. H. Williams, *Acc. Chem. Res.*, **32,** 869 (1999).
[9] N. Arulsamy, D. S. Bohle, J. A. Butt, J. Irvine, P. A. Jordan, and E. J. Sagan, *J. Am. Chem. Soc.*, **121,** 7115 (1999).
[10] D. L. H. Williams, *Chem. Commun.*, 1085 (1996).
[11] D. Nikitovic and A. Holmgren, *J. Biol. Chem.*, **271,** 19180 (1996).
[12] J. E. Freedman, B. Frei, G. N. Welch, and J. Loscalzo, *Am. Soc. Clin. Inv.*, **96,** 394 (1995).
[13] Y. C. Hou, Z. M. Guo, J. Li, X. Zhu, Z. Yin,and P. G. Wang, *Biochem. Biophys. Res. Commun.*, **228,** 88 (1996).
[14] M. Asahi, J. Fujii, T. Takao, T. Kuzuya, M. Hori, Y. Shimonishi, and N. Taniguchi, *J. Biol. Chem.*, **272,** 19152 (1997).
[15] H. Sies, V. S. Sharov, L.-O. Klotz, and K. Briviba, *J. Biol. Chem.*, **272,** 27812 (1997).
[16] H. Sies and G. E. Arteel, *Free Rad. Biol. & Medicine*, **28,** 1451 (2000).
[17] G. E. Arteel, K. Briviba, and H. Sies, *FEBS Letters*, **445,** 226 (1999).
[18] G. E. Arteel, K. Briviba, and H. Sies, *Chem. Res. in Tox.*, **12,** 264 (1999).
[19] I. Wagner, W.-W. du Mont, S. Pohl, and W. Saak, *Chem. Ber.*, **123,** 2325 (1990).
[20] W.-W. du Mont and I. Wagner, *Chem. Ber.*, **121,** 2109 (1988).
[21] J. Jeske, P. G. Jones, and W.-W. du Mont, *Chem. Ber.*, **126,** 1335 (1993).
[22] C. Wismach and W.-W. du Mont, *7. Int. Conf. on Selenium in Biology and Medicine*, Venezia, Italy (2000). Abstract P-188.
[23] C. Wismach, *Ph.D. Thesis*, Technology University Braunschweig (2004).
[24] K. Goto, Y. Hino, T. Kawashima, E. Yano, G. Yamamoto, N. Takagi, and S. Nagase, *Tetrahedron Lett.*, **41,** 8479 (2000).
[25] K. Goto, Y. Hino, G. Yamamoto, and T. Kawashima, *Abstract C14 19. Int. Symp. on the Org. Chem. of Sulfur,* Sheffield, UK (2000).
[26] W.-W. du Mont, G. Mugesh, C. Wismach, and P. G. Jones, *Angew. Chem.*, **113,** 2547 (2001).
[27] W. W. du Mont, G. Mugesh, C. Wismach, and P. G. Jones, *Angew. Chem. Int. Ed. Engl.*, **40,** 2486 (2001).
[28] A. Panda, G. Mugesh, H. B. Singh, and R. J. Butcher, *Organometallics*, **18,** 1986 (1999).
[29] G. Mugesh, A. Panda, H. B. Singh, and R. J. Butcher, *Chem. Eur. J.*, **5,** 1411 (1999).
[30] G. Mugesh, H. B. Singh, and R. J. Butcher, *Tetrahedron: Asymmetry*, **10,** 237 (1999).
[31] Z. Majeed, W. R. McWhinnie, and P. R. Lowe, *Acta Cryst. B., Cryst. Struct. Commun.*, **56,** 105 (2000).
[32] C. Wismach, W.-W. du Mont, P. G. Jones, L. Ernst, U. Papke, K. D. Becker, W. Kaim, and M. Wanner, *submitted for publication* (2004).
[33] R. W. Ware Jr. and S. B. King, *J. Org. Chem.*, **65,** 8725 (2000).
[34] M. Cavero, W. B. Motherwell, and P. Potier, *Tetrahedron Lett.*, **42,** 4377 (2001).