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Seleno Compounds and Glutathione Peroxidase Catalyzed Decomposition of *S*-Nitrosothiols

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Seleno compounds such as selenocystamine and seleno-D, L-cystine were found to catalyze the decomposition of *S*-nitrosothiols (e.g. *S*-nitroso-glutathione and *S*-nitroso-*N*-acetyl-D, L-penicillamine) in the presence of different thiols (e.g. glutathione, *N*-acetyl-D-penicillamine and 2-mercaptoethanol), and liberate nitric oxide. It was also found that glutathione peroxidase itself can catalyze the decomposition of *S*-nitroso-glutathione without the presence of any thiol or H_2O_2. © 1996 Academic Press, Inc.

Nitric oxide (NO) has been implicated in diverse physiological processes, including vasodilatory and antiplatelet effects, macrophage-induced cytotoxicity, and neurotransmission (1). *S*-nitrosothiols play an important role in storing, transporting and releasing NO (2-3). As endogenous NO donors, *S*-nitrosothiols can decompose to produce NO and disulfides (4). For most commercial *S*-nitrosotiols such as *S*-nitroso-*N*-acetyl-D, L-penicillamine (SNAP) and *S*-nitroso-glutathione (GSNO), the rate of this decomposition is rather slow in de-ionized aqueous solution, but it is greatly enhanced in the presence of a trace amount of metal ions such as Cu^{II} (5-7). Recently, the function of glutathione peroxidase (EC 1.11.1.9, GPx), an essential *selenium*-containing antioxidant enzyme (8), has been linked with nitric oxide. It is well documented that selenium is an essential mineral in the mammalian diet and its deficiency is tightly associated with many heart diseases such as myocardial necrosis and atherosclerosis (9). Man and coworkers observed that acetylcholine-induced endothelium dependent relaxation was enhanced in aortic rings from rats after receiving *selenium* supplements as compared to control rats (10). Interestingly, Freedman and coworker reported that GPx potentiated the inhibition of platelet function by *S*-nitrosothiols (11). Their report showed that adding GSNO at concentrations that alone did not inhibit platelet function to platelet-rich plasma, together with GPx, led to a dose-dependent inhibition of platelet aggregation. Freedman further suggested that GPx catalyzed the metabolism of endogenous GSNO to liberate NO in the presence of H_2O_2. Prütz reported that some simple selenium compounds such as selenocystamine can activate the decomposition of H_2O_2 by glutathione (GSH) (12). Here we report our latest finding that diselenides catalyze the decomposition of *S*-nitrosothiols to produce NO in the presence of thiols. We also found that GPx alone can catalyze the decomposition of *S*-nitroso-glutathione without the presence of thiols or H_2O_2.

MATERIALS AND METHODS

Materials and chemicals. Selenocystamine, seleno-D, L-cystine, *N*-acetyl-D-penicillamine, 2-mercaptoethanol, glutathione and glutathione peroxidase were purchased from Sigma Chemical Co. *S*-nitroso-*N*-acetyl-D, L-penicillamine and *S*-nitrosoglutathione were prepared by the methods of Field et al (13) and Hart et al (14) respectively. All other chemicals were purchased from Aldrich Chemical Co.

Monitoring of the decomposition of S-nitrosothiols. The decomposition of *S*-nitrosothiols, SNAP or GSNO was

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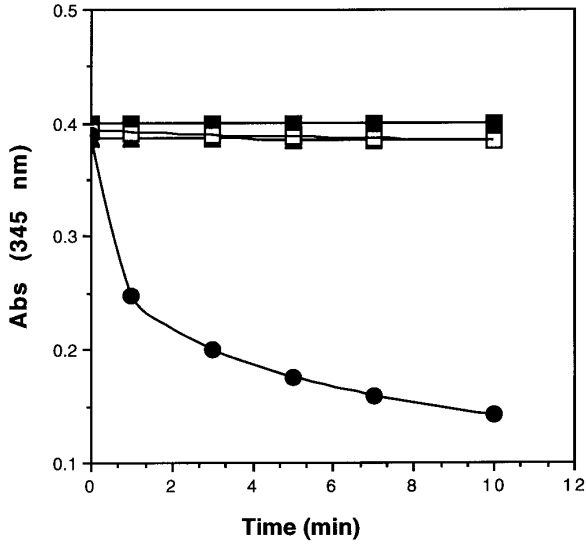


FIG. 1. Selenocystamine catalyzed decomposition of SNAP in the presence of *N*-acetyl-D-penicillamine. The concentration of SNAP was monitored at 345 nm. (■) SNAP control (400 μM); (□) SNAP (400 μM) + selenocystamine (200 μM); (▲) SNAP (400 μM) + *N*-acetyl-D-penicillamine (200 μM); (●) SNAP (400 μM) + *N*-acetyl-D-penicillamine (200 μM) + selenocystamine (200 μM).

monitored by following the reduction in absorbance at 345 nm using a *GENESYS 2* spectrometer. All the experiments were carried out at room temperature (22°C) in a phosphate buffer at pH 7.4 (50 mM Na_2HPO_4-NaH_2PO_4 buffer, 2 mM EDTA).

Detection of nitric oxide (NO). NO generated from the decomposition of GSNO or SNAP was measured with a commercial ISO-NO Mark II Isolated Nitric Oxide Meter, using a Duo.18 Interface & Software to process the data (both manufactured by World Precision Instruments, Inc. Sarasota, Florida). All the solutions used were deoxygenated using high purity nitrogen gas (N_2) and the concentrations of SNAP, N-acetyl-D-penicillamine and selenocystamine were adjusted to keep NO response within a linear range.

RESULTS AND DISCUSSION

1. Selenocystamine or Seleno-D,L-cystine Catalyzes the Decomposition of S-Nitrosothiols (e.g. SNAP or GSNO)

Fig. 1 shows a typical result of selenocystamine (SeCA) catalyzed decomposition of SNAP by monitoring the characteristic RS-NO absorption at 345 nm. SNAP (400 μM) control, as well as its mixture with N-acetyl-D-penicillamine (200 μM), was stable in 50 mM sodium phosphate buffer (pH 7.4, 2 mM EDTA). When selenocystamine (SeCA, 200 μM) was incubated with SNAP (400 μM), no apparent decomposition of SNAP was detected. However, SNAP decomposed quickly when *N*-acetyl-D-penicillamine (AP) was present in the mixture of SNAP and selenocystamine. The decomposition proceeded abruptly in the initial 2 min, then at a relatively slower rate. The dependence on both SeCA and AP suggested that a reactive intermediate was generated from the reaction of the diselenide SeCA and the thiol AP. Subsequent reaction of the intermediate with SNAP led to the decomposition of SNAP.

The above diselenide catalyzed *S*-nitrosothiol decompostion in the presence of thiols was reproducibly observed with different *S*-nitrosothiols (e.g. SNAP and GSNO), and different thiols (e.g. glutathione, *N*-acetyl-D-penicillamine and 2-mercaptoethanol) and different diselenides (e.g. selenocystamine and seleno-D, L-cystine). For example (Fig. 2), GSNO (500 μM), either with or without glutathione or 2-mercaptoethanol (200 μM), was very stable in the 50 mM phosphate buffer. Addition of selenocystamine (200 μM) into a GSNO (200 μM) solution,

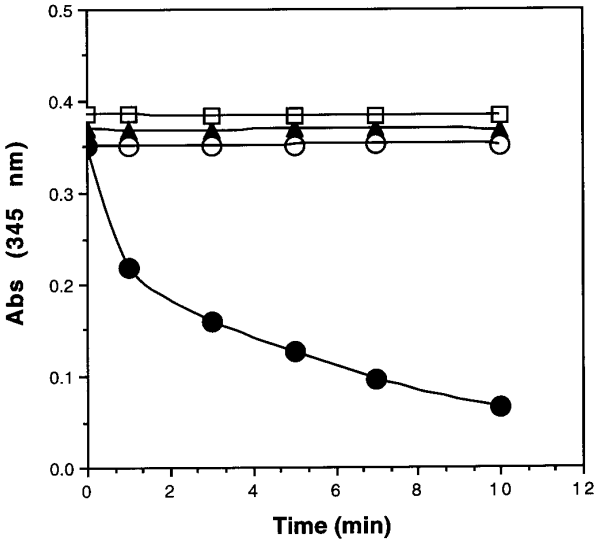


FIG. 2. Selenocystamine catalyzed decomposition of GSNO in the presence of glutathione. The concentration of GSNO was monitored at 345 nm. (■) GSNO control (500 μM); (□) GSNO (500 μM) + selenocystamine (200 μM); (▲) GSNO (500 μM) + glutathione (200 μM); (●) GSNO (500 μM) + glutathione (200 μM) + selenocystamine (200 μM).

did not promote decomposition of the GSNO. However, a quick decomposition of GSNO was observed in the simultaneous presence of both glutathione and selenocystamine. The rate of the decomposition of *S*-nitrosothiols depends on the concentration of diselenides and thiols. The higher concentration of the diselenide and thiol, the faster the decomposition. Similar to selenocystamine, seleno-D, L-cystine remarkably catalyzed the decomposition of either SNAP or GSNO in the presence of thiols such as glutathione, or *N*-acetyl-D- penicillamine or 2-mercaptoethanol.

2. Selenocystamine or Seleno-D,L-cystine Catalyzes the Formation of Nitric Oxide (NO) from S-Nitrosothiols (e.g. SNAP or GSNO)

Using a NO detector, we measured the generated NO in the above systems catalyzed by the seleno compounds. Fig. 3 shows a typical NO measurement result. SNAP (10 μM), its mixture with *N*-acetyl-D-penicillamine (5 μM) as well as its mixture with selenocystamine (5 μM), was stable in 50 mM sodium phosphate buffer (pH 7.4, 2 mM EDTA) and no NO response was detected. However, rapid generation of NO was detected when *N*-acetyl-D-penicillamine was present in the mixture of SNAP and selenocystamine. The NO was generated quickly in the first few minutes, then at a relatively slower rate. This result together with the results in Fig. 1 and Fig. 2 demonstrated clearly that selenocystamine catalyzed the NO generation with the decomposition of SNAP. Furthermore, we found that the similar NO generation was observed with different diselenides (e.g. selenocystamine and seleno-D, L-cystine), with different *S*-nitrosothiols (e.g. SNAP and GSNO), and different thiols.

3. Proposed Mechanism

To interpret the function of seleno compounds in the decomposition of the *S*-nitroso compounds, we propose a mechanism incorporating diselenide(R′Se-SeR′), *S*-nitrosothiol (RS-NO) and thiol (R-SH) as shown in Scheme 1. It is assumed that the initial activation involves the interchange reaction of the diselenide and the thiol. The diselenide reacts with the free

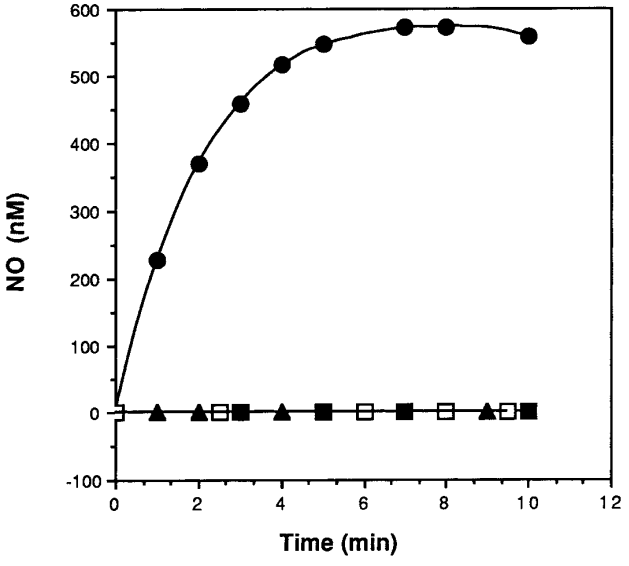
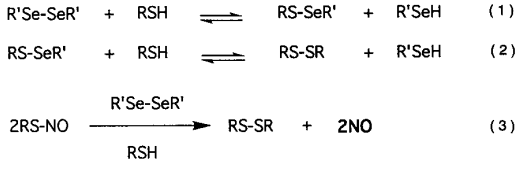


FIG. 3. Detection of NO generated from the decomposition of SNAP catalyzed by selenocystamine in the presence of *N*-acetyl-D-penicillamine. The NO generation was monitored by using a NO detector. (■) SNAP control (10 μM); (□) SNAP (10 μM) + selenocystamine (5 μM); (▲) SNAP (10 μM) + *N*-acetyl-D-penicillamine (5 μM); (●) SNAP (10 μM) + *N*-acetyl-D-penicillamine (5 μM) + selenocystamine (5 μM).

thiol to produce the selenosulfide (RS-SeR′) and the selenol (R′SeH) (eq. 1 and eq. 2). These reactions have been studied in detail by Whitesides (15-16), Rabenstein (17) and Engman (18). The rate of the exchange reaction is normally very fast. The selenol generated from these exchange reactions reacts with RS-NO to release nitric oxide. The overall reaction was that *S*-nitrosothiol was decomposed and NO was liberated in the presence of diselenide and thiol (eq. 3). To support this mechanism, a pure selenol, 2-aminoethaneselenol, prepared from reduction of selenocystamine with sodium borohydride (15), was added to the solution of SNAP, a similar decomposition of SNAP was observed and NO generation was detected without the presence of any thiol. This result was consistent with proposed mechanism. In summary, selenol generated from exchange reactions between diselenides and thiols was found to serve as an active intermediate and catalyze the decomposition of *S*-nitrothiols to generate NO. This finding may provide some insights into the mechanism of seleno compounds in the functions of nitric oxide.

4. Glutathione Peroxidase Catalyzes the Decomposition of GSNO

Using photometric method, we found that GPx catalyzed the decomposition of GSNO in the presence of glutathione. As shown in Fig. 4, when GSNO (500 μM) was incubated with



SCHEME 1. A proposed mechanism for diselenides-catalyzed formation of nitric oxide from *S*-nitrosothiols in the presence of thiols. Abbreviations: diselenide (R′Se-SeR′), *S*-nitrosothiol (R-NO), thiol (R-SH), selenol (R′SeH), selenosulfide (RS-SeR′), disulfide (RS-SR).

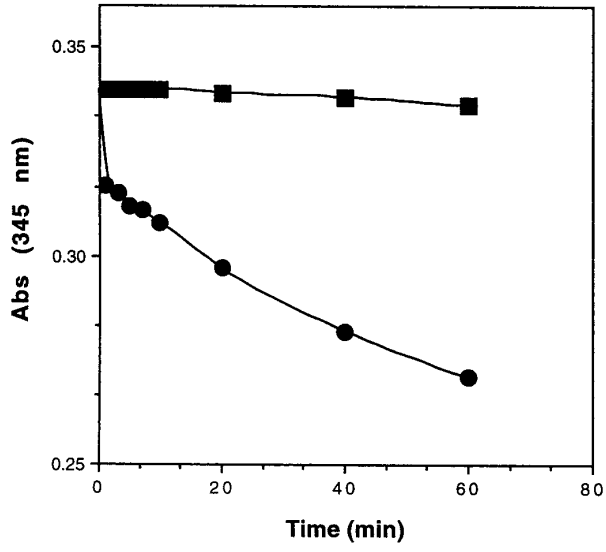


FIG. 4. Glutathione peroxide (GPx) catalyzed decomposition of GSNO in the presence of glutathione. The concentration of GSNO was monitored at 345 nm. (■) GSNO control (500 μM) + glutathione (200 μM); (●) GSNO (500 μM) + glutathione (200 μM) + GPx (0.20 mg/mL, 130 units/mg).

glutathinoe (200 μM), only a slow decomposition of GSNO was observed. However, when the GPx (0.25 mg/mL, 130 units/mg) was present in the system, GPx clearly catalyzed the decomposition of GSNO. The result was also reproducibly observed with other thiol such as *N*-acetyl-D-penicillamine or 2-mercaptoethanol. More interestingly, we also found that GPx

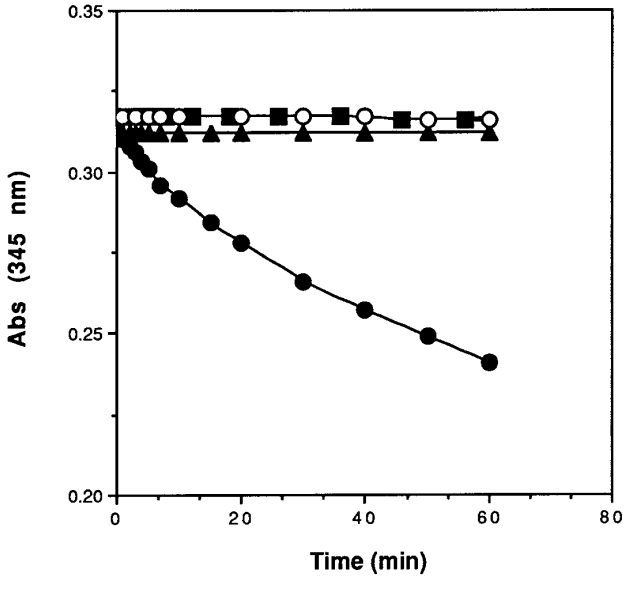


FIG. 5. Glutathione peroxide (GPx) catalyzed decomposition of GSNO without the presence of glutathione or H_2O_2. The concentration of GSNO was monitored at 345 nm. (▲) GSNO control (500 μM); (■) GSNO (500 μM) + H_2O_2 (500 μM); (○) GSNO (500 μM) + H_2O_2 (500 μM) + GPx (0.25 mg/mL, 130 units/mg); (●) GSNO (500 μM) + GPx (0.25 mg/mL, 130 units/mg).

alone can catalyze the decomposition of GSNO. As shown in Fig. 5, glutathione peroxidase (GPx) (0.25 mg/mL, 130 units/mg) clearly catalyzed the decomposition of GSNO (500 μM) without the presence of any thiol or H_2O_2. Meanwhile, it was found that GPx can not catalyze the decomposition of SNAP. Since the concentration of glutathione *in vivo* can be as high as 1-15 mM (19) and GSNO is the major form of *S*-nitrosothiols *in vivo* (20-21), our finding may have important implications on the role of glutathione peroxidase in the biological function of GSNO.

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