A Mechanism for the Oxidation of Glutathione to Glutathione Disulfide with Organotellurium(IV) and Organoselenium(IV) Compounds. A Stepwise Process with Implications for Photodynamic Therapy and Other Oxidative Chemotherapy

Michael R. Detty,*,† Alan E. Friedman,*,‡ and Alan R. Oseroff§

Office Imaging Research and Technical Development and Clinical Diagnostics Research Laboratories, Eastman Kodak Company, Rochester, New York 14650-2129 and Roswell Park Cancer Institute, Elm and Carlton Street, Buffalo, New York 14263

Received August 16, 1994®

The reactions of telluroxides or their hydrates **3**–**5** with glutathione to give telluropyrylium dyes **1**, **2** or diphenyl telluride, respectively, and glutathione disulfide have at least two discrete steps. A fast reaction, which is first-order in both substrate and glutathione, is observed with second-order rate constants of 2.30×10^7 L mol^{-1} s^{-1} at 285.4 K for **3**, 1.66×10^7 L mol^{-1} s^{-1} at 293.2 K for **4**, and 5.2×10^6 L mol^{-1} s^{-1} at 285.5 K for **5**. This reaction is followed by a slower reaction, which is first-order in both substrate and glutathione, with second-order rate constants of 2.65×10^5 L mol^{-1} s^{-1} at 293.5 K for **3**, 3.34×10^5 L mol^{-1} s^{-1} at 293.2 K for **4**, and 7.64×10^3 L mol^{-1} s^{-1} at 285.5 K for **5**. The slow reaction is accompanied by the generation of the corresponding tellurium(II) compound. Diphenyl selenoxide hydrate (**6**) displays similar behavior, although the rate constants associated with the fast (2.26×10^2 L mol^{-1} s^{-1}) and slow (6.62×10^1 L mol^{-1} s^{-1}) reactions are many orders-of-magnitude less than observed for the tellurium analogues.

Introduction

Selenium is an essential trace element to humankind primarily as the element source for the selenium-containing enzymes—the glutathione peroxidases.[1] These enzymes reduce hydrogen peroxide, fatty-acid peroxides, and phospholipid and cholesterol hydroperoxides in the body.[2] All aerobic cells, including aerobic transformed (cancerous) cells, rely on a variety of antioxidative systems, which include the glutathione peroxidases, to protect against the potentially lethal effects of lipid peroxidation and other oxidative damage.[3–6] These processes work against the therapeutic oxidative stress created by the generation of singlet oxygen in photodynamic therapy (PDT).[7] PDT is a relatively recent cancer treatment in which light, oxygen, and a photosensitizer combine at a tumor site to produce singlet oxygen, which is cytotoxic.

No specific inhibitors of glutathione peroxidases are known. Consequently, manipulation of selenium levels has been utilized to examine the involvement of these enzymes in cytoprotection against photooxidative stress initiated by PDT.[8] In human and murine leukemia cell lines, cells fed a selenium-deficient diet were more susceptible to photoperoxidation and photokilling than selenium-sufficient control populations. In the human leukemia cell lines, 5- to 10-fold lower glutathione peroxidase activity was observed in the selenium-deficient cells. One important conclusion from these studies is that impairment of the glutathione–glutathione peroxidase repair cycle in transformed cells might lead to more effective treatment with singlet-oxygen-generating photosensitizers.

How might such an impairment be designed into sensitizers or codrugs used in PDT? While selenium deficiency in the patient is an extreme condition to achieve prior to treatment with PDT, attacking the repair cycle by depleting glutathione levels may be a viable alternative—one that has been suggested for conventional chemotherapy.[9] Several classes of materials have been described as having thiol peroxidase activity including selenium-containing compounds Ebselen,[10] diaryl diselenides,[11] α-(phenylselenenyl)acetophenone deriva-

†Office Imaging Research and Technical Development.
‡Clinical Diagnostics Research Laboratories.
§Roswell Park Cancer Institute.

® Abstract published in *Advance ACS Abstracts,* November 15, 1994.

(1) Schwarz, K.; Foltz, C. M. *J. Am. Chem. Soc.* **1957**, *79*, 3292.
(2) Wendel, A. *Phosphorus Sulfur Silicon* **1992**, *67*, 405.
(3) Flohe, L. In *Free Radicals in Biology*; Pryor, W. A., Ed., Academic Press: New York, 1982; Vol. 5, pp 223–253.
(4) Ursini, F.; Bindoli, A. *Chem. Phys. Lipids* **1984**, *44*, 255.
(5) Thomas, J. P.; Maiorino, M.; Ursini, F.; Girotti, A. W. *J. Biol. Chem.* **1990**, *265*, 454.
(6) Nathan, C.; Arrick, B. A.; Murray, H. W.; DeSantis, N. M.; Cohn, Z. A. *J. Exp. Med.* **1980**, *153*, 766.
(7) (a) *Future Directions and Applications of Photodynamic Therapy*, Gomer, C. J., Ed.; SPIE Optical Engineering Press: Bellingham, WA, 1990. (b) *Photodynamic Therapy of Neoplastic Disease*, D. Kessel, Ed.; CRC Press: Boca Raton, FL, 1990.
(8) Lin, F.; Geiger, P. G.; Girotti, A. W. *Cancer Res.* **1992**, *52*, 5282.
(9) (a) Siemann, D. W.; Beyers, K. L. *Br. J. Cancer* **1993**, *68*, 1071. (b) Lee, F. Y. F.; Allalunis-Turner, M. J.; Siemann, D. W. *Br. J. Cancer* **1987**, *56*, 33. (c) Bellomo, G.; Mirabelli, F.; DiMonte, D.; Richelmi, P.; Thor, H.; Orrenius, C.; Orrenius, S. *Biochem. Biopharmacol.* **1987**, *36*, 1313. (d) Tomashefsky, P.; Astor, M.; DeVere White, R. *J. Natl. Cancer Inst.* **1985**, *74*, 1233.
(10) Sies, H. *Free Radical Biol. Med.* **1993**, *14*, 313.
(11) Wilson, S. R.; Zucker, P. A.; Huang, R.-R. C.; Spector, A. *J. Am. Chem. Soc.* **1989**, *111*, 5936.
(12) Cotgreave, I. A.; Moldéus, P.; Brattsand, R.; Hallberg, A.; Andersson, C. M.; Engman, L. *Biochem. Pharmacol.* **1992**, *43*, 793.
(13) (a) Wu, Z.-P., Hilvert, D. *J. Am. Chem. Soc.* **1990**, *112*, 5647. (b) House, K. L.; Dunlap, R. B.; Odom, J. D.; Wu, Z. P.; Hilvert, D. *J. Am. Chem. Soc.* **1992**, *114*, 8573.
(14) (a) Soda, K. *Phosphorus Sulfur Silicon* **1992**, *67*, 461. (b) Esaki, N.; Oikawa, T.; Tanaka, H.; Soda, K. *Biomed. Res. Trace Elem.* **1990**, *1*, 261.
(15) Reich, H. J.; Jasperse, C. P. *J. Am. Chem. Soc.* **1987**, *109*, 5549.
(16) Engman, L.; Stern, D.; Cotgreave, I. A.; Andersson, C. M. *J. Am. Chem. Soc.* **1992**, *114*, 9737.
(17) Engman, L.; Stern, D.; Pelcman, M.; Andersson, C. M. *J. Org. Chem.* **1994**, *59*, 1973.
(18) Detty, M. R.; Gibson, S. L. *Organometallics* **1992**, *11*, 2147.
(19) (a) Powers, S. K.; Walstad, D. L.; Brown, J. T.; Detty, M. R.; Watkins, P. J. *J. Neuro-Oncol.* **1989**, *7*, 179. (b)Detty, M. R. In *Proc. Fourth Int. Symp. Uses Selenium Tellurium* **1989**, 448. (c) Detty, M. R.; Merkel, P. B.; Hilf, R.; Gibson, S. L.; Powers, S. K. *J. Med. Chem.* **1990**, *33*, 1108. (d) Detty, M. R.; Merkel, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 3845. (e) Detty, M. R.; Merkel, P. B.; Gibson, S. L.; Hilf, R. *Oncology Res.* **1992**, *4*, 367.

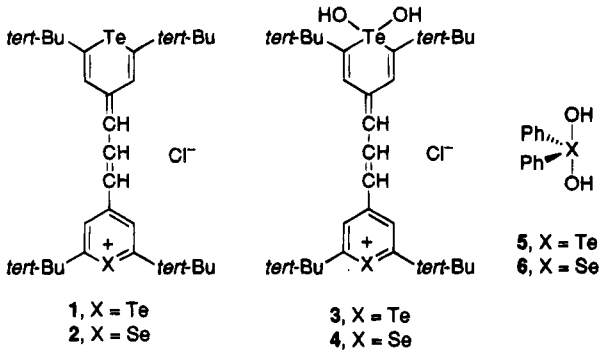
0022-3263/94/1959-8245$04.50/0

Table 1. Observed Pseudo First-Order Rate Constants (k_{obs}) and Calculated Second-Order Rate Constants (k_{calc}) in the Reaction of 36 with Glutathione[a]

compd	conc, M	[GSH], M	T, K	λ,[b] nm	k_{obs}, s^{-1}	k_{calc}, L mol^{-1} s^{-1}	Reaction
3	1.5×10^{-5}	1.5×10^{-5}	285.4	320	345 ± 7[c]	2.30×10^{7}	fast
	1.5×10^{-5}	5.0×10^{-4}	293.5	510	258 ± 3	–	slow
	1.5×10^{-5}	3.33×10^{-4}	293.5	510	205.4 ± 1.5	2.64×10^{5}[d]	slow
	1.5×10^{-5}	1.67×10^{-4}	293.5	510	146.7 ± 0.2	–	slow
4	7.5×10^{-6}	7.5×10^{-6}	293.2	270	124.3 ± 0.3[c]	1.66×10^{7}	fast
	7.5×10^{-6}	5.0×10^{-4}	293.5	650	188 ± 10	3.76×10^{5}	slow
	7.5×10^{-6}	5.0×10^{-4}	293.5	500	171.6 ± 1.2	–	slow
	7.5×10^{-6}	3.33×10^{-4}	293.5	500	134.6 ± 0.4	3.33×10^{5}[d]	slow
	7.5×10^{-6}	1.67×10^{-4}	293.5	500	83.5 ± 0.5	–	slow
5	5×10^{-5}	1.0×10^{-4}	285.5	270	260 ± 30[c]	5.2×10^{6}	fast
	5×10^{-5}	5.0×10^{-4}	285.5	370	3.82 ± 0.07	7.64×10^{3}	slow
6	5×10^{-5}	5.0×10^{-4}	293.1	270	0.113 ± 0.002	2.26×10^{2}	fast
	5×10^{-5}	5.0×10^{-4}	293.1	310	0.0331 ± 0.0002	6.62×10^{1}	slow

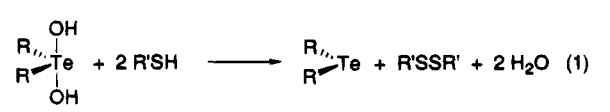
[a] The observed rate constants, k_{obs}, are the average of 10 independent kinetic traces. The deviations given are $\pm 2\sigma$ for the average. The second-order rate constants, k_{calc}, were determined by dividing k_{obs} by [glutathione] except for those values calculated by the method of footnote *d*. [b] Wavelength of measurement for kinetic trace. [c] Second-order curve fit. [d] Rate constant is equal to the slope of the line defined by k_{obs} plotted as a function of [glutathione].

tives,[12] selenosubtilisin,[13] glutaselenone (γ-glutamylselenocysteinylglycine),[14] and a few isoselenazolidin-3-ones.[15] Tellurium-containing compounds including diaryl ditellurides,[16] diaryl tellurides,[17] and telluropyrylium dyes **1** and **2** have also shown thiol peroxidase activity.[18]



Telluropyrylium dyes **1** and **2** have been shown to be effective sensitizers for PDT in both *in vitro*[19] and *in vivo*[20] studies, presumably via the generation of singlet oxygen. Epifluorescence microscopy of human glioma cells treated with dyes **1** and **2** and light shows the yellow-green fluorescence characteristic of photooxidized dyes **3** and **4**, which are produced by the reaction of singlet oxygen and water with the tellurium atom of **1** and **2**.[19c] If the oxidized telluropyrylium dyes were to react with glutathione, then the *in vitro* or *in vivo* formation of **3** or **4** becomes a potential means for depleting treated cells and tumors of glutathione.

The overall stoichiometry for the oxidation of thiols to disulfides with tellurium(IV) oxides is shown in eq 1.[17,18] In this process, a tellurium(IV) species is reduced to a tellurium(II) species. In the presence of hydrogen peroxide or singlet oxygen and water, a tellurium(IV)–tellurium(II)–tellurium(IV) catalytic shuttle can convert thiols to disulfides. While these oxidations are assumed to be fast, little is known about either the rate constants for oxidation of thiols to disulfides with tellurium(IV) oxides or the mechanistic steps and order of the reaction leading to the disulfide products.



Herein, we describe the reactions of tellurium(IV) oxides **3–5** and selenium(IV) oxide **6** with glutathione to give glutathione disulfide and tellurium(II) compounds **1**, **2**, and diphenyl telluride or diphenyl selenide. These reactions were monitored by stopped-flow spectroscopy and consisted of at least two kinetically distinct steps: a fast initial reaction followed by a slower reaction.

Results and Discussion

Preparative Studies. The addition of 2.1 equiv of glutathione to 5.0×10^{-5} M solutions of **3** or **4** in phosphate buffered saline (PBS, pH 7.4) gave 5.0×10^{-5} M solutions of the corresponding tellurium(II) dyes **1** (λ_{max} 810 nm, ϵ 1.5×10^{5} M^{-1} cm^{-1}) or **2** (λ_{max} 770 nm, ϵ 1.25×10^{5} M^{-1} cm^{-1}), respectively, as determined spectrophotometrically. The addition of 2.1 equiv of glutathione to 1.0×10^{-3} M solutions of **5** or **6** in PBS gave diphenyl telluride or diphenyl selenide, respectively, which were sparingly soluble in PBS. Both diphenyl telluride and diphenyl selenide were recovered in >95% isolated yield following ether extraction of the PBS mixtures. The preparative reactions of **3–5** with other thiols (thiophenol, *tert*-butyl mercaptan) to give disulfides and tellurium(II) compounds have been described.[17,18]

Stopped-Flow Kinetics and Spectroscopy. The reductions of organotellurium(IV) oxides **3** and **4** with glutathione in PBS were monitored via stopped-flow spectroscopy (Table 1). At a concentration of 1.5×10^{-5} M in each reagent for **3** and 7.5×10^{-6} M in each reagent for **4**, a fast reaction was observed with a second-order rate constant of 2.30×10^{7} L mol^{-1} s^{-1} at 285.4 K for **3** (Figure 1) and a second-order rate constant of 1.66×10^{7} L mol^{-1} s^{-1} at 293.2 K for **4**. At 5.0×10^{-5} M concentrations in both glutathione and **3** or **4**, second-order rate constants of 2.3 x 10^{7} L mol^{-1} s^{-1} for **3** at 285.4 K and 1.71×10^{7} L mol^{-1} s^{-1} for **4** at 293.5 K were calculated, which are indicative of a first-order dependence on the concentration of each reagent in both reactions. Transient spectra for the reactions of **3** and **4** with glutathione are found in the supplementary material.

The wavelengths at which these reactions were monitored were critical to the successful isolation of the reaction. At λ_{max} for **3** and **4** (510 and 500 nm, respectively, in PBS), very small spectral changes were observed during the time frame of the fast reaction, which suggest that a change in tellurium(IV) oxidation state is not involved in the fast reaction. However, at 320 nm

(20) Detty, M. R.; Powers, S. K. Photodynamic Therapy Using Seleno- or Telluropyrylium Salts. U.S. Patent 5,047,419 (1991).

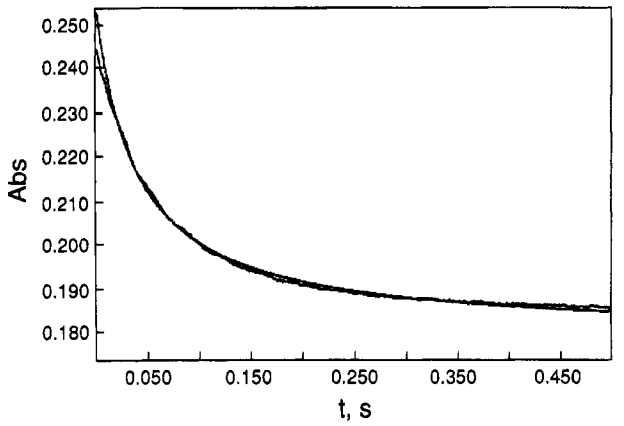


Figure 1. A kinetic trace obtained at 320 nm for the fast reaction of **3** (1.5×10^{-5} M) with glutathione (1.5×10^{-5} M) at 285.4 K. Second-order curve fitting (identified as the upper trace in the first 20 ms) was indicative of k_{obs} of 345 ± 7 s^{-1} for 10 independent runs.

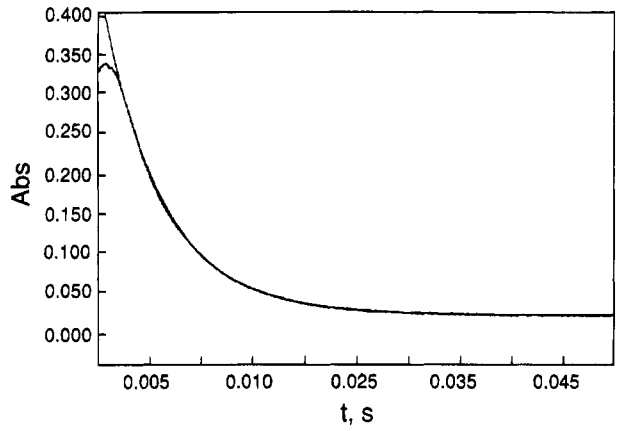


Figure 2. A kinetic trace obtained at 510 nm for the slow reaction of **3** (1.5×10^{-5} M) with glutathione (5×10^{-4} M) at 285.4 K. The curved region in the first 2 ms of reaction is an artifact associated with the response time of the instrument. First-order curve fitting was indicative of k_{obs} of 172.7 ± 0.6 s^{-1} for 10 independent runs.

for **3** and 270 nm for **4**, good second-order curves were obtained (Figure 1).

At glutathione concentrations in excess of substrate concentration, a second, slower process was also observed (Figure 2). This process was followed at λ_{max} for **3** and **4** and was characterized by nearly complete loss of absorbance at these wavelengths. Under pseudo first-order conditions, the calculated second-order rate constants for this second process were 2.64×10^5 L mol^{-1} s^{-1} for **3** at 293.4 K and 3.33×10^5 L mol^{-1} s^{-1} for **4** at 293.5 K (Table 1 and Figure 3).

The loss of absorption at these wavelengths was accompanied by increases in absorption at wavelengths where the reduced dyes **1** and **2** absorb. As indicated in Table 1 for **4**, the rate of increase at 650 nm (k_{obs} = 188 ± 10 at 5×10^{-4} M glutathione) at 293.5 K was nearly identical to the rate of decrease at 500 nm (k_{obs} = 171.6 ± 1.2 at 5×10^{-4} M glutathione). These data suggest that the slow reaction involves formal reduction of tellurium(IV) to tellurium(II).

Similar results were obtained with telluroxide hydrate **5** (Table 1). An inital fast reaction was followed by a slower reaction to give diphenyl telluride with the spectral changes shown in Figure 4. The rate constants

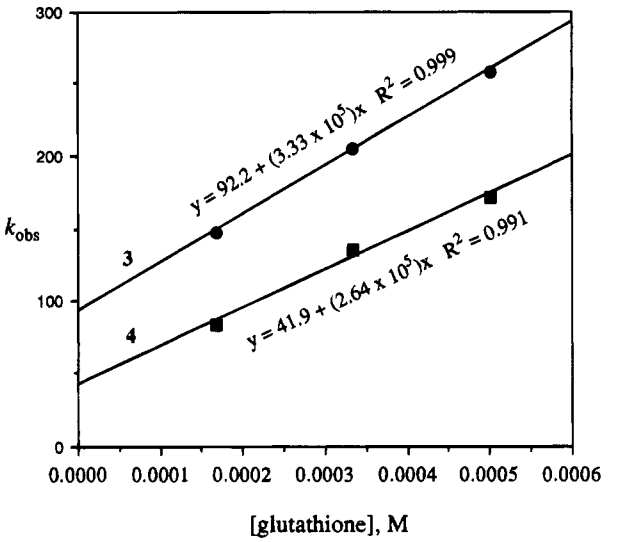


Figure 3. A plot of k_{obs} as a function of glutathione concentration under pseudo-first-order conditions for **3** and **4**. The slope of the line defines the second-order rate constant: 2.64×10^5 for **3** and 3.33×10^5 M^{-1} s^{-1} for **4**. Glutathione concentrations employed were 5.0×10^{-4}, 3.33×10^{-4}, and 1.67×10^{-4} M.

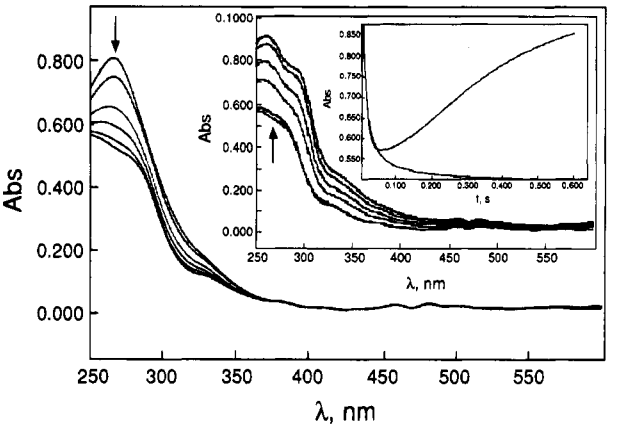


Figure 4. Transient spectra for the reaction of diphenyl telluroxide hydrate (**5**, 5×10^{-5} M) with glutathione (1.0×10^{-4} M) at 285.5 K. The fast reaction is shown with decreasing absorbance from the arrow with transient spectra recorded (from the arrow) at 0.005, 0.0085, 0.014, 0.019, 0.029, and 0.057 s. The larger inset, which shows transient spectra associated with the slow reaction, displays increasing absorbance (from the arrow) at 0.057, 0.075, 0.30, 0.45, 0.80, and 7.85 s. The smaller inset shows a kinetic trace obtained at 270 nm at 285.5 K. A rapid, initial decrease in absorption (shown with second-order curve fitting to a base line of 0.500, k_{obs} of 260 ± 30 s^{-1} for 10 independent runs) is followed by a slower increase in absorption (second-order curve fitting not shown, k_{obs} of 0.38 ± 0.02 for 10 independent runs).

associated with these processes in **3** and **4** were somewhat greater than those for **5** (a factor of 3–5 for the fast process and a factor of 30–50 for the slow process). However, the observed rates for these reactions with all three tellurium(IV) compounds were orders-of-magnitude faster than the two processes observed for selenium(IV) compound **6**.

The two reactions of **6** with glutathione that were followed by stopped-flow spectroscopy could both be observed at 270 nm . However, the similarity in rate for the two processes made kinetic isolation difficult at one wavelength. No absorption changes were observed at 310 nm for the first reaction as shown in the kinetic trace of

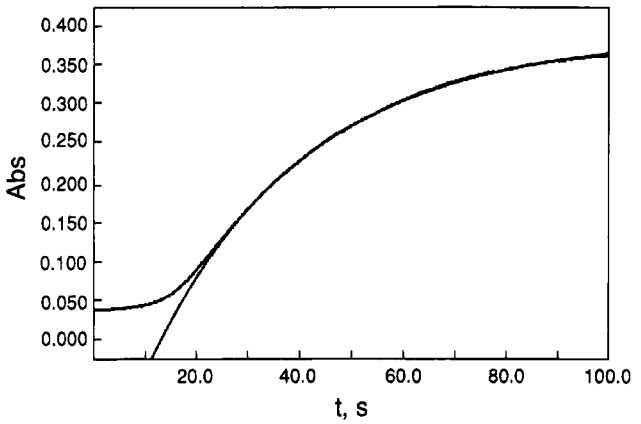


Figure 5. A kinetic trace obtained at 310 nm for the slow reaction of diphenyl selenoxide hydrate (**6**, 5×10^{-5} M) with glutathione (5×10^{-4} M) at 293.1 K. The initial fast reaction is not observed at 310 nm as indicated by the flat response in the initial 10 ms. First-order curve fitting to the slow reaction was indicative of k_{obs} of 0.0331 ± 0.0002 s^{-1} for 10 independent runs.

Scheme 1

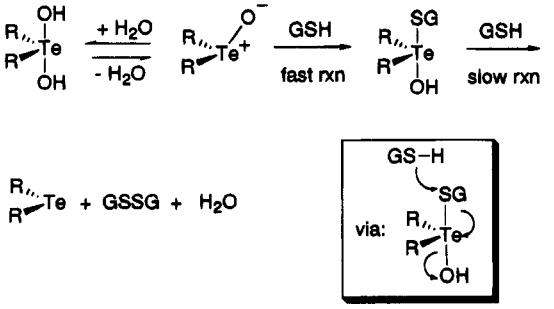
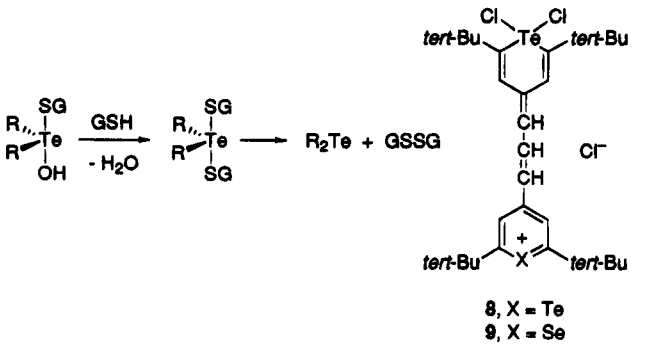


Figure 5, which allowed the second, slower reaction to be observed unambiguously. At 5×10^{-4} M glutathione and 5×10^{-5} M **6** as shown in Table 1, k_{obs} for the faster reaction at 270 nm was 0.113 ± 0.002 s^{-1} and k_{obs} for the slower reaction at 310 nm was 0.0331 ± 0.0002 s^{-1}, which are 10^4–10^5 times slower than the fast and slow processes observed for **3**–**5**. The rate constants for these two processes are similar in magnitude as opposed to the roughly 10^2 difference in rates observed for the two processes in **3**–**5**.

Mechanistic Implications. In an aqueous environment, the hydrated forms of telluroxides and selenoxides should form readily and may be the predominant chalcogen(IV) species in solution. In several examples, telluroxide hydrates have been isolated from the oxidation of diorganotellurides with *N*-chlorosuccinimide or *tert*-butyl hypochlorite and aqueous base.[21] Compound **5** can be dehydrated with heat and vacuum to the telluroxide. However, the telluroxide is hygroscopic and picks up water upon standing to regenerate **5**. This suggests that the hydration of the telluroxide is reversible as shown in Scheme 1. Thiols, including glutathione, are excellent nucleophiles and should enter into the ligand sphere of tellurium(IV) to form a tellurium(IV) hydroxide thiolate as proposed by Engman *et al.*[17] This process would not involve a change in oxidation state at tellurium and is probably the fast reaction observed by stopped-flow spectroscopy as shown in Scheme 1. The pK_a of GSH

Scheme 2



is 8.9 in water,[22] which suggests that GSH is not extensively dissociated in PBS at pH 7.4.

The slow step observed by stopped-flow spectroscopy is associated with reduction of tellurium(IV) to tellurium(II). Nucleophilic attack of a second glutathione at the sulfur atom of the Te–S bond would lead to glutathione disulfide, tellurium(II), and water as shown in the inset of Scheme 1.

Another mechanistic possibility as proposed by Engman *et al.* is shown in Scheme 2.[17] Attack of a second glutathione at tellurium would displace the second hydroxide to form a tellurium(IV) dithiolate **7**, which would then lose glutathione disulfide via a reductive elimination reaction.[18,23] The formation of **7** would not involve a change in oxidation state at tellurium, and reductive elimination to give the tellurium(II) state would follow formation of **7**. In this study, the rate of appearance of tellurium(II) species **2** was identical to the rate of disappearance of the tellurium(IV) species derived from **4** and was consistent with an overall second-order process that was first-order in both **4** and glutathione. From the kinetic analysis of the reaction, reductive elimination of glutathione disulfide via a first-order process from **7** would have to be much faster than k_{obs} for the slow step.

Dithiolate complexes of tellurium(IV) are known[23] and reductive elimination reactions of tellurium(IV) dithiolates have been implicated in the formation of disulfides.[24] However, kinetic data regarding the stability of the tellurium(IV) derivatives has not been described. Reductive elimination reactions of this type have been examined for **3** and **4** and their corresponding tellurium(IV) dichloride analogues **8** and **9**. At 298 K, the rate constants for reductive elimination to generate the tellurium(II) center are $< 10^{-5}$ s^{-1},[18,25] which is at least 10^7 smaller than necessary to fit the kinetic data of this study.

The selenium(IV) derivative **6** also oxidizes glutathione to glutathione disulfide with at least two steps observable by stopped-flow spectroscopy. However, this process need not follow the same mechanism as that of the tellurium(IV) derivatives. Both steps of the reaction of **6** with glutathione are much slower than those observed for the tellurium(IV) derivatives **3**–**5**. The faster of the two reactions is 10^4–10^5 times slower than the fast reaction of the tellurium(IV) derivatives while the slower reaction to generate diphenyl selenide is 10^2–10^4 times slower than the slow reaction of the tellurium(IV) derivatives. Furthermore, the observed rate constants for the two

(21) Detty, M. R. *J. Org. Chem.* **1980**, *45*, 274.
(22) Chen, W.-J.; Graminski, G. F.; Armstrong, R. N. *Biochemistry* **1988**, *27*, 647.
(23) Abed-Ali, S. S.; McWhinnie, W. R. *J. Organomet. Chem.* **1984**, *277*, 365.
(24) Wieber, M.; Kaunzinger, E. *J. Organomet. Chem.* **1977**, *129*, 339.
(25) Detty, M. R.; Frade, T. M. *Organometallics* **1993**, *12*, 2496.

steps in the reaction of **6** with glutathione are comparable in magnitude, which one might expect for sequential replacement of two hydroxide ligands with two sulfide ligands. Very little is known about reductive elimination from selenium(IV) derivatives relative to tellurium(IV) derivatives. The discrepancies between the oxidation of glutathione with the selenium(IV) derivative **6** and with tellurium(IV) derivatives **3**–**5** are sufficiently large to warrant further investigation before drawing mechanistic conclusions with respect to the oxidation of thiols with selenium(IV) compounds.

Conclusions

The reactions of tellurium(IV) derivatives **3**–**5** with glutathione to give glutathione disulfide and tellurium(II) compound involve at least two steps: (a) a fast reaction to give a new tellurium(IV) derivative and (b) a slower reaction to generate a tellurium(II) compound and glutathione disulfide. Although one reaction is roughly 2 orders-of-magnitude faster than the other, both reactions are relatively fast with second-order rate constants $> 7 \times 10^3$ L mol^{-1} s^{-1}.

In reaction schemes where a tellurium(II) or tellurium(IV) derivative is employed in a catalytic cycle to oxidize thiols to disulfides, the rate-determining step will be a function of the rate of oxidation of tellurium(II) to tellurium(IV). Hydrogen peroxide in PBS oxidizes **1** and **2** with second-order rate constants on the order of 2–4 L mol^{-1} s^{-1} at 298 K.[18] In reactions where peroxides produce **3** and **4** from **1** and **2**, respectively, the oxidation of tellurium(II) is rate determining. Similar behavior should be observed with other diaryl tellurides.[17]

In applications such as PDT, where singlet oxygen in the presence of water is the oxidant for converting tellurium(II) to tellurium(IV), the incorporation of thiol into the coordination sphere of tellurium can become rate limiting. In PBS, the oxidations of **1** and **2** with singlet oxygen to give **3** and **4**, respectively, have second-order rate constants $> 10^8$ L mol^{-1} s^{-1} at 298 K.[19d]

On the basis of kinetic observations of this study, the depletion of glutathione levels in tissues treated with telluropyrylium dyes via the dye-sensitized generation of singlet oxygen should be possible through the intermediacy of tellurium(IV) derivatives such as **3** and **4**. Alternatively, tellurium-containing substituents on other sensitizers or organotellurium codrugs might be utilized to deplete glutathione. If glutathione depletion were to lead to impairment of the glutathione–glutathione peroxidase repair cycle in transformed cells, more effective treatment with singlet-oxygen-generating photosensitizers might be realized. We are currently exploring *in vitro* and *in vivo* studies to test this possibility.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a General Electric QE-300 spectrometer or on a Varian Gemini-200 spectrometer. UV-visible–near infrared spectra were recorded on a Perkin-Elmer Lambda 9 spectrophotometer. Infrared spectra were recorded on a Beckman IR 4250 instrument. Microanalyses were performed on a Perkin-Elmer 240 C, H, and N Analyzer. Selenium and tellurium analyses were determined by atomic absorption analysis. Distilled water was purified with a Milli-Q Water System made by Millipore Corp. to a resistance of 16–17 MΩ cm^{-1} before use in the preparation of phosphate buffered saline from the standard recipe. Diphenyl selenide and diphenyl telluride were obtained from various commercial sources and were distilled prior to use. These materials were oxidized with *N*-chlorosuccinimide to **5** and **6** according to ref 21. Telluropyrylium dyes **1** and **2** were prepared according to ref 19c.

Preparation of 5 and Dehydration to Diphenyl Telluroxide. Diphenyl telluride (1.41 g, 5.00 mmol) was oxidized with *N*-chlorosuccinimide (NCS, 0.74 g, 5.3 mmol) as described in ref 21. The initial product when air-dried was characterized as the telluroxide "hydrate", mp 176–181 °C (dec): ^{1}H NMR ($CDCl_3$) δ 7.65 (m, 4 H), 7.45 (m, 6 H), 2.10 (br s, 2 H); IR (KBr) 3068, 3056, 3044, 2990, 1570, 1476, 1435, 1059, 1020, 999, 731, 721, 691, 685 cm^{-1}. (In reference 21, we had assumed the product was "wet".) Anal. Calcd for $C_{12}H_{12}O_2Te$: C, 45.63; H, 3.83; Te, 40.40. Found: C, 46.02; H, 4.00; Te, 41.12.

The sample was dried at 80 °C at 5 torr for 48 h to give a white solid, which is presumably the telluroxide, mp 186–189 °C: ^{1}H NMR ($CDCl_3$) δ 7.65 (m, 4 H), 7.37 (m, 6 H); IR (KBr) 2990, 1570, 1430, 728, 718, 682 cm^{-1}. Anal. Calcd for $C_{12}H_{10}OTe$: C, 48.40; H, 3.36; Te, 42.85. Found: C, 48.51; H, 3.69; Te, 42.70.

Preparation of 6 and Dehydration to Diphenyl Selenoxide. Diphenyl selenide (2.33 g, 10.0 mmol) was treated with NCS (1.41 g, 10.5 mmol) as described in reference 21. Product yield was 2.03 g (79%) of an off white solid, which was presumably the selenoxide "hydrate" following air-drying, mp 98–100 °C: ^{1}H NMR ($CDCl_3$) δ 7.70 (m, 4 H), 7.44 (m, 6 H), 2.10 (br s, 2 H); IR (KBr) 3046, 2991, 1570, 1475, 1439, 824, 749, 736, 691, 485 cm^{-1}. Anal. Calcd for $C_{12}H_{12}O_2Se$: C, 53.94; H, 4.53; Se, 29.55. Found: C, 54.13; H, 4.55; Se, 30.01.

The sample was dried at 80 °C at 5 torr for 48 h to give a white solid, which was presumably the selenoxide, mp 111.5–113 °C: ^{1}H NMR ($CDCl_3$) δ 7.70 (m, 4 H), 7.47 (m, 6 H); IR (KBr) 2990, 1570, 1470, 1440, 820, 745, 730, 685 cm^{-1}. Anal. Calcd for $C_{12}H_{10}OSe$: C, 57.84; H, 4.05; Se, 31.69. Found: C, 57.71; H, 3.96; Se, 31.60.

Preparation of Stock Solutions of 3 and 4 in Phosphate Buffered Saline. A 2.04-mg (3.00 μmol) sample of **1** was dissolved in 100.0 mL of PBS. The resulting solution was irradiated through the Pyrex walls of a 125-mL Erlenmeyer flask with a General Electric100-W incandescant bulb at a distance of 15 cm until the absorbances associated with **1** had disappeared (≈5 min). The resulting 1.5×10^{-5} M solution of **3** was used in the stopped-flow experiments.

A 1.90-mg (3.00 μmol) sample of **2** was dissolved in 200.0 mL of PBS. The resulting solution was irradiated through the Pyrex walls of a 250-mL Erlenmeyer flask with a General Electric100-W incandescant bulb at a distance of 15 cm until the absorbances associated with **2** had disappeared (≈12 min). The resulting 1.5×10^{-5} M solution of **4** was used in the stopped-flow experiments.

Preparative Studies of Glutathione with 3 and 4. For the preparative studies, a 3.40-mg (5.00 μmol) sample of **1** was dissolved in 100 mL of PBS and irradiated as described above. To the resulting 5.0×10^{-5} M solution of **3** was added 3.22 mg (10.5 μmol) of glutathione. The resulting solution was blue-green in color with λ_{max} of 810 nm and an absorbance of 0.75 at 810 nm in 0.1-cm cells. Based on an extinction coefficient of 1.5×10^5 M^{-1} cm^{-1} for authentic **3**,[18] the resulting concentration of **1** is 5.00×10^{-5} M. The addition of smaller amounts of glutathione followed the stoichiometry of eq 1 while the addition of excess glutathione had no effect on the concentration of **1**.

Similarly, a 3.17-mg (5.00 μmol) sample of **1** was dissolved in 100 mL of PBS and irradiated as described above. To the resulting 5.0×10^{-5} M solution of **4** was added 3.22 mg (10.5 μmol) of glutathione. The resulting solution had an absorbance of 0.63 at 770 nm in 0.1-cm cells. Based on an extinction coefficient of 1.25×10^5 M^{-1} cm^{-1} for authentic **4**,[18] the resulting concentration of **2** is 5.04×10^{-5} M.

Preparative Studies of Glutathione with 5 and 6. A 31.6-mg (100 μmol) sample of **5** was dissolved in 100.0 mL of PBS. To the resulting solution was added 64.5 mg (210 μmol) of glutathione. The solution became cloudy within a minute

of addition and diphenyl telluride began oiling out of solution. The reaction mixture was extracted with ether (3 × 20 mL). The combined ether extracts were washed with brine, were dried over magnesium sulfate, and concentrated to give 27.0 mg (96%) of diphenyl telluride.

Similarly, a 26.7-mg (100 μmol) sample of **6** was dissolved in 100 mL of PBS. To the resulting solution was added 64.5 mg (210 μmol) of glutathione. The solution became cloudy within several minutes of addition and yellow diphenyl selenide began oiling out of solution. The reaction mixture was extracted with ether (3 × 20 mL). The combined ether extracts were washed with brine, were dried over magnesium sulfate, and concentrated to give 23.0 mg (95%) of diphenyl selenide.

Stopped-Flow Experiments. All stopped-flow experiments were performed on a Sequential DX17 MV Stopped-Flow Spectrometer (Applied Photophysics, Leatherhead, UK). All experiments incorporated the instrument in stopped-flow mode only. The sample handling unit was fitted with two drive syringes that are mounted inside a thermostated-bath compartment, which allowed for variable temperature experimentation. The optical-detection cell was set up in the 10-mm pathlength. First- and second-order curve fitting and rate constants used a Marquardt algorithm[26] based on the routine Curfit.[27] Absorption spectra at indicated time points were calculated through software provided by Applied Photophysics. This consisted of slicing the appropriate time points across a series of kinetic traces (at different wavelengths) and then splining the points of a specific time group. Stock solutions of substrates and glutathione at appropriate concentrations in PBS described in the text were utilized in the stopped-flow experiments.

Supplementary Material Available: Figures 6 and 7, which show transient spectra for the reactions of **3** and **4**, respectively, with glutathione (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(26) Marquardt, D. W. *J. Soc. Indust. Appl. Mathematics* **1963**, *11*, 431.

(27) Curfit is found in Bevington, P. R. *Data Reduction and Error Analysis for the Physical Sciences*; McGraw-Hill: New York, 1969.