Tellurium Compounds: Selective Inhibition of Cysteine Proteases and Model Reaction with Thiols

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Received November 18, 1997

Ammonium trichloro(dioxoethylene-*O*,*O*′)tellurate (AS101) is an organotellurium(IV) compound that exhibits immunomodulation activity. In light of the unique Te(IV)−thiol chemistry, it was tested as a selective cysteine protease inhibitor. Although no inhibitory activity of serine-, metallo-, or aspartic proteases was observed, AS101 exhibited time- and concentration-dependent inactivation of cysteine proteases. The kinetic parameters of inactivation of papain were $K_i = 3.5 \pm 2.0\ \mu M$ and $k_i = (5.1 \pm 0.4) \times 10^{-2}\ min^{-1}$. The enzymatic activity could be recovered by treatment with thiols, indicating that the inactivation involves oxidation of the active-site thiol to a disulfide bond (Enz−S−S−R) or to a species containing a Te−S bond such as Enz−S−Te−S−R. Gel permeation chromatography established that the R group is a small molecule and excludes the possibility of dimerization of the enzyme itself. It was further established that some other Te(IV) derivatives could also inactivate cysteine proteases, while Te(VI) derivatives did not exhibit any such inhibitory activity. In order to understand the chemistry underlying the cysteine protease inactivation by AS101 and other organotellurium(IV) compounds, their interaction with the model compound cysteine was studied. While the Te(VI) derivatives did not interact with cysteine, all of the Te(IV) compounds interacted with 4 equiv of cysteine. The kinetics of this interaction is first order in Te and second order in thiol, yielding a third-order rate constant of $\sim 10^6\ M^{-2}\ s^{-1}$, as determined for the interaction between AS101 with cysteine. The interactions between Te derivatives and cysteine in DMSO were followed by ^{125}Te and ^{13}C NMR. While Te(VI) compounds did not undergo any changes upon interaction with cysteine, on the basis of their ^{125}Te NMR, the Te(IV) derivatives interacted with 4 equiv of cysteine, yielding new stable Te(IV) compounds. These compounds were tentatively designated as Te(cysteine)$_4$ or its high-valence complex with other components in the reaction mixture. These results expand our understanding of tellurium chemistry and correlate well with its biological activity. Such knowledge can be applied for the development of novel biologically active tellurium compounds.

Introduction

Ammonium trichloro(dioxoethylene-*O*,*O*′)tellurate (AS101), an organotellurium(IV) compound, is a potent immunomodulator (both in vitro and in vivo) with a variety of potential therapeutic applications.[1] Some other related organotellurium compounds also possess different levels of immunomodulating activity.[2] AS101 was found to be effective in treatment of AIDS[3] and cancer.[4] It confers protection against side effects of both radiotherapy[5] and chemotherapy,[5b,6] such as protection of the bone marrow and prevention of alopecia.[7] It also exhibits synergistic effects with a variety of other drugs such as Taxol,[8] a well-recognized advantage in chemotherapy. AS101 was also found effective against systemic lupus erythematosus (SLE)[9] and psoriasis.[10] A number of studies addressed mechanistic aspects of the biological activity of AS101, mainly on a cellular

(1) Sredni, B.; Caspi, R. R.; Klein, A.; Kalechman, Y.; Danziger, Y.; Ben Ya'akov, M.; Tamari, T.; Shalit, F.; Albeck, M. *Nature* **1987**, *330*, 173−176.

(2) (a) Sredni, B.; Albeck, M. US Patents 4,929,739 and 4,946,437, 1990. (b) Sredni, B.; Albeck, M. US Patent 5,093,135, 1992. (c) Albeck, M.; Sredni, B. European Patent 0 311 138, 1994.

(3) (a) Ruiz-Palacios, G. M.; Ponce de León, S.; Alarcón-Segovia, D.; Alcocer Varela, J.; Cruz, A.; Nares, F.; Ponce de León, A.; Rojas, G.; Sredni, B. *IV International Conference on AIDS*, Stockholm, Sweden, 1988; International AIDS Society: Stockholm, Sweden; Abstr. 3038, p 229. (b) Laporte, J. Ph.; Frottier, J.; Dormont, D.; Imbert, J. C.; Albeck, M.; Najman, A.; et al. *V International Conference on AIDS*, Montréal, Canada, 1989; International AIDS Society: Stockholm, Sweden; Abstr. WB P320, p 405. (c) Vansover, A.; Kalechman, Y.; Oron, M.; Shalit, F.; Albeck, M.; Sredni, B.; et al. *V International Conference on AIDS*, Montréal, Canada, 1989; International AIDS Society: Stockholm, Sweden; Abstr. TC P142, p 590. (d) Vansover, A.; Sredni, B.; Loya, S.; Horwith, G.; Kirsch, T. M.; Albeck, M.; Gotlieb-Stematsky, T.; Hizi, A. *AIDS Res. Human Retroviruses* **1992**, *8*, 613−623.

(4) Shani, A.; Tichler, T.; Catane, R.; Gurwith, M.; Rozenszajn, L. A.; Gezin, A.; Levi, E.; Schlesinger, M.; Kalechman, Y.; Michlin, H.; Shalit, F.; Engelsman, E.; Farbstein, H.; Farbstein, M.; Albeck, M.; Sredni, B. *Nat. Immun. Cell Growth Regul.* **1990**, *9*, 182−190.

(5) (a) Kalechman, Y.; Albeck, M.; Oron, M.; Sobelman, D.; Gurwith, M.; Sehgal, S. N.; Sredni, B. *J. Immunol.* **1990**, *145*, 1512−1517. (b) Sredni, B.; Albeck, M.; Kazimirsky, G.; Shalit, F. *Int. J. Immunopharmacol.* **1992**, *14*, 613−619.

(6) (a) Kalechman, Y.; Albeck, M.; Oron, M.; Sobelman, D.; Gurwith, M.; Horwith, G.; Kirsch, T.; Maida, B.; Sehgal, S. N.; Sredni, B. *Cancer Res.* **1991**, *51*, 5614−5620. (b) Kalechman, Y.; Sotnik-Barkai, I.; Albeck, M.; Sredni, B. *Cancer Res.* **1993**, *53*, 1838−1844. (c) Kalechman, Y.; Shani, A.; Sotnik-Barkai, I.; Albeck, M.; Sredni, B. *Cancer Res.* **1993**, *53*, 5962−5969. (d) Kalechman, Y.; Albeck, M.; Sredni, B. *Exp. Hematol.* **1995**, *23*, 1358−1366.

(7) (a) Sredni, B.; Albeck, M.; Tichler, T.; Shani, A.; Shapira, J.; Bruderman, I.; Catane, R.; Kaufman, B.; Kalechman, Y. *J. Clin. Oncol.* **1995**, *13*, 2342−2353. (b) Sredni, B.; Xu, R. H.; Albeck, M.; Gafter, U.; Gal, R.; Shani, A.; Tichler, T.; Shapira, J.; Bruderman, I.; Catane, R.; Kaufman, B.; Whisnant, J. K.; Mettinger, K.; Kalechman, Y. *J. Cancer* **1996**, *65*, 97−103.

(8) (a) Sredni, B.; Kalechman, Y.; Shalit, F.: Albeck, M. *Immunology* **1990**, *69*, 110−116. (b) Kalechman, Y.; Albeck, M.; Sredni, B. *Cell. Immunol.* **1992**, *143*, 143−153. (c) Kalechman, Y.; Shani, A.; Dovrat, S.; Whisnant, J. C.; Mettinger, K.; Albeck, M.; Sredni, B. *J. Immunol.* **1996**, *156*, 1101−1109.

S0020-1669(97)01456-0 CCC: $15.00
Published on Web 03/31/1998

level.[11] It was noticed that the in vitro expression of its biological activity is thiol-dependent, and mercaptoethanol is routinely added to the incubation solution in all such experiments.[12]

Several other tellurium compounds exhibit different biological activities. Compounds of the form R_2TeCl_n exhibit anthelmintic activity[13] while tellurite ions (TeO_3^{2-}) induce alterations of the erythrocyte membrane.[14] The latter activity is also thiol-dependent and probably involves intermediates with thiol−tellurium covalent bonds and various tellurium oxidation states. Thus, accumulated evidence suggests that much of the biological activity of organotellurium compounds is directly related to their specific chemical interactions with endogenous thiols. Such thiol−tellurium compounds may be important for transportation of tellurium species to its target location and/or for the manifestation of the biological function itself.

Te(IV) compounds such as TeX_4 or $Te(OR)_4$ interact readily with nucleophiles such as alcohols, thiols, carboxylates, etc., yielding $(Nu)_4Te$ products.[15] The latter may further exchange ligands or, eventually, hydrolyze to TeO_2 in aqueous solution. Contrary to the general rule, $Te(SR)_4$ compounds undergo an oxidation−reduction disproportionation reaction according to eq 1.[16] $Te(SR)_2$ may further react to form a second disulfide and metallic tellurium.

$$Te(SR)_4 \rightarrow Te(SR)_2 + RSSR \qquad (1)$$

To understand the mechanisms of interaction of tellurium compounds with biological systems at a molecular level, it is essential to expand our understanding of the tellurium−thiol chemistry. In the present study we explore a few aspects of this chemistry. Furthermore, the tellurium−thiol chemistry may be applied to other biological functions that have not been explored yet. As part of our interest in developing selective inhibitors of proteases in general and of cysteine proteases in particular,[17] we study here the interaction of AS101 and other related tellurium compounds with these enzymes. Proteases catalyze the hydrolysis of amide bonds in proteins and peptides. They are divided into four families, based on their catalytic residues and mechanism.[18] Whereas serine proteases utilize a nucleophilic hydroxyl of a serine residue and aspartic and metalloproteases possess carboxylates as active functionalities, the cysteine proteases have an active-site thiol-nucleophile. Proteases are responsible for many biological activities, by the activation or degradation of essential proteins, enzymes, and peptides. Excessive uncontrolled enzymatic activity, on the other hand, is implicated in many diseases such as emphysema, osteoporosis, inflammation, and high blood pressure.[19] Furthermore, control over some viral infections, from AIDS to the common cold, may also be achieved by inhibition of the corresponding viral protease. Thus, inhibition of such proteolytic activities is of major interest. In the present study we demonstrate the feasibility of selective inhibition of cysteine proteases by taking advantage of the unique chemistry of organotellurium compounds with thiols.

Experimental Section

General. Enzymes and their substrates were obtained from Sigma Chemical Company and used without further purification. [3-^{13}C]-L-Cysteine was from Cambridge Isotope Laboratories (Andover, MA). $TeCl_4$ was from Merck. Ammonium trichloro(dioxoethylene-*O*,*O*′)-tellurate (AS101) and 1,4,6,9-tetraoxa-5-telluraspiro[4,4]nonane (AS102) were synthesized as previously described.[20] All other Te(IV) and Te(VI) were prepared by Ms. Iris Elyashiv, Department of Chemistry, Bar Ilan University. Gel permeation chromatography was carried out on either Sephadex G-15 or Superdex-75 HR from Pharmacia. ^{125}Te and ^{13}C NMR spectra were recorded at 94.8 and 75.4 MHz, respectively, in DMSO-d_6. Chemical shifts are reported with diphenyl telluride as a secondary reference for the ^{125}Te NMR and solvent resonance as an internal reference in the ^{13}C NMR measurements.

Enzymatic Assays. All enzymatic assays were carried out at 25 °C, by following substrate hydrolysis spectrophotometrically (unless otherwise specified). A 20 μL substrate solution (in the indicated concentration and solvent) was dissolved in 960 μL of buffer. The catalytic reaction was initiated by addition of 20 μL enzyme solution. The concentration of the enzymes was set such that, under substrate saturation (V_{max}) conditions, the initial velocity of hydrolysis was about 10^{-3} OD/s.

Chymotrypsin (from bovine pancreas, EC 3.4.21.1) was assayed in 100 mM potassium phosphate buffer, pH 7.0, by following the hydrolysis of BTEE (20 mM in DMSO) at 256 nm.[21]

Subtilisin (Sigma Type XXVII) was assayed in 100 mM potassium phosphate buffer, pH 7.0, by following the hydrolysis of Succ-AAPF-pNA (5 mM in DMSO) at 404 nm.[22]

Elastase (from porcine pancreas, EC 3.4.21.36) was assayed in 100 mM Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl, by following the hydrolysis of Succ-AAA-pNA (4.4 mM in the same buffer) at 412 nm.[23]

(9) (a) Alcocer Varela, J.; Alarcon-Segovia, D.; Sredni, B.; Albeck, M . *Clin. Exp. Immunol.* **1989**, *77*, 319−323. (b) Blank, M.; Sredni, B.; Albeck, M.; Mozes, E.; Shoenfeld, Y. *Clin. Exp. Immunol.* **1990**, *79*, 443−447.

(10) Shohat, B.; Kozenitzki, L.; David, M.; Albeck, A.; Sredni, B. *Nat. Immun.* **1993**, *12*, 50−55.

(11) (a) Kalechman, Y.; Sotnik-Barkai, I.; Albeck, M.; Horwith, G.; Sehgal, S. N.; Sredni, B. *Cancer Res.* **1991**, *51*, 1499−1503. (b) Kalechman, Y.; Gafter, U.; Sotnik-Barkai, I.; Albeck, M.; Gurwith, M.; Horwith, G.; Kirsch, T.; Maida, B.; Sehgal, S. N.; Sredni, B. *Exp. Hematol.* **1993**, *21*, 150−155. (c) Kalechman, Y.; Zuloff, A.; Albeck, A.; Sredni, B. *Blood* **1995**, *85*, 1555−1561. (d) Sredni, B.; Tichler, T.; Shani, A.; Catane, R.; Kaufman, B.; Strassmann, G.; Albeck, M.; Kalechman, Y. *J. Natl. Cancer Inst.* **1996**, *88*, 1276−1284. (e) Kalechman, Y.; Sredni, B. *J. Immunol.* **1996**, *157*, 589−597.

(12) Albeck, M.; Sredni, B.; Kalechman, Y. Unpublished results.

(13) Ordyntseva, A. P.; Sivovolova, I. D.; Abakarov, G. M.; Sadekova, E. I. *Khim. Farm. Zh.* **1988**, *22*, 1098−1100.

(14) Deuticke, B.; Lütkemeier, P.; Poser, B. *Biochim. Biophys. Acta* **1992**, *1109*, 97−107 and references therein.

(15) For a general review of tellurium(IV) chemistry, see: (a) Irgolic, K. J. In *Organotellurium Compounds* (Vol. E12b of *Methods of Organic Chemistry* (Houben-Weyl), Additional and Supplementary Volumes of the 4th ed.); Klamann, D., Ed.; Georg Thieme Verlag: Stuttgart, 1990. (b) *The Chemistry of Organic Selenium and Tellurium Compounds*; Patai, S.; Rappoport, Z. Eds.; John Wiley & Sons: Chichester, 1986; Vol. 1. (c) Irgolic, K. J. *The Organic Chemistry of Tellurium*; Gordon and Breach Science Publishers: New York, 1974.

(16) (a) Stukalo, E. A.; Yur'eva, E. M.; Markovskii, L. N. *Zh. Org. Khim.* **1983**, *19*, 343−346; (*Russ J. Org. Chem.*, 301−303). (b) Mazurek, W.; Moritz, A. G.; O'Connor, M. J. *Inorg. Chim. Acta* **1986**, *113*, 143−146.

(17) (a) Albeck, A.; Kliper, S. *Biochem. J.* **1997**, *322*, 879−884. (b) Albeck, A.; Fluss, S.; Persky, R. *J. Am. Chem. Soc.* **1996**, *118*, 3591−3596. (c) Albeck, A.; Persky, R.; Kliper, S. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1767−1772.

(18) (a) Fersht, A. In *Enzyme Structure and Mechanism*, 2nd ed.; W. H. Freeman and Co.: New York, 1985; pp 405−426. (b) Walsh, C. In *Enzymatic Reaction Mechanisms*; W. H. Freeman and Co.: New York, 1979; pp 53−107.

(19) (a) Rich, D. H. in *Comprehensive Medicinal Chemistry*; Hansch, C., Sammes, P. C., Taylor, J. B., Eds.; Pergamon Press: Oxford, 1990; Vol. 2, pp 391−441. (b) Fischer, G. *Nat. Prod. Rep.* **1988**, 465−495. (c) Powers, J. C.; Harper, J. W. In *Proteinase Inhibitors;* Research Monographs in Cell and Tissue Physiology 12; Barrett, A. J., Salvesen, G., Eds.; Elsevier: Amsterdam, 1986; pp 55−152.

(20) (a) Albeck, M.; Tamari, T.; Sredni, B. *Synthesis* **1989**, 635−636. (b) Holmes, R. R. *Acc. Chem. Res.* **1979**, *12*, 257−265.

(21) Hummel, B. C. W. *Can. J. Biochem. Physiol.* **1959**, *37*, 1393−1399.

(22) Del Mar, E. G.; Langman, C.; Brodrick, J. W.; Geckas, M. C. *Anal. Biochem.* **1979**, *99*, 316−320.

(23) Bieth, J.; Spiess, B.; Wermuth, C. G. *Biochem. Med.* **1974**, *11*, 350−357.

Trypsin (EC 3.4.21.4) was assayed in 100 mM Tris-HCl buffer, pH 8.3, containing 10 mM $CaCl_2$, by following the hydrolysis of BAEE (10 mM in DMSO) at 254 nm.[24]

Carboxypeptidase A (from bovine pancreas, EC 3.4.17.1) was dissolved in 10% aqueous LiCl for stock solution and was assayed in 25 mM Tris-HCl buffer, pH 7.5, containing 0.5 M NaCl, by following the hydrolysis of hyppuryl-L-phenylalanine (2 mM in the same buffer) at 254 nm.[25]

Pepsin (pepsin A from porcine stomach mucosa, EC 3.4.23.1) was assayed for its ability to digest hemoglobin, according to a published procedure.[26]

Papain (EC 3.4.22.2) and cathepsin B (from bovine spleen, EC 3.4.22.1) were activated at 25 °C for 1 h in 100 mM potassium phosphate buffer, pH 7.0, containing 0.5 mM cysteine and 2 mM EDTA, followed by gel filtration to remove excess cysteine (see below). They were assayed in 100 mM potassium phosphate buffer, pH 7.0, by following the hydrolysis of Cbz-Gly-ONp (1.25 mM in CH_3CN) at 404 nm.[27]

Inactivation Studies. The discontinuous method was applied.[28] Typically, the enzyme studied was incubated at 25 °C with the particular inhibitor dissolved in DMSO (volume of organic solvent not exceeding 10% of the total volume). Aliquots were removed periodically and diluted into assay solution containing the substrate, and the residual enzymatic activity was measured. A control preincubation solution, containing all of the ingredients except for the inhibitor itself, was run and assayed in parallel. Values of k_{obs} (the apparent inactivation rate constant) were calculated from semilog plots of percentage residual enzymatic activity vs time (as ln $2/t_{1/2}$, or by fitting the graphs to the exponential equation $\%\text{activity}_t = 100\ e^{-k_{obs}t}$). Replot of $1/k_{obs}$ vs 1/[I] yielded the inactivation kinetic parameters k_i and K_i.[28] For AS101 inhibition of papain, the experiment was repeated three times and average values of the kinetic parameters were calculated.

Gel Permeation Chromatography. A 1 mL solution of activated papain was loaded on a 1 × 15 cm Sephadex G-15 column, preequilibrated with 10 mM potassium phosphate buffer, pH 7.0, containing 0.1 mM EDTA. The enzyme was eluted with the same buffer (degassed) at 0.5 mL/min, and 1 mL fractions were collected. The A_{280} of each fraction was measured. Subsequently, aliquots of 300 μL were removed and mixed with DTNB[29] (20 μL of 10 mM aqueous solution), and A_{412} was measured.

For estimation of the inactivated enzyme molecular weight, a solution of the inactivated enzyme was loaded on a Superdex-75 HR column, preequilibrated with 100 mM potassium phosphate buffer, pH 7.0, and eluted with the same buffer at a rate of 0.5 mL/min. Fractions of 0.5 mL were collected, and their A_{280} were determined. The same procedure was applied to activated papain. The results of the two runs were compared to the data obtained from a calibration run, under identical conditions, consisting of blue dextran (M_r 2 000 000), BSA (M_r 68 000), chicken ovalbumin (M_r 45 000), and chymotrypsinogen (M_r 25 000).

Cysteine/Tellurium Compound Stoichiometry in Model Reactions. To a 0.5 mM solution of cysteine in DMSO was added an equal volume of 0.05 mM solution of a tellurium compound in DMSO. The solution was kept at room temperature. Aliquots of 50 μL were removed periodically and mixed with DTNB (450 μL of 0.5 mM aqueous solution), and their A_{412} were determined.

Kinetics of Cysteine−AS101 Interaction. The reaction order in cysteine was measured at room temperature, at constant AS101 concentration (80 μM) and varying thiol concentrations (30−70 μM). The reaction was initiated by the addition of 500 μL aqueous solution of cysteine at twice the desired final concentration (60−140 μM) to 500 μL of a 160 μM aqueous solution of AS101. The reaction was followed by periodically removing aliquots and determination of their free thiol content by DTNB, as described above. The reaction was followed until 10%, at the most, of the tellurium compound was consumed. The data were fit to both a first-order and a second-order equation. The corresponding pseudo-first- and pseudo-second-order rate constants were determined from these graphs. In addition, the overall rate constant was determined by a similar experiment in which a constant cysteine concentration (70 μM) was reacted with varying AS101 concentrations (40−70 μM) and the data were fit to the appropriate function.

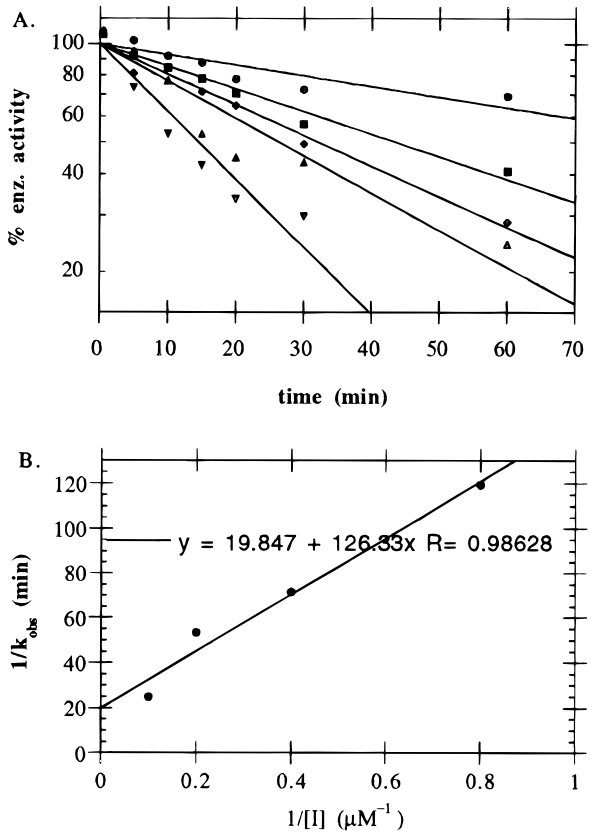


Figure 1. (A) Representative time course of inactivation of papain by AS101 in 100 mM phosphate buffer containing 10% (V/V) DMSO and 2 mM EDTA, at 25 °C. Inhibitor concentration: (●) 0 μM; (■) 1.25 μM; (◆) 2.5 μM; (▲) 5 μM; (▼) 10 μM. The solid lines are calculated exponential fit to the experimental data points. (B) Replot of $1/k_{obs}$ vs 1/[I].

Results

AS101 as an Inhibitor of Proteolytic Enzymes. AS101 was tested for its possible inhibitory activity toward all four families of proteases. No inhibition of serine proteases (chymotrypsin, trypsin, elastase, and subtilisin, characterized by different substrate selectivities), metalloproteases (carboxypeptidase A), or aspartic proteases (pepsin) was detected even upon long incubation (1 h) at AS101 concentrations as high as 10 mM.

Contrary to its inertness toward serine, metallo-, and aspartic proteases, AS101 was found to be a time- and concentration-dependent inhibitor of the cysteine proteases papain and cathepsin B (Figure 1).

Kinetic analysis of the minimal inhibition scheme

$$E + I \overset{K_i}{\rightleftharpoons} E{\cdot}I \xrightarrow{k_i} E{-}I$$

yields eq 2.[28] Analysis of our data by using eq 2 leads to the

$$1/k_{obs} = (K_i/k_i)(1/[I]) + 1/k_i \qquad (2)$$

following kinetic parameters for the inhibition process: K_i =

(24) Schwert, G. W.; Takenaka, Y. *Biochim. Biophys. Acta* **1955**, *16*, 570−575.
(25) Folk, J. E.; Schirmer, E. W. *J. Biol. Chem.* **1963**, *238*, 3884−3894.
(26) Tang, J. *Methods Enzymol.* **1970**, *19*, 406−421.
(27) Giordano, C.; Gallina, C.; Consalvi, V.; Scandurra, R. *Eur. J. Med. Chem.* **1990**, *25*, 479−487.
(28) Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245−3249.

3.5 ± 2.0 μM, $k_i = (5.1 \pm 0.4) \times 10^{-2}$ min^{-1}, and $k_i/K_i = 310 \pm 126$ M^{-1} s^{-1} for papain inactivation and $k_i/K_i = 37$ M^{-1} s^{-1} for cathepsin B inactivation.

The inhibition of papain by AS101 was shown to be covalent by gel permeation chromatography (Sephadex G-15) of inhibited papain, upon which no enzymatic activity was regained. Activated papain retained most of its enzymatic activity under identical chromatographic conditions. The inhibited enzyme could be fully reactivated by incubation with thiols (1 mM cysteine, room temperature, 1 h).

The inhibition product was characterized by gel permeation chromatography (on Superdex-75 HR column). It eluted at 12−13 mL, the same volume at which active papain elutes (Figure 2).

Organo-Te(IV) and -Te(VI) as Inhibitors of Cysteine Proteases. The interaction of several other organo-Te(IV) and -Te(VI) compounds with cysteine proteases was also studied. Each of these Te compounds was incubated with papain, and the residual enzymatic activity as a function of incubation time was determined. The observed second-order rate constants of inactivation (expressed as $k_{obs}/[I]$) clearly demonstrate that the different Te(IV) compounds are papain inhibitors while the Te(VI) compounds do not inhibit the enzyme to any extent (Table 1).

Model Reaction between Organotellurium and Thiols. Stoichiometry. The stoichiometry of interaction between cysteine and a variety of Te(IV) and Te(VI) compounds was determined by following the free thiol concentration in an aqueous reaction mixture containing a 10:1 initial molar ratio of cysteine/Te. It was shown that while Te(VI) compounds did not interact with cysteine at all, the different Te(IV) compounds all interacted with 4 molar equiv of the thiol (Figure 3 and Table 2).

Kinetics. The reaction between AS101 and cysteine was further studied in order to determine the reaction order and its kinetic parameters. Its order in cysteine was determined by carrying out the reaction at a constant Te concentration (80 μM) and varying concentrations of cysteine (30−70 μM). Progress of the reaction was followed by periodic titration of the residual free thiol with DTNB.[29] The results are plotted according to a pseudo-first-order reaction in cysteine (eq 3, Figure 4A) and a pseudo-second-order reaction in cysteine (eq 4, Figure 4B). The experimental data points were fitted to the corresponding theoretical curves. The calculated rate constants for the first-order reaction, extracted from the data and the corresponding graph (Figure 4A), span the range of 0.034−0.060 min^{-1}, with an average of 0.047 ± 0.011 min^{-1}. The corresponding fit to a second-order reaction yields rate constants ranging from 39 to 45 M^{-1} s^{-1}, averaging 42 ± 2 M^{-1} s^{-1}. These results indicate that the reaction between AS101 and cysteine is second order in [cysteine].

1st-order reaction: $v = k'[\text{cys}]$,

$$\ln\{[\text{cys}]_t/[\text{cys}]_0\} = -k_1 t \quad (3)$$

2nd-order reaction: $v = k'[\text{cys}]^2$,

$$1/[\text{cys}]_t = k_2 t + 1/[\text{cys}]_0 \quad (4)$$

In a second set of experiments, the reaction between cysteine and AS101 was followed at a constant thiol concentration (70 μM) and varying Te concentrations (40−70 μM). Assuming a reaction of the type

$$4\text{A} + \text{B} \rightarrow \text{P}$$

where A ≡ cysteine, B ≡ AS101, and P ≡ products, with an overall third-order rate constant corresponding to a reaction rate equation

$$v = k[\text{A}]^2[\text{B}]$$

the data were fit to eq 5,[30]

$$4(1/[\text{A}]_t - 1/[\text{A}]_0)/(4[\text{B}]_0 - [\text{A}]_0) + 4\ln\{([\text{B}]_0[\text{A}]_t)/([\text{A}]_0[\text{B}]_t)\}/(4[\text{B}]_0 - [\text{A}]_0)^2 = kt \quad (5)$$

which yielded parallel lines corresponding to a rate constant $k = (1.22 \pm 0.15) \times 10^6$ M^{-2} s^{-1} (Figure 4c).

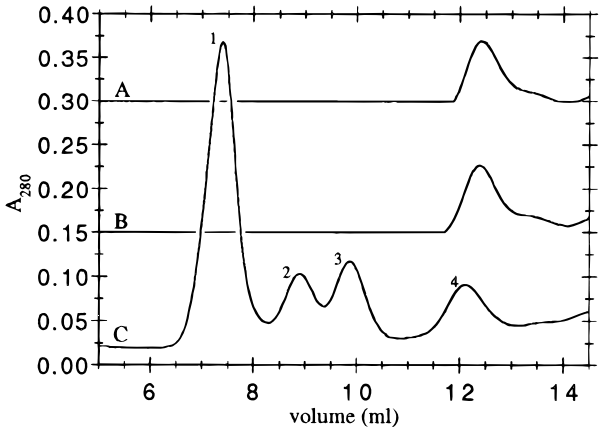
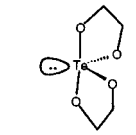
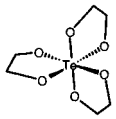
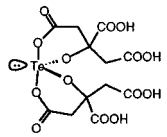
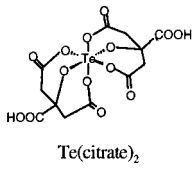
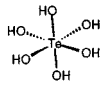
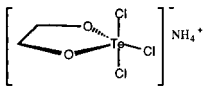


Figure 2. Gel permeation chromatography. (A) AS101-inactivated papain. (B) Active papain. (C) Calibration of column with blue dextran (1), BSA (2), ovalbumin (3), and chymotrypsinogen (4).

Table 1. Kinetic Parameters[a] for the Inactivation of Papain by Organotellurium Compounds

Te (IV) derivative	$k_{obs}/[I]$ M^{-1} s^{-1}	Te (VI) derivative	$k_{obs}/[I]$ M^{-1} s^{-1}
 Te(ethylene glycol)$_2$	5.5	 Te(ethylene glycol)$_3$	no inhibition
 Te(citrate)$_2$	20.2	 Te(citrate)$_2$	no inhibition
 tellurium oxide	3.0	 telluric acid	no inhibition
 AS101	310[b]		

[a] Due to experimental limitations, only estimated second-order rate constants ($k_{obs}/[I]$) are given. [b] For AS101, the result represents the actual second-order rate constant, k_i/K_i.

(29) Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70−77.

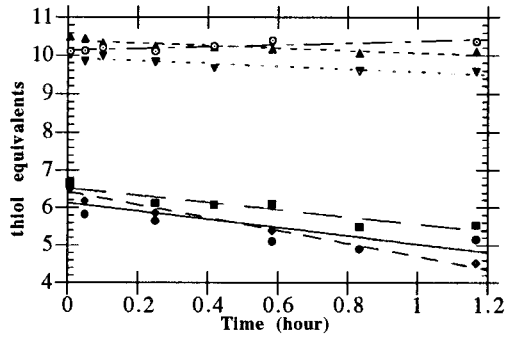


Figure 3. Stoichiometry of the interaction between the various organotellurium compounds and 10 equiv of cysteine. (○) Reference; (●) AS101; (■) $Te^{IV}(citrate)_2$; (◆) Te(ethylene glycol)$_2$; (▼) $Te^{VI}(citrate)_2$; (▲) Te(ethylene glycol)$_3$.

Table 2. Stoichiometry of Interaction of Cysteine with Organotellurium Compounds

compound	no. of thiol equiv ± sd	
	Te(IV)	Te(VI)
Te(ethylene glycol)$_2$	3.7 ± 0.7	–
Te(ethylene glycol)$_3$	–	0
Te(citrate)$_2$	3.9 ± 0.4	0
telluric acid	–[a]	0
AS101	4.0 ± 0.5	–

[a] Tellurium dioxide also reacts with cysteine, but the stoichiometry of the reaction could not be accurately determined due to the low solubility of the former.

^{125}Te NMR. The interaction between a thiol model compound (cysteine) and Te(IV) or Te(VI) compounds was studied by ^{125}Te NMR. Each of the three Te(IV) compounds, AS101, Cl_2Te(ethylene glycol), and Te(citrate)$_2$, was incubated with increasing stoichiometric ratios (0, 1, 2, and 4 equiv) of cysteine in DMSO-d_6, and the ^{125}Te NMR spectra of the reaction solutions were taken after a few minutes. In the reactions with the first two tellurium compounds, the original resonance of the tellurium starting material (at 1680 and 1706 ppm, respectively) decreased as cysteine was added and a single new resonance appeared at 1807 and 1800 ppm, respectively (Figure 5). The exchange was completed with the addition of 4 equiv of cysteine, and the new resonance was stable at room temperature for at least 8 h. $Te^{IV}(citrate)_2$, on the other hand, exhibited an upfield shift from 1445 to 1282 ppm upon addition of up to 4 equiv of cysteine. Contrary to the above interaction and in agreement with the spectrophotometric results (Figure 3 and Table 2), the two Te(VI) compounds, $Te^{VI}(citrate)_2$ and Te^{VI}(ethylene glycol)$_3$, did not exhibit any change in their ^{125}Te NMR resonance (787 and 933 ppm, respectively) upon incubation with 4 equiv of cysteine.

^{13}C NMR. In a complementary experiment, the reaction between AS101 and a specifically labeled [3-^{13}C]cysteine was followed by ^{13}C NMR spectroscopy. Addition of 4 equiv of cysteine to a 12.5 mM solution of AS101 in DMSO-d_6 at room temperature produced a resonance at 38.5−39.0 ppm. Further addition of up to 12 equiv of cysteine did not increase the integration of this peak but produced an additional resonance at 35−36 ppm. The product resonance at ∼39 ppm was stable under the experimental conditions for several hours.

Discussion

Organotellurium Compounds as Inhibitors of Proteases. Selectivity. As described above, Te(IV) compounds interact with nucleophiles, yielding $(Nu)_4Te$ products.[15] The latter may further interact with nucleophiles (or undergo hydrolysis in aqueous solution). $Te(SR)_4$ are unique in the family of Te(IV) compounds in that they undergo an oxidation−reduction disproportionation reaction according to eq 1.[16] On the basis of the chemistry of Te(IV) compounds described above, we expected that, while they might interact nonspecifically and reversibly with a variety of functional groups in enzymes and other proteins, they should oxidize proteinous thiols. Therefore, we decided to study the interaction between reactive Te(IV) compounds and the four families of proteases described above. We expected that active-site thiol oxidation should lead to an irreversible inhibition of cysteine proteases, while specific interactions with proteases representing the other three families were not anticipated. Indeed, AS101 did not inactivate any of the enzymes from the three protease families serine-, aspartic, and metalloproteases, even after long incubation time. On the other hand, time- and concentration-dependent inhibition was

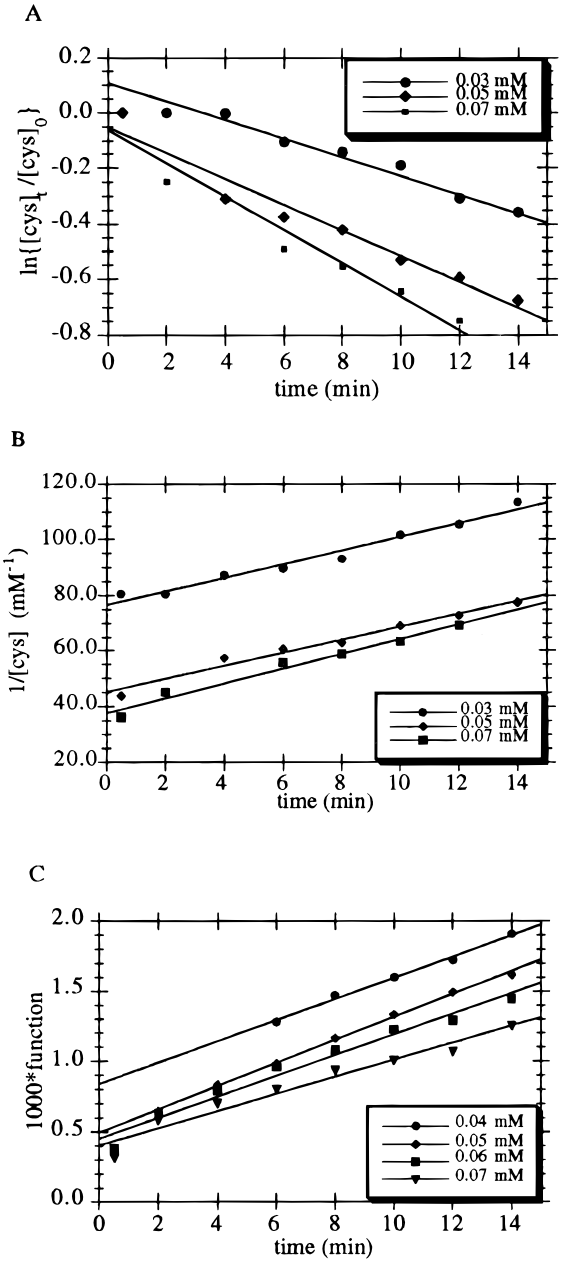


Figure 4. Time course of the interaction between AS101 and cysteine in water. (A) Fitting the kinetics to a pseudo-first-order reaction in [cysteine]. (B) Fitting the kinetics to a pseudo-second-order reaction in [cysteine]. (C) Fitting the kinetics to an overall third-order reaction.

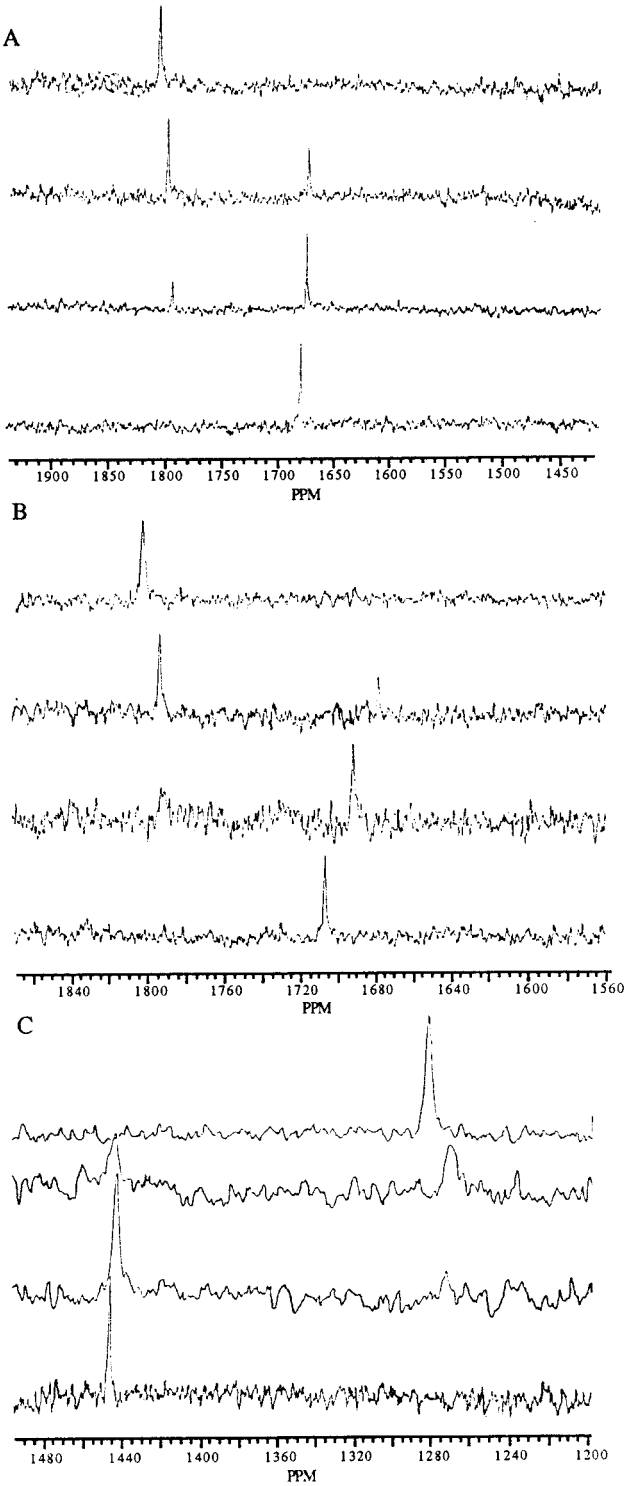


Figure 5. ^{125}Te NMR of the reaction mixture of (A) AS101, (B) Cl_2Te(ethylene glycol), and (C) $Te^{IV}(citrate)_2$ with cysteine in DMSO-d_6. The traces from bottom to top correspond to 0, 1, 2, and 4 equiv of cysteine, relative to the Te compound. (Note the different frequency range of the spectra of the three compounds).

observed upon incubation of the two cysteine proteases, papain and cathepsin B, with AS101. This is indicative of an irreversible, covalent inhibition due to the thiol oxidation. In support of this assumption, the cysteine protease papain that was inactivated by AS101 remained inactive after gel permeation chromatography. It is expected that a noncovalent enzyme−inhibitor complex would dissociate under such conditions and thus enzymatic activity would resume. In the absence of AS101, active papain retained its enzymatic activity upon undergoing similar treatment.

Inactivation Characterization. The inactive papain could be reactivated by incubation with low molecular weight thiols, such as cysteine. This suggests that the inactive species is of the form Enz−S−TeL_3, where the enzyme's active-site thiol has replaced one of the Te ligands. Alternatively, the inactive species could be Enz−S−S−R, where R−S comes from a residual trace amount of the cysteine used for the initial activation of papain.[31] In both cases, enzymatic activity would be recovered by a thiol exchange reaction which releases the enzyme's active-site thiol. The possibilities of enzyme dimerization, Enz−S−S−Enz, due to the oxidation−reduction disproportionation reaction of $Te(SR)_4$, or inactivated species of the form $(Enz-S)_2Te$ or $(Enz-S)_2TeL_2$ were excluded since the elution volume of the inactivated enzyme in gel permeation chromatography was very similar to that of the active papain (M_r 23 400, ca. 12.5 mL), implying a similar molecular weight. Since these other possible forms have M_r >46 000, they would be expected to elute at a volume ≤10 mL, the elution volume of ovalbumin (Figure 2).

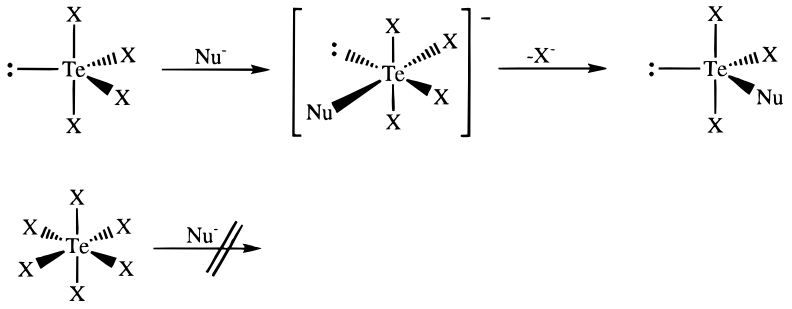
Kinetics. Saturation kinetics was observed in the inactivation of papain by AS101, demonstrating that the inactivation process is not a simple bimolecular reaction, but must proceed via a noncovalent enzyme−inactivator complex.[32] The nature of this noncovalent complex is unknown at present. The other Te(IV) compounds tested also inactivated papain, but with second-order rate constants 1−2 orders of magnitude lower than that of AS101.[33] Contrary to the observed inactivation of papain by Te(IV) compounds, the enzyme was not inactivated by three different Te(VI) compounds (Te(ethylene glycol)$_3$, telluric acid, and $Te^{VI}(citrate)_2$), even after long incubation time with high concentration of the Te compounds. Thus, the inactivation process is selective in respect to the oxidation state of the tellurium compound. Such selectivity stems from the different reactivity of Te(IV) vs Te(VI) compounds toward thiol nucleophiles (see below).

Reaction between Organotellurium and Thiols. Stoichiometry. Table 2 and Figure 3 reveal that all Te(IV) compounds tested interacted very rapidly (within less than 10 s under our experimental conditions) with 4 equiv of cysteine. The interaction is presumably through the cysteine thiol and therefore it was followed by titration with DTNB, a thiol specific reagent.[29] The observed further slow disappearance of titratable thiol was probably due to air oxidation to form cystine. It should be noted that tellurium dioxide (TeO_2) also interacted rapidly with cysteine, but the stoichiometry of this interaction could not be determined accurately due to its low solubility in the aqueous

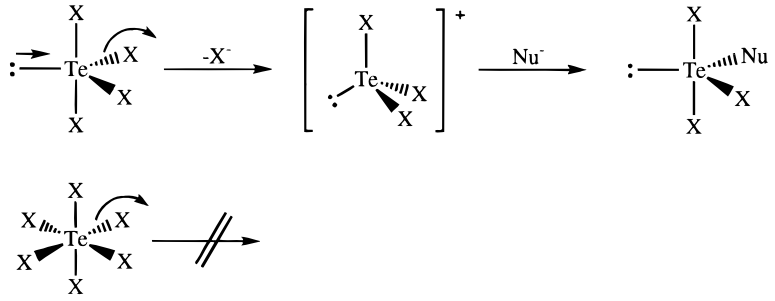
(30) Frost, A. A.; Pearson, R. G. *Kinetics and Mechanism*, 2nd ed.; John Wiley & Sons: New York, 1961; pp 20−21.
(31) Prior to the inhibition studies of cysteine proteases it was necessary to remove the excess activating thiol (used to activate the cysteine proteases) since Te(IV) compounds interact readily with thiols (see text). This separation of the active protein from the low-molecular weight thiol was carried out by gel filtration chromatography, though we could not ensure complete separation. Thus, small quantities of free cysteine, below the sensitivity of our DTNB assay, could be present in the protein fractions.
(32) Segel, I. H. *Enzyme Kinetics*; John Wiley & Sons: New York, 1975; pp 18−99.
(33) Due to experimental limitations, only the second-order rate constant approximation values, k_{obs}/[I], were determined. Thus we cannot attribute the differences in inactivation rate between the different Te(IV) compounds either to binding energy in the noncovalent complex (K_i) or to the chemical reaction (k_i).

Scheme 1. Interactions of Te(IV) and Te(VI) Compounds with Nucleophiles According to (A) Associative and (B) Dissociative Mechanisms

A. Associative nucleophilic substitution



B. Dissociative nucleophilic substitution



medium.[34] On the other hand, the three different Te(VI) compounds examined in this study did not interact with cysteine to any appreciable extent (under the conditions specified in the Experimental Section) even after 3.5 h of incubation.

Thus, there appears to be a clear distinction between the interaction of Te(IV) and Te(VI) compounds with nucleophiles such as the cysteine thiol in the present study. This difference in chemical reactivity is manifested also in the in vitro biological activity of tellurium compounds, where Te(IV) compounds are inhibitors of cysteine proteases while the corresponding Te(VI) compounds are inert.

From the mechanistic point of view, the different reactivity of Te(IV) and Te(VI) toward nucleophiles can be explained in terms of both associative (analogous to S_N2-type reactions) and dissociative (analogous to S_N1-type reactions) mechanisms (Scheme 1). The mechanism by which Te(IV) interacts with nucleophiles is still poorly understood, but it was pointed out that this interaction bears some similarities to the corresponding phosphorus chemistry.[20b,35−37] Less is known about the chemistry of Te(VI). Considering an associative mechanism, it can be argued that while the Te(IV) compounds are formally pentavalent (including the equatorial lone pair electrons) and thus addition of another ligand in the equatorial plane is possible, the Te(VI) compounds are hexavalent with an octahedral structure. Therefore, association of an additional ligand in the latter case is sterically hindered (Scheme 1A). In a dissociative mechanism, a Te(IV) compound can "compensate" for the developing positive charge during the dissociation of an equatorial ligand by the equatorial lone pair electrons. This process can repeat itself four times, exchanging all of the ligands (including the axial ones) either directly or utilizing a pseudorotation mechanism[35b] (also operative in phosphorus chemistry[38]). Te(VI) compounds, on the other hand, do not have the necessary two electrons to support the developing positive charge upon ligand dissociation (Scheme 1B). AS101 is formally a Te(IV) compound and it has an equatorial nonbonding electron pair, but unlike other Te(IV) compounds (and rather similar to the Te(VI) compounds) it is octahedral.[20a] Thus, the fact that it interacts readily with thiols seems to support a dissociative mechanism, where its equatorial lone electron pair can support the loss of a ligand, while its octahedral structure prevents introduction of a new ligand in an associative mechanism.

Kinetics. The reaction between the organotellurium(IV) compound AS101 and cysteine was further analyzed kinetically. The order of the reaction in [cysteine] was determined by following the reaction between a constant high Te concentration and varying cysteine concentrations, thus reducing the overall reaction order. Technical limitations such as AS101 solubility and spectrometer sensitivity restricted the concentration range of the experiment. Nevertheless, since Te(IV) interacts with 4 equiv of thiol at an increasing rate (i.e. the order of the rate of addition of the four thiols is 4th > 3rd > 2nd > 1st, see below), even at high thiol concentration only about 10% of the Te compound was consumed upon long incubation. We therefore treat [Te] as constant during the course of the reaction, reducing the reaction order to pseudo-first or second order. The obtained

(34) TeO_2 was shown to interact with 4 equiv of thiols under different conditions. See ref 16b.

(35) (a) Houalla, D.; Wolf, R.; Gagnaire, O.; Robert, J. B. *Chem. Commun.* **1969**, 443−444. (b) Gottlieb, H. E.; Hoz, S.; Elyashiv, I.; Albeck, M. *Inorg. Chem.* **1994**, *33*, 808−811.

(36) For a general review of nucleophilic attacks at phosphorus centers see: (a) Kirby, A. J.; Warren, S. G. *The Organic Chemistry of Phosphorus*; Elsevier: Amsterdam, 1967; pp 250−364. (b) Westheimer, F. H. In *Rearrangements in Ground and Excited States;* de Mayo, P., Ed.; Academic Press: New York, 1980; Vol. 2, pp 229−271.

(37) For a general review of biochemical phosphoryl transfer reactions, see: Knowles, J. *Annu. Rev. Biochem.* **1980**, *49*, 877−919.

(38) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70−78.

data were fit to both eqs 3 and 4 (first- and second-order reactions, respectively) (Figure 4). Clearly the linear curve fit is better for the pseudo-second-order equation than for the pseudo-first-order reaction, as the lines obtained for the different cysteine concentrations are parallel for the former but not for the latter. This is also evident from the numerical data, as the calculated first-order rate constants span a larger range (with higher standard deviation) than the second-order rate constant. Thus, it is concluded that the reaction between AS101 and cysteine is second order in the thiol concentration (with $k' = 42 \pm 2\ M^{-1}\ s^{-1}$) and an overall third-order rate constant according to the equation $v = k[\mathrm{Te}][\mathrm{thiol}]^2$ (therefore $k = k'[\mathrm{AS101}] = (5.25 \pm 0.25) \times 10^5\ M^{-2}\ s^{-1}$).

In a second set of experiments, the initial thiol concentration was kept constant and the initial tellurium(IV) concentration was varied. Based on the stoichiometry of

$$4(\text{cysteine}) + \text{AS101} \rightarrow \text{products}$$

determined above, and an expected reaction rate equation of

$$v = k[\mathrm{AS101}][\text{cysteine}]^2$$

the results were fit to eq 5. As can be seen in Figure 4c, the data at four AS101 concentrations fit very well to four parallel lines, representing an overall third-order rate constant $k = (1.22 \pm 0.15) \times 10^6\ M^{-2}\ s^{-1}$, in good agreement with the rate constant obtained from the pseudo-second-order reaction described above.

^{125}Te NMR. ^{125}Te NMR was used to follow the interaction between cysteine (as a thiol model compound) and Te(IV) or Te(VI) compounds. Although ^{125}Te NMR resonances are highly sensitive to the environment (e.g., solvent, concentration, temperature, etc.),[39] they can be divided into frequency ranges corresponding to the tellurium oxidation state.[40] Most TeX_4 compounds (where X's are different heteroatoms or strong electron-withdrawing groups such as CF_3) resonate at 1100−2000 ppm, while the corresponding Te(II) resonate at $\delta < 1000$ ppm. More specifically, the chemical shift of $Te(SR)_2$ ranges between 50 and 700 ppm, strongly depending on the parent thiol's pK_a.[16b] Therefore, $Te(cysteine)_4$ is expected to resonate at 1500−2000 ppm while the resonance of $Te(cysteine)_2$ is expected at about 400 ppm. The data presented here demonstrate a clear distinction in reactivity toward thiol nucleophiles between the Te(IV) and the Te(VI) compounds (in agreement with the spectrophotometric results described above, Figure 3 and Table 2). While the latter do not interact with the thiols, as is evident from the lack of any shift in their ^{125}Te NMR spectra, all three Te(IV) compounds exhibit a significant shift in their spectrum upon interaction with cysteine. All the new resonances are still well within the range of Te(IV) compounds. AS101 and Cl_2Te(ethylene glycol) exhibit a downfield shift from ~1700 ppm to 1807 and 1800 ppm, respectively. This is attributed to the formation of $Te(cysteine)_4$. The small difference in chemical shifts of the corresponding products is probably due to some differences in the corresponding NMR solutions arising from the nature of the tellurium reactants. Upon incubation of $Te^{IV}(citrate)_2$ with cysteine, an upfield shift to 1282 ppm is obtained. Although it is in the Te(IV) range, it is clearly different from the shift observed for the other two compounds, and therefore it must be associated with a different product. We tentatively attribute this resonance to a tellurium complex involving both 4 equiv of cysteine (as was clearly demonstrated by the spectrophotometric assay) and citrate. This complex should be further characterized. Other complexes of higher coordination of Te(IV) are well documented.[41] It should be noted that addition of citrate to the $Te(SR)_4$ obtained from the interaction of AS101 and cysteine did not affect its 1807 ppm spectrum. Therefore the difference in the ^{125}Te NMR of the two products cannot be attributed to a simple equilibrium of the form

$$\mathrm{Te(SR)_4} + n(\text{citrate}) \rightleftharpoons \mathrm{Te(SR)_4 \cdot citrate}_n$$

Two important points should be emphasized.

(a) The Te(IV) compounds exhibit a downfield chemical shift even upon addition of only 1 equiv of cysteine, and this new resonance does not shift further upon addition of up to 4 equiv of cysteine. This strongly indicates that under the experimental conditions the original Te(IV) compound exchanges its ligands in an increasing rate, the fourth ligand being the fastest to be exchanged (if the exchange reaction is practically irreversible). Alternatively, the final product, $Te(cysteine)_4$, is thermodynamically more stable than any of the other intermediates, $TeX_n(cysteine)_{4-n}$ (if the reaction is reversible and in equilibrium). Otherwise, one would expect to observe different resonances for the different species formed at different Te/thiol ratios. If the reaction is indeed kinetically controlled (irreversible), then the observed order of ligand exchange rate supports a dissociative ("S_N1") mechanism, with the thiol ligands better stabilizing the developing positive charge of the following ligand exchange. This point will be further elaborated in a separate study.

(b) The ^{125}Te NMR resonance of the new $Te(cysteine)_4$ was stable under the experimental conditions (in DMSO at room temperature) for a few hours, and no decomposition to Te(II) was detected.

^{13}C NMR. The interaction between AS101 and cysteine in DMSO-d_6 was further probed by ^{13}C NMR, following the C-3 methylene resonance of specifically labeled cysteine. A single major product, resonating at ca. 39 ppm, was formed immediately. This resonance, assigned to $Te(cysteine)_4$, was stable for a few hours and did not change upon further addition of up to 12 equiv of cysteine. The excess free cysteine resonates at the expected range (35−36 ppm) and was not influenced by the $Te(cysteine)_4$/cysteine ratio, suggesting that there is no exchange of the Te ligands with the free thiol in the NMR time scale under the experimental conditions.

Conclusions

In the present study we have explored the possibility of using organotellurium compounds as protease inhibitors. The bio-

(39) (a) Kalabin, G. A.; Valeev, R. B.; Kushnarev, D. F. *Zh. Org. Khim.* **1981**, *17*, 947−953. (b) Luthra, N. P.; Odom, J. D. In *The Chemistry of Organic Selenium and Tellurium Compounds*; Patai, S., Rappoport, Z., Eds.; John Wiley & Sons: Chichester, 1986; Vol. 1. (c) Granger, P.; Chapelle, S. *J. Magn. Reson.* **1980**, *39*, 329−334. (d) Eliashev I. M.Sc. Thesis, Bar Ilan University, Ramat Gan, Israel, 1992.

(40) (a) Rodger, C.; Sheppard, N.; McFarlane, C.; McFarlane, W. In *NMR and the Periodic Table*; Harris, R. K., Mann, B. E., Eds.; Academic Press: London, 1978; pp 383−419. (b) Lutz, O. In *The Multinuclear Approach to NMR Spectroscopy*; Lambert, J. B., Riddell, F. G., Eds.; D. Reidel Publishing Company: Dordrecht, 1982; pp 389−403. (c) McFarlane, H. C. E.; McFarlane, W. In *NMR of Newly Accessible Nuclei;* Laszlo, P., Ed.; Academic Press: New York, 1983; Vol. 2, pp 275−299.

(41) (a) Hargittai, I.; Rozsondai, B. In *The Chemistry of Organic Selenium and Tellurium Compounds*; Patai, S., Rappoport, Z., Eds.; John Wiley & Sons: Chichester, 1986; Vol. 1, pp 63−155. (b) Rout, G. C.; Seshasayee, M.; Aravamudan, G.; Radha, K. *Acta Crystallogr. Sect. C* **1984**, *40*, 1142−1145. (c) Schnabel, W.; Von Deuten, K.; Klar, G. *Phosphorus Sulfur* **1982**, *13*, 345−355. (d) Schnabel, W.; von Deuten, K.; Klar, G. *Cryst. Struct. Commun.* **1981**, *10*, 1405−1411.

chemical studies showed that as opposed to Te(VI) compounds, the Te(IV) compounds are selective cysteine protease inhibitors, while exhibiting no inhibitory activity toward other families of proteases. Some mechanistic aspects of the cysteine protease inhibition by AS101 and other Te(IV) compounds, as revealed in this study, are as follows.

(1) The inhibition is time- and concentration-dependent.

(2) It shows saturation kinetics, characteristic of formation of an initial noncovalent enzyme−inhibitor complex. Kinetic parameters vary for different Te(IV) compounds.

(3) Enzymatic activity could be restored by incubation with thiols, indicative of a structure such as Enz−S−S−R or Enz−S−TeX_3 of the inhibited enzyme.

(4) The inhibited enzyme has a molecular weight similar to that of the active enzyme, ruling out dimerization of the form Enz−S−S−Enz, $(Enz-S)_2TeX_2$, or $(Enz-S)_2Te$.

To understand the different reactivity of Te(IV) and Te(VI) toward cysteine proteases and the different reactivity of the former toward the four families of proteases, we investigated their chemistry with thiols. We report that

(1) Te(IV) compounds interact rapidly with 4 equiv of thiols.

(2) Different Te(IV) compounds may give different products upon interaction with 4 equiv of the same thiol, probably due to specific stable high-valence complexation.

(3) Only the product, $Te(SR)_4$, but not any of the intermediates, $TeX_n(SR)_{4-n}$, could be detected, even upon incubation with limiting amounts of the thiol.

(4) The product $Te(SR)_4$ is stable in DMSO at room temperature for at least several hours.

(5) The reaction proceeds with an overall third-order rate constant according to the equation

$$v = k[\text{Te(IV)}][\text{thiol}]^2$$

(6) Te(VI) compounds are inert toward thiols.

(7) ^{125}Te NMR data of a variety of Te(IV) compounds with heteroatom ligands are provided.

These findings show a good correlation between biological activity of organotellurium(IV) compounds (cysteine protease inhibition) and their chemical reactivity toward thiols. This may improve our understanding, at the molecular level, of the in vivo biological activity of AS101 and other related tellurium compounds as strong immunomodulators.

Acknowledgment. We thank Ms. Iris Elyashiv for a generous gift of tellurium(IV) and tellurium(VI) compounds and for her assistance with the ^{125}Te NMR. This work was partially supported by a grant from the Raoul Wallenberg Chair, Bar Ilan University.

IC971456T