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Pseudo-Enzymatic Dealkylation of Alkyltins by Biological Dithiols

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Abstract

We investigated the time dependence of the degradation of three alkyltin derivatives by a nine amino acid linear peptide (I₁LGCWCYLR₉) containing a CXC motif derived from the primary sequence of stannin, a membrane protein involved in alkyltin toxicity. We monitored the reaction kinetics using the intrinsic fluorescence of the tryptophan residue in position 5 of the peptide and found that all of the alkyltins analyzed are progressively degraded to dialkyl derivatives, following a pseudo-enzymatic reaction mechanism. The end-point of the reactions is the formation of a covalent complex between the disubstituted alkyltin and the peptide cysteines. These data agree with the speciation profiles proposed for poly-substituted alkyltins in the environment and reveal a possible biotic degradation pathway for these toxic compounds.

Keywords

Organotin compounds; alkyltins; kinetics of dealkylation; fluorescence spectroscopy; stannin; metal binding peptides

Introduction

Organotins (or alkyltins) are xenobiotic compounds that are introduced in the environment through human activities due to their use as anti-fouling agents and fungicides [1–4]. Unlike other heavy metals and organometallic compounds, alkyltins display highly selective activity, damaging specific areas of the brain [5–7]. This selective cytotoxicity makes them potential anticancer drugs [8–12]. Incidental exposures to alkyltins in humans and testing on rats have both resulted in severe behavioral changes and in some cases, death [13–16]. For

humans and rodents, a single exposure to trimethyltin chloride ($((\text{CH}_3)_3\text{SnCl})$) causes the destruction of neuronal cells located within the hippocampal pyramidal band and the fascia dentata, while triethyltin chloride ($(\text{CH}_3\text{CH}_2)_3\text{SnCl}$) intoxication causes brain and spinal cord edema, and damage to the peripheral nervous system [16–19].

In spite of the relatively high dissociation energy ($\sim 190\text{--}220\text{ kJ/mol}$), environmental degradation (speciation) and metabolization of alkyltin compounds by prokaryotic and eukaryotic organisms are very common. For instance, the covalent Sn-C bond can be cleaved by chemical attacks (nucleophilic or electrophilic), UV radiation, and dealkylation by bacteria [20]. Interestingly, in both bacteria and mammals (including humans) alkyltins are progressively de-alkylated [21]. Arakawa *et al.* (1981) [21] showed that tetra-alkyltins undergo a progressive loss of organic groups from the Sn(IV) center, with the extent of the conversion from tetra-alkyltins to tri-alkyltins correlating with the length of their alkyl chains, i.e., the extent of formation of tri-alkyltin derivatives decreases as both the size and the stability of the organotin increase. These authors proposed that the dealkylation process explains the delayed toxicity in mammals of highly substituted alkyltins [21–23].

While several papers describe the environmental speciation of alkyltins due to physical and chemical agents [1, 3, 4, 24–32], the data on their interactions with biological targets are rather scarce. A few reports point out the interactions of alkyltin with hemoglobin, H^+ -ATPase, the xenobiotic efflux pump PDR5, and the ion channel of the F-ATP synthase [33–36]. Additionally, a few studies point out that alkyltins target enzymes carrying reactive sulphhydryl groups, inhibiting their function [20, 37–40]. However, there are no reports on molecular causes of the speciation of alkyltins *in vivo*.

One interesting study was conducted by Walsh and co-workers in the late 80s [41–43]. These authors demonstrated that the bacterial organomercurial lyase present in organomercurial-resistant bacteria is capable of dealkylating several organotin substrates [43], proposing a possible enzymatic mechanism of degradation of alkyltins in the environment [43]. Their observations are also supported by the existence of microorganisms resistant to alkyltins that are able to metabolize alkyltins. For the organomercurial lyase, the dealkylation reaction is carried out by the three cysteine residues present in the active sites of the organomercurial lyase, which are able to turnover tetramethyltin and trimethyltin fluorides into the corresponding dimethyltin derivatives to yield methane [42]. The dialkyl derivatives bind the enzyme, forming a dead-end complex.

Recently, we showed that a synthetic peptide (SNN-PEP, $\text{I}_1\text{LGCWCYLR}_9$) derived from the primary sequence of stannin (SNN), a mitochondrial membrane spanning protein that mediates the neurotoxic activity of TMT in mammals [44–46], containing only two vicinal cysteine residues (CXC motif) is able to dealkylate several trialkyltin compounds to their corresponding dialkyltin derivatives [20, 37, 38, 47]. In this paper, we analyzed the kinetics of dealkylation of tetramethyltin ($((\text{CH}_3)_4\text{Sn})$ or TetraMT) and two of the most common trisubstituted alkyltins by SNN-PEP: trimethyltin ($((\text{CH}_3)_3\text{SnCl})$ or TMT) and triethyltin ($((\text{CH}_3\text{CH}_2)_3\text{SnCl})$ or TET) chlorides. We monitored the kinetics of dealkylation of the alkyltins, exploiting the intrinsic fluorescence of the tryptophan residue in position 5 of the SNN-derived peptide. We propose a pseudo-enzymatic mechanism for the interpretation of the dealkylation process similar to that predicted by Walsh and co-workers [41–43]. The SNN-PEP degrades the polysubstituted alkyltins to more toxic species that bind and deactivate the peptide. These data give new insights into the biological fate and delayed toxic effects of the polysubstituted alkyltins.

Methods

Tetramethyltin, trimethyltin chloride and triethyltin dichloride were purchased from Strem Chemicals (Newburyport, MA). All chemicals were used without further purification.

Peptide Preparation

SNN-PEP was synthesized by the Microchemical Facility at the University of Minnesota; Minneapolis, MN. Crude SNN-PEP was dissolved in a 50:50 acetonitrile/water solution and purified by reverse phase high-performance liquid chromatography (HPLC), using a Waters Delta Pak 15 C18 column (Milford, MA). A linear gradient of 0.085% TFA (trifluoroacetic acid) in CH₃CN (v/v) and 0.1% TFA in H₂O was used in the HPLC runs. The fractions containing pure peptide were combined and the residual solvent was removed under gentle stream of N₂. The peptide was then dissolved in 2 ml of double distilled H₂O and lyophilized to a powder. Molecular weight and purity of peptide were assessed by electrospray ionization mass spectrometry (ESI-MS).

Fluorescence Spectroscopy

Steady-state fluorescence measurements were carried out on a LS-55 Fluorescence spectrometer (Perkin Elmer). The excitation wavelength was set to follow the tryptophan fluorescence at 295 nm and the emission spectra were recorded from 300 to 450 nm, with an excitation and emission bandpass set to 5.0 nm. At wavelengths above 290 nm, the absorption of tyrosine is minimal and can be neglected [48]. Kinetic studies were carried out in time course of 300 s and recorded at time intervals of 1.0 s for TMT and TET whereas a time course of 40 min with recording at 60s of time interval were used for tetramethyltin. The spurious background spectrum of the buffer was subtracted from the spectrum of the peptide.

Determination of binding affinity

For binding studies, SNN-PEP (approximately 0.2 mM) was dissolved in 20 mM phosphate buffer (PBS) at pH 6.5. TMT and TET were solubilized in 99% ethanol and added to the peptide solution from an initial concentration of 1.0×10^{-5} M up to a final concentration of 2.6×10^{-4} M. To ensure the completion of the dealkylation reaction and measure the dissociation constants, the samples were incubated for approximately 20 minutes at room temperature. All spectra were acquired in triplicate and the errors calculated from the average of the three runs. The fluorescence contribution from buffer components was subtracted from the peptide spectrum, while emission intensities have been corrected for sample dilution.

Steady-State Kinetic Studies

For steady-state kinetic studies, SNN-PEP 0.2 mM was dissolved into a solution containing 0.5 ml of 20 mM sodium phosphate buffer at pH 6.5 and 4.0×10^{-5} M dithiothreitol. Dealkylation kinetics were followed by monitoring the changes of the emission wavelength at 356 nm with excitation at 295 nm. Dealkylation reactions were started by titrating alkyltin compounds starting at a concentration of 1.0×10^{-6} up to 1.1×10^{-5} M. Rate constants were determined using the initial rates approximation, and the kinetic constants were calculated using a double reciprocal plot (initial velocities vs concentrations). The initial rates at each substrate concentration were measured in triplicate and averaged to estimate the experimental errors. The presence of the dialkyltin-peptide complexes at the end of each reaction was assessed by ESI-MS as reported previously [38].

Results

Monitoring the dealkylation kinetics using fluorescence spectroscopy

The kinetics of dealkylations were followed by the intrinsic fluorescence of Trp in position 5 of the SNN-PEP using selective excitation at $\lambda=295$ nm, which avoids the interference from tyrosine or phenylalanine side chains [48]. As the dealkylation progressed, we observed a decrease in the Trp fluorescence. The decay of fluorescence can be attributed to intramolecular charge-transfer [49–54] (internal mechanism), and/or the interaction between the excited indole group and the polar solvent and the charge transfer to the solvent [54] (external mechanism). Upon binding of the two alkyltins, SNN-PEP undergoes a conformational change from unfolded to type I β -turn structure [38]. Specifically, two nuclear Overhauser effect (NOE) contacts detected between the indole side chain and the methyl groups of the bound alkyltins indicate that the binding event brings the Trp indole ring in close proximity to the alkyl groups of the dialkyltin (Figure 1A). These conformational changes are also supported by circular dichroism analysis [37, 38] and are responsible for the gradual decay of the Trp fluorescence. Figure 1B shows the fluorescence intensity of the Trp5 decreases as a function of trimethyltin concentrations. We exploited this decrease in fluorescence to determine the apparent dissociation constants (Figures 1C and 1D). The variations of fluorescence intensity can be fitted to the following equation [55]:

$$\Delta F_{obs} = \Delta F_{max} \frac{[S]}{K_d + [S]}$$

where ΔF_{obs} is the variation in fluorescence intensity, ΔF_{max} is the maximum change in fluorescence intensity, S is the concentration of the organotin compounds, and K_d the apparent dissociation constant (Figures 1C and 1D). The apparent dissociation constants calculated for TMT and TET are $K_d = 22 \pm 5 \mu\text{M}$ and $K_d = 50 \pm 9 \mu\text{M}$, respectively. These values underscore a slightly higher affinity of SNN-PEP for TMT than TET. Since the binding curve was obtained after the completion of the dealkylation reaction, the obtained K_d values are in quantitative agreement with the binding of the SNN-PEP to dimethyltin and diethyltin chlorides [37]. The sluggish kinetics of dealkylation of the tetramethyltin did not allow us to determine the value of the apparent dissociation constant.

Kinetic model for alkyltin dealkylation by the SNN-PEP

The decay of fluorescence intensity for tetramethyltin, and the two trisubstituted alkyltins (trimethyltin and triethyltin chlorides) are reported in Figure 2. To interpret the kinetics of dealkylation, we assumed the following scheme [38]:

Based on 1:1 SNN-PEP:alkyltin stoichiometry detected by ESI-MS [37], we have interpreted the reaction mechanism using the pre-equilibrium approximation, assuming the formation of a transition state that evolves to the final SNN-PEP/dialkyltin complex with the production of methane:

Here P represents the free peptide, S is the tetra-alkyltin compound, and PS^* is the SNN-PEP/tetra-alkyltin complex, PS is the SNN-PEP/three-alkyltin complex and PI is the SNN-PEP/di-alkyltin complex. If P , S , and PS^* are assumed to be in rapid equilibrium defined by

the dissociation constant, K_S , the velocity of the reaction under these conditions can be expressed as:

$$v = d[PI]/dt = k_3 [PS] \quad (2)$$

The solution to the equation (2) is given by:

$$v = \frac{\left(\frac{k_2 k_3}{k_2 + k_3}\right)([P_o] - [PI])[S]}{\left(\frac{k_3}{k_2 + k_3}\right)K_S + [S]} = \frac{k_{cat}([P_o] - [PI])[S]}{K + [S]} \quad (3)$$

Where $k_{cat} = \left(\frac{k_2 k_3}{k_2 + k_3}\right)$ and $K = \left(\frac{k_3}{k_2 + k_3}\right)K_S$ are the catalytic constant and the pseudo Michaelis constant, respectively. For TMT and TET k_{cat} is simplified to k_3 and K comes the classical form of a Michaelis constant. The maximal velocity (V_{max}) is equal to $k_3([P_o] - [PI])$ for TMT and TET and to $k_{cat}([P_o] - [PI])$ for TetraMT. The data were fit using equation (3) and the kinetic parameters obtained using a double reciprocal plot ($1/v_o$ vs $1/[S]$) (see Figure 2). The data are summarized in Table 1. The estimate of the ratio k_3/K was obtained from the first order time constant, following the reaction progress curve [56].

For TetraMT (Figure 2), we found a rather slow reaction kinetics with $V_{max} = 2.4 \pm 0.6$ (significant figures) $I \text{ min}^{-1}$ ($0.039 I/s$) (where I is the fluorescence intensity), $K = 5.5 \pm 0.5 \mu\text{M}$, $k_{cat} = 0.003 \pm 0.001 \text{ min}^{-1}$ ($6.0 \times 10^{-5} \text{ s}^{-1}$) and the ratio $(k_{cat}/K) = 0.00001 \pm 0.00001$ ($\text{s}^{-1}/\mu\text{M}^{-1}$). For TMT, we found a $V_{max} = 0.05 \pm 0.01 I \text{ s}^{-1}$, $K = 4.7 \pm 0.4 \mu\text{M}$, while for TET we determined $V_{max} = 0.12 \pm 0.01 I \text{ s}^{-1}$, $K = 88.0 \pm 0.9 \mu\text{M}$. The rates (k_3) for the elimination step of the reaction (i.e. dealkylation) were $0.14 \pm 0.03 \text{ s}^{-1}$ and $0.11 \pm 0.03 \text{ s}^{-1}$ for TMT and TET, respectively. The k_3 values for both alkyltin compounds are remarkably similar. This indicates that once the peptide binds the trialkyltin compound, it cleaves the alkyl groups (methyl or ethyl) at approximately the same rate. The catalytic efficiencies of the reactions expressed as the ratio k_3/K were $0.034 \pm 0.005 \text{ s}^{-1} \mu\text{M}^{-1}$ and $0.0013 \pm 0.001 \text{ s}^{-1} \mu\text{M}^{-1}$ for TMT and TET, respectively, showing a marked preference of the peptide for TMT depending essentially on the affinity, measured by K , between the organotin compound and the peptide. The reduced catalytic efficiency (k_3/K) for TetraMT with respect to the TMT and TET is probably due to the presence of two demethylation steps, decreasing the overall rate of reaction.

Discussion

In the environment, polysubstituted organometallic compounds such as organomercurial, organolead, and organotin compounds undergo degradation or speciation due to both chemical and physical phenomena [1, 2]. Specifically, it has been shown that the cleavage of the Hg-C, Pb-C, as well as Sn-C bonds can be catalyzed by physical or chemical agents. However, the biochemical fate of these compounds is still under investigation. Walsh and co-workers hypothesized that these organometallic compounds could undergo enzymatic degradation (i.e. dealkylation) in a manner similar to the dealkylation of methyl mercury by the bacterial organomercurial lyase (MerB) [41, 42]. The mechanism of organotin degradation by MerB involves three essential cysteine residues located in the binding pocket of the enzyme. These cysteines carry out a S_E2 -type protonolytic cleavage of the Sn-C bond [41, 42]. The authors concluded that other enzymes similar to MerB could be in other resistant microbial organisms with high specificity for organotin compounds. To date, no specific organotin lyase has been identified. Progressive dealkylation of organotin compounds has been also detected in mammals including humans [21]. However, the

biochemical pathways of the organotin toxicity have not been fully elucidated. A complicating factor is that chemically similar alkyltins (TMT and TET) display remarkably different toxicological effects with different biodegradation pathways [28, 46, 57–66].

Another atypical feature of alkyltins is the delayed toxic effects [21]. This delayed toxicity has been correlated to either the activation of apoptotic pathways or to degradation of alkyltins from more substituted to less substituted compounds that elicit the toxic function. In fact, unlike other organometallic compounds, alkyltins cause apoptosis rather than necrosis [67].

In the late 1990s, Toggas et al. discovered a small membrane protein stannin (SNN, from latin *stannum*, which means tin), which is involved in the selective organotin toxicity and apoptosis [68]. SNN is mostly expressed in the brain hippocampus and it has two conserved vicinal cysteines (Cys-32 and Cys-34) that may constitute a TMT binding site [68]. Our designed peptide (SNN-PEP) corresponding to residues 29–37 of the SNN sequence and incorporating the CXC motif [37, 38] preferentially binds TMT and TET, with lower affinity for organotin compounds with longer alkyl chains [37, 38]. Unlike dithiothreitol, mercaptoethanol ($\text{CH}_3\text{CH}_2\text{SH}$), and glutathione. The peptide vicinal cysteines dealkylate the trisubstituted organotin compounds to the corresponding disubstituted analogs.

In this work, we tested whether vicinal cysteine residues could be responsible for the progressive dealkylation of alkyltins in proteins. Analogous to the work by Walsh and coworkers [41, 42], we found that vicinal cysteines are sufficient to carry out the dealkylation reaction. The reaction mechanism can be interpreted using a pseudo-enzymatic kinetics that terminates into a dead-end complex formed by the dialkylated species covalently bound to SNN-PEP. Our model is derived from our previous theoretical studies carried out on the peptide bound to the dialkyltin compound, suggesting a possible formation of a transition state with hydrolytic proteolysis of a Sn-C bond after addition of one or two cysteine thiolate groups [37, 38]. The transition state which resulted from a reoptimization of the SNN-PEP structure bound to the dialkyltin compound is described as a concerted addition/elimination (with general acid catalysis) at the trigonal bipyramidal center as the incoming thiolate removes the trans-diaxial CH_3 group that is being protonated. The steady-state kinetic studies agree with our theoretical predictions, indicating that this small linear peptide dealkylates polysubstituted alkyltin compounds with a pseudo-enzymatic mechanism. The kinetic data show a marked difference in dealkylation efficiency between tetraalkyltins and trialkyltins. For both TMT and TET the kinetic constants k_3 are very similar, suggesting that the longer alkyl group does not affect the dealkylation step, while K encoding the binding affinity between the peptide and the alkyltin is remarkably different. The elimination step (i.e. production of methane or ethane) proceeds at the same rate for both TMT and TET. It is conceivable that the substrate binding is the rate determination step. In the case of TMT and TET, the dialkylated alkyltin forms a dead-end complex with the peptide, inhibiting the turnover. TetraMT is progressively dialkylated by SNN-PEP with a substantially slower kinetics. These data are in agreement with the findings by Arawata and co-workers, who correlated the delayed toxicity of poly-substituted alkyltins with the progressive degradation of these compounds in more active and less substituted analogs. [21].

Taken all together, these results support Arakawa's original hypothesis that the hydrophobic TetraMT and TMT cross the cell membranes, interact with vicinal thiols, and are dealkylated prior to forming a covalent complex with SNN (or other biological dithiols) [21]. The delayed toxicity is explained on the basis of the dealkylation process that must occur prior to formation of the dead-end SNN-dimethyltin complex. The selectivity toward TMT is explained in terms of the binding affinity rather than the elimination (dealkylation)

step, which proceeds at the same rate for both TMT and TET. Since TMT causes selective damage in the central nervous system and TET is specific to the peripheral nervous system, it is possible to speculate that other proteins containing dithiols might bind TET in the peripheral nervous system more specifically.

In summary, we report a kinetic analysis of the dealkylation of tetraalkyl and trialkyltin compounds (TetraMT, TMT and TET) by a synthetic peptide containing a CXC motif. The peptide shows a marked affinity of binding for TMT over TET. However, once the peptide/alkyltin complex is formed, the dealkylation of the trialkyltin to the corresponding dialkyltin derivative proceeds at the same rate. The dialkylated alkyltin compounds form dead-end complexes with the peptide that cannot turn over other substrates. Taken together with the findings of the studies of Stridh et al. [65] on CXXC motifs and alkyltin interactions, these results could explain the delayed toxic effects of these organotin in mammals with a major role of dithiols in the degradation pathways of these xenobiotic compounds. Our future endeavor will include the effects of the spacing of the two cysteine residues as well as the importance of the β -turn in the reactivity kinetics and mechanism

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Abbreviations

TetraMT	Tetramethyltin
TMT	Trimethyltin chloride
TET	Triethyltin dichloride
SNN	stannin
ACN	acetonitrile
TFA	trifluoroacetic acid
ESI-MS	Electrospray Ionization Mass Spectrometry.

REFERENCES

1. Fent K. Toxicology. 2004; 205:223–240. [PubMed: 15464632]
2. Fent K. Crit Rev Toxicol. 1996; 26:1–117. [PubMed: 8833456]
3. Gadd GM. Sci Total Environ. 2000; 258:119–127. [PubMed: 11007284]
4. Hoch M. Appl Geochem. 2001; 16:119–127.
5. Philbert MA, Billingsley ML, Reuhl KR. Toxicol Pathol. 2000; 28:43–53. [PubMed: 10668990]
6. Ogita K, Nishiyama N, Sugiyama C, Higuchi K, Yoneyama M, Yoneda Y. J Neurosci Res. 2005; 82:609–621. [PubMed: 16273549]
7. Shintani N, Ogita K, Hashimoto H, Baba A. Yakugaku Zasshi. 2007; 127:451–461. [PubMed: 17329931]
8. Gielen M. Met Based Drugs. 1995; 2:99–103. [PubMed: 18472751]
9. Gielen M. Met Based Drugs. 1994; 1:213–219. [PubMed: 18476233]
10. Gielen M, Biesemans M, de Vos D, Willem R. J Inorg Biochem. 2000; 79:139–145. [PubMed: 10830858]
11. Costa MA, Gulino L, Pellerito L, Fiore T, Pellerito C, Barbieri G. Oncol Rep. 2009; 21:593–599. [PubMed: 19212616]

12. Costa MA, Pellerito L, Izzo V, Fiore T, Pellerito C, Melis M, Musmeci MT, Barbieri G. *Cancer Lett.* 2006; 238:284–294. [PubMed: 16140459]
13. Boyer JJ. *Toxicology.* 1989; 55:253–298. [PubMed: 2655175]
14. Feldman RG, White RF, Eriator II. *Arch Neurol.* 1993; 50:1320–1324. [PubMed: 8257310]
15. Kreyberg S, Torvik A, Bjorneboe A, Wiik-Larsen W, Jacobsen D. *Clin Neuropathol.* 1992; 11:256–259. [PubMed: 1424320]
16. Hwang CH. *Neurol Sci.* 2009
17. Aschner M, Aschner JL. *Neurosci Biobehav Rev.* 1992; 16:427–435. [PubMed: 1480339]
18. Aschner M, Gannon M, Kimelberg HK. *Brain Res.* 1992; 582:181–185. [PubMed: 1356582]
19. Milaeva E, Petrosyan V, Berberova N, Pimenov Y, Pellerito L. *Bioinorg Chem Appl.* 2004:69–91. [PubMed: 18365070]
20. Buck-Koehntop BA, Porcelli F, Lewin JL, Cramer CJ, Veglia G. *J Organomet Chem.* 2006; 691:1748–1755.
21. Arakawa Y, Wada O, Yu TH, Iwai H. *J Chromatogr.* 1981; 216:209–217. [PubMed: 7320105]
22. Karpiak VC, Bridges RJ, Eyer CL. *J Toxicol Environ Health A.* 2001; 63:273–287. [PubMed: 11437060]
23. Karpiak VC, Eyer CL. *Cell Biol Toxicol.* 1999; 15:261–268. [PubMed: 10696825]
24. Diez S, Jover E, Albaiges J, Bayona JM. *Environ Int.* 2006; 32:858–865. [PubMed: 16806472]
25. Gallert C, Winter J. *Water Res.* 2002; 36:3130–3140. [PubMed: 12171412]
26. Harino H, Yamamoto Y, Eguchi S, Kawai S, Kurokawa Y, Arai T, Ohji M, Okamura H, Miyazaki N. *Arch Environ Contam Toxicol.* 2007; 52:179–188. [PubMed: 17165102]
27. Horiguchi T, Kojima M, Hamada F, Kajikawa A, Shiraishi H, Morita M, Shimizu M. *Environ Health Perspect.* 2006; 114(Suppl 1):13–19. [PubMed: 16818241]
28. Mundy WR, Freudenrich TM. *Neurotoxicology.* 2006; 27:71–81. [PubMed: 16181675]
29. Rudel H, Muller J, Steinhilber J, Schroter-Kermani C. *Chemosphere.* 2007; 66:1884–1894. [PubMed: 17005234]
30. Voulvoulis N, Lester JN. *Sci Total Environ.* 2006; 371:373–382. [PubMed: 17049584]
31. Wuertz S, Miller CE, Pfister RM, Cooney JJ. *Appl Environ Microbiol.* 1991; 57:2783–2789. [PubMed: 1746939]
32. Zhai G, Liu J, He B, Zhang J, Zhou Q, Jiang G. *Chemosphere.* 2008; 72:389–399. [PubMed: 18433831]
33. von Ballmoos C, Brunner J, Dimroth P. *Proc Natl Acad Sci U S A.* 2004; 101:11239–11244. [PubMed: 15277681]
34. Golin J, Ambudkar SV, Gottesman MM, Habib AD, Sczepanski J, Ziccardi W, May L. *J Biol Chem.* 2003; 278:5963–5969. [PubMed: 12496287]
35. Golin J, Barkatt A, Cronin S, Eng G, May L. *Antimicrob Agents Chemother.* 2000; 44:134–138. [PubMed: 10602734]
36. Hughes G, Harrison MA, Kim YI, Griffiths DE, Finbow ME, Findlay JB. *Biochem J.* 1996; 317(Pt 2):425–431. [PubMed: 8713068]
37. Buck B, Mascioni A, Que L Jr, Veglia G. *J Am Chem Soc.* 2003; 125:13316–13317. [PubMed: 14583001]
38. Buck BA, Mascioni A, Cramer CJ, Veglia G. *J Am Chem Soc.* 2004; 126:14400–14410. [PubMed: 15521759]
39. Pitts KE, Summers AO. *Biochemistry.* 2002; 41:10287–10296. [PubMed: 12162744]
40. Cardiano P, De Stefano C, Giuffre O, Sammartano S. *Biophys Chem.* 2008; 133:19–27. [PubMed: 18069116]
41. Begley TP, Walts AE, Walsh CT. *Biochemistry.* 1986; 25:7192–7200. [PubMed: 3542022]
42. Walsh CT, Distefano MD, Moore MJ, Shewchuk LM, Verdine GL. *FASEB J.* 1988; 2:124–130. [PubMed: 3277886]
43. Walts AE, Walsh CT. *J Am Chem Soc.* 1988; 110:1950–1953.
44. Thompson TA, Lewis JM, Dejneka NS, Severs WB, Polavarapu R, Billingsley ML. *J Pharmacol Exp Ther.* 1996; 276:1201–1216. [PubMed: 8786553]

45. Figiel I, Fiedorowicz A. *Neurotoxicology*. 2002; 23:77–86. [PubMed: 12164551]
46. Gunasekar P, Li L, Prabhakaran K, Eybl V, Borowitz JL, Isom GE. *Toxicol Sci*. 2001; 64:83–89. [PubMed: 11606804]
47. Buck-Koehntop BA, Mascioni A, Buffy JJ, Veglia G. *J Mol Biol*. 2005; 354:652–665. [PubMed: 16246365]
48. Lackowicz, JR. *Principles of Fluorescence Spectroscopy*. 2nd ed.. Plenum Publisher, NY: 1999.
49. Cowgill RW. *Biochim Biophys Acta*. 1967; 133:6–18. [PubMed: 6047662]
50. Cowgill RW. *Biochim Biophys Acta*. 1967; 140:552–554. [PubMed: 6058131]
51. Ricci RW, Nesta JM. *J Phys Chem*. 1976; 80:974–980.
52. Chang MC, Petrich JW, McDonald DB, Fleming GR. *J Am Chem Soc*. 1983; 105:3819–3824.
53. Saito I, Sugiyama H, Yamamoto A, Muramatsu S, Matsuura T. *J Am Chem Soc*. 1984; 106:4286–4287.
54. Shizuka H, Serizawa M, Shimo T, Saito I, Matsuura T. *J Am Chem Soc*. 1988; 110:1930–1934.
55. Wada A, Mie M, Aizawa M, Lahoud P, Cass AE, Kobatake E. *J Am Chem Soc*. 2003; 125:16228–16234. [PubMed: 14692764]
56. Wahl RC. *Anal Biochem*. 1994; 219:383–384. [PubMed: 8080100]
57. Dopp E, Hartmann LM, von Recklinghausen U, Florea AM, Rabieh S, Shokouhi B, Hirner AV, Obe G, Rettenmeier AW. *Toxicology*. 2007; 232:226–234. [PubMed: 17316952]
58. Nakatsu Y, Kotake Y, Ohta S. *Toxicol Sci*. 2007; 97:438–447. [PubMed: 17341481]
59. Dong W, Muramoto W, Nagai Y, Takehana K, Stegeman JJ, Teraoka H, Hiraga T. *J Vet Med Sci*. 2006; 68:573–579. [PubMed: 16820714]
60. Ohhira S, Enomoto M, Matsui H. *Toxicology*. 2006; 228:171–177. [PubMed: 16978758]
61. Cristofol RM, Gasso S, Vilchez D, Pertusa M, Rodriguez-Farre E, Sanfeliu C. *Toxicol Lett*. 2004; 152:35–46. [PubMed: 15294345]
62. Seibert H, Morchel S, Gulden M. *Cell Biol Toxicol*. 2004; 20:273–283. [PubMed: 15685930]
63. Costa LG, Guizzetti M, Lu H, Bordi F, Vitalone A, Tita B, Palmery M, Valeri P, Silvestrini B. *Toxicology*. 2001; 160:19–26. [PubMed: 11246120]
64. Stridh H, Cotgreave I, Muller M, Orrenius S, Gigliotti D. *Chem Res Toxicol*. 2001; 14:791–798. [PubMed: 11453724]
65. Stridh H, Orrenius S, Hampton MB. *Toxicol Appl Pharmacol*. 1999; 156:141–146. [PubMed: 10198279]
66. Stridh H, Gigliotti D, Orrenius S, Cotgreave I. *Biochem Biophys Res Commun*. 1999; 266:460–465. [PubMed: 10600525]
67. Billingsley ML, Yun J, Reese BE, Davidson CE, Buck-Koehntop BA, Veglia G. *J Cell Biochem*. 2006; 98:243–250. [PubMed: 16453279]
68. Toggas SM, Krady JK, Billingsley ML. *Mol Pharmacol*. 1992; 42:44–56. [PubMed: 1635553]

Figure 1.

(A) Structure of the SNN-PEP/DMT complex. (B) Changes in intrinsic tryptophan fluorescence emission spectrum of SNN-PEP upon TMT binding ($\lambda_{ex} = 295$ nm) (C) TMT chloride binding curve ($\lambda_{ex} = 295$ nm, $\lambda_{em} = 352$ nm) from three independent titrations (error bars are smaller than the data points) (D) The percentage of fluorescence changes plotted as a function of TMT additions.

Figure 2.

Time course of fluorescence intensity upon addition of TetraMT (A), TMT (C), TET (D) and double reciprocal plot of initial velocities against concentrations of TetraMT (B), TMT (D) and TET (F) at pH 6.5 and $T = 25^\circ\text{C}$. Error bars are smaller than the data points.

Scheme 1.

Plausible reaction mechanism for the proteolytic cleavage of a Sn-C bond after addition of one or two cysteine thiolate groups

Table 1

Kinetic parameters of the dealkylation of TetraMT, TMT and TET by SNN-PEP. Pseudo Michaelis–Menten parameters (K , k_{cat}) from the initial reaction velocity V_o derived from the first 30 s for $(CH_3)_3Sn$ and $(CH_3CH_2)_3Sn$ and from the first 5 min for $(CH_3)_4Sn$.

	K (μM)	k_{cat} (s^{-1})	k_{cat}/K ($s^{-1}\mu M^{-1}$)
$(CH_3)_4Sn$	5.5 ± 0.5	0.00006 ± 0.00001	0.00001 ± 0.00001
$(CH_3)_3Sn$	4.7 ± 0.4	0.16 ± 0.04	0.036 ± 0.005
$(CH_3CH_2)_3Sn$	88.0 ± 4.0	0.12 ± 0.04	0.0013 ± 0.0001