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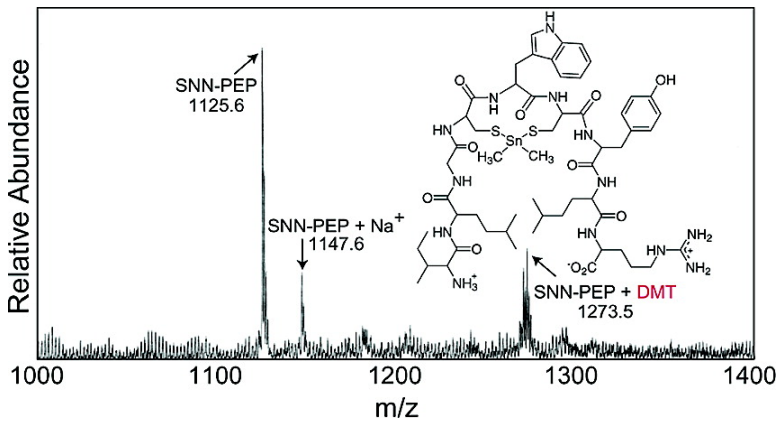

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Dealkylation of Organotin Compounds by Biological Dithiols: Toward the Chemistry of Organotin Toxicity

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Alkyltin salts or organotins have been widely used in material sciences and agriculture as antifouling agents and fungicides.[1] Because of their specific cytotoxicity, these compounds have also seen limited use as potential anticancer drugs.[2] Although banned, considerable amounts of organotins are still being introduced into the environment, causing growing concern about their impact on health.[3] Several human cases of organotin intoxication have been documented that resulted in severe health complications, and in some instances death.[4]

Organotins are characterized by a tetravalent structure with at least one carbon−tin bond and are classified as mono-, di-, tri-, and tetraalkyltins, depending on the number of alkyl moieties. Their toxicity correlates with the number and length of alkyl groups bound to tin, while the counterions do not affect organotin toxicity.[5] Trialkyltin compounds with short carbon chains are the most toxic with organotin toxicity diminishing from the methyl derivative to *n*-hexyl, with *n*-octyl being nontoxic.[5]

Unlike other environmental neurotoxins (i.e., methylmercury, lead, *m*-dinitrobenzene, or polychlorinated biphenyls, etc.), organotins possess a high specificity of action.[4,6] While trimethyltin chloride (TMT) causes lesions in specific regions of the hippocampus and neocortex, triethyltin chloride (TET) damage is localized within the spinal cord.[7] Interestingly, it has been found that in mammalian organs such as brain, liver, and kidneys, organotins are progressively dealkylated to inorganic Sn(IV).[4,5,8] Arakawa et al.[5] showed that the extent of this dealkylation correlates inversely with the length and stability of alkyl chains. Furthermore, delayed toxic action of polysubstituted organotins has been observed and associated with the rate of in vivo conversion of highly substituted organotins into their metabolites.[5,9] Organotin dealkylation has also been detected in the environment,[10] where alkyl group removal has been attributed to the action of UV light, chemical cleavage, and biological degradation by bacteria. Although the chemistry of organotin degradation has never been elucidated, organotin reactivity has been attributed to the nature of the C−Sn bond that can be attacked by both nucleophilic and electrophilic reagents.[10]

Recent studies have shown that cysteine and histidine residues are the primary biological ligands for organotin compounds[11] and that vicinal dithiols rather than monothiols constitute a general target for organotins.[12] Billingsley and co-workers have identified a small membrane protein, stannin (SNN), containing vicinal dithiols at the membrane interface, which mediates the selective neurotoxic activity of TMT in mammalians by triggering neuronal apoptosis in the hippocampus.[13]

To study the chemistry of organotin binding to biological dithiols, we synthesized a nine-residue peptide (SNN-PEP) corresponding to amino acids 29−37 of stannin (ILGCWCYLR) and examined its binding with different organotin compounds. Using circular dichroism (CD) and electrospray ionization mass spectrometry (ESI-MS) as probes, we determined the affinity and the stoichiometry of the SNN-PEP/organotin complexes formed.

CD spectra of SNN-PEP show that it undergoes a distinct structural change upon addition of different organotins. As with other linear peptides, this feature was exploited to measure the equilibrium dissociation constants (K_d) for the various organotin compounds.[14] SNN-PEP free in solution at pH 4.0 adopts mostly a random coil conformation, exhibiting a broad negative CD band with a minimum at 208 nm (Figure 1). Upon addition of increasing amounts of TMT, dimethyltin (DMT), TET, diethyltin (DET), and tri-*n*-propyltin (TPrT) chlorides, the CD spectra of the peptide showed a substantial dichroic shift with a large negative band centered at ∼222 nm and a positive band centered at ∼205 nm, representative of a transition to a β-turn-type structure (Figure 1). This dichroic transition is similar to the one observed for some β-amyloid peptides.[15] Based on the depth of the minimum at 222 nm, the extent of the conformational change was DMT ≈ TMT > DET > TET > TPrT, while the addition of TBT, MMT, and $SnCl_4$ resulted in no dichroic change.

From the titration curves, K_d values for the different organotins were calculated (Table 1). SNN-PEP displays the following order of affinity: DMT > DET > TPrT > TET > TMT. The affinity of SNN-PEP for DMT was approximately 1.5 times higher than that for DET, 2 times higher than that for TPrT, 5 times higher than that for TET, and 24 times higher than that for TMT (Table 1). For the trialkyltins analyzed, the increase of $\Delta G_{binding}$ from TMT was 0.9 kcal/mol for TET and 1.4 kcal/mol for TPrT, respectively. These results show that at pH 4.0 SNN-PEP (a) discriminates between different alkyl chains (it does not bind organotins with alkyl chains of more than three carbons), (b) shows a marked preference for tri- and dialkyltins (does not bind either MMT or $SnCl_4$), and (c) displays a noticeably higher affinity for DMT. At pH 6.5, where deprotonation makes cysteines more reactive toward the organotins,[16] all of the SNN-PEP−organotin complexes are more insoluble, hampering accurate measurement of the K_d values. Nonetheless, K_d values were obtained for DMT, DET, and TMT, in which SNN-PEP exhibited a 1.3-fold higher affinity for DET and a 3-fold higher affinity for DMT and TMT than at pH 4.0. In addition, the peptide was able to bind MMT and TBT, which were not observed at pH 4.0. While the selectivity of SNN-PEP was somewhat decreased at pH 6.5, the trend of affinities for the organotin compounds remained unchanged, with DMT still exhibiting the highest affinity.

The stoichiometry of the peptide/organotin complexes was analyzed by ESI-MS. SNN-PEP has a theoretical mass of 1125.7 and appeared at *m*/*z* 1125.6. Based on previous binding studies between trialkyltin compounds and mono- and dithiols,[12] we expected to observe an *m*/*z* of 1289.4 for the SNN-PEP/TMT complex (*m*/*z* 1125.6 plus 163.8 for the peptide and TMT cation, respectively). Instead, the complex was detected at *m*/*z* 1273.4, corresponding to the SNN-PEP/TMT complex minus 16 mass units, or methane. A control experiment with SNN-PEP plus DMT confirmed that the complex formed was SNN-PEP/DMT at *m*/*z*

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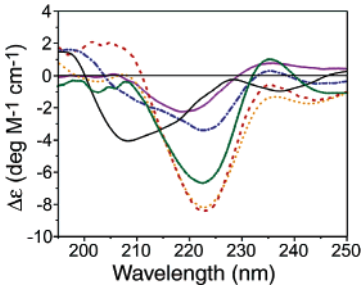

Figure 1. CD spectra of SNN-PEP free (black) and titrated with DMT (red), TMT (orange), DET (green), TET (blue), and TPrT (purple) chlorides (see Supporting Information for experimental details).

Table 1. Dissociation Constant (K_d) and ESI-MS Fragments for SNN-PEP Titrated with Various Organotins

organotin	K_d (μM) pH 4.0	K_d (μM) pH 6.5	ESI-MS (*m*/*z*)
DMT	60 ± 4	19 ± 5	1273.5
DET	89 ± 12	67 ± 7	1301.5
TPrT[a]	126 ± 5	NM	1329.4
TET[a]	311 ± 9	NM	1301.5
TMT[a]	1420 ± 9	439 ± 2	1273.4
TBT[b]	ND	NM	ND
MMT[b]	ND	NM	ND
$SnCl_4$[b]	ND	NM	ND

[a] Apparent dissociation constants (K_d^{app}). [b] TBT, tributyltin chloride; MMT, monomethyltin trichloride; $SnCl_4$, tin(IV) chloride; ND, nondetectable; NM, not measurable.

1273.5. These results indicate that SNN-PEP causes TMT demethylation and binds DMT. Identical results were obtained for TET and TPrT, which were dealkylated to DET and DPrT, respectively, and complexed by SNN-PEP (Supporting Information). Although there was a dichroic shift for TBT and MMT binding at pH 6.5, ESI-MS did not detect the formation of TBT/ or MMT/SNN-PEP complexes. Neither CD nor ESI-MS detected the binding of $SnCl_4$ to the peptide.

To test the role of the dithiols in organotin binding and dealkylation, the cysteines at positions 4 and 6 of SNN-PEP were substituted individually with serine. ESI-MS analysis showed that both of these mutants were unable to dealkylate the trialkyltin compounds under investigation. In addition, glutathione (GSH) and dithiothreitol (DTT) were also analyzed in the presence of organotin compounds, and no organotin dealkylation was observed by ESI-MS. In sum, CD and ESI-MS data show that (a) SNN-PEP dealkylates not only TMT, but also TET and TPrT, forming a 1:1 complex with the dealkylated species, while organotins with alkyl chains longer than three carbons were not affected, and (b) both thiols from the cysteine residues are required for organotin dealkylation. Consequently, the dealkylation reaction can be represented as:

$$R_3Sn^+ + SNN - PEP \rightarrow SNN - PEP{\cdot}SnR_2 + H^+ + RH$$

where the peptide thiols form two Sn−S bonds with the alkyltin cation displacing one alkyl group.[17] Therefore, the dissociation constants for the trialkyltin compounds should be referred to as apparent (K_d^{app}, Table 1).

The organotin dealkylation carried out by SNN-PEP may be similar to the degradation of organomercurials into Hg(II) and corresponding alkanes by the cysteine residues of the organomercurial lyase (MerB).[18,19] MerB, which contains four cysteines in the binding site, also dealkylates organotins with a V_{max} that is sluggish as compared to that of organomercurial compounds.[19] Taken together with the marked resistance to organomercurials displayed by organotin-resistant bacteria,[20] these findings suggest that cysteine-rich proteins may be responsible for organotin resistance mechanisms. Dealkylation, which accounts for the delayed toxicity of dialkylmercury compounds in humans,[4,9,21] may also play a critical role in delayed TMT toxicity. It is also known that mono- and dialkyltins are less toxic to organisms, despite the higher affinity displayed by SNN-PEP for DMT (see Table 1). Thus, we speculate that highly substituted, more lipophilic organotins cross cellular membranes more easily and are subsequently dealkylated into more reactive metabolites that further inhibit essential enzymes.[19,22]

In conclusion, we showed that a short linear peptide containing a CXC motif is sufficient to bind and degrade trialkyltin compounds with alkyl chains up to three carbon atoms. Our results suggest that dithiols found in proteins of the central nervous system can account for the chemistry of these selective neurotoxins. Because SNN is selectively activated by TMT, these results may indicate that, while this small linear peptide possesses a certain degree of discrimination for different ligands, it may not fully retain the specificity found for the native protein in vivo.[23]

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Supporting Information Available: Experimental details for CD spectroscopy and ESI-MS (including spectra) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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