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Methylation of (2-methylethanethiol-bis-3,5-dimethylpyrazolyl)methane zinc complexes and coordination of the resulting thioether: relevance to zinc-containing alkyl transfer enzymes

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Methylation of the coordinated thiolate in pseudotetrahedral Zn complexes of the form [(L3S)ZnX] by a variety of alkylating agents appears to occur *via* a nondissociative route and the resulting thioether can remain coordinated to the metal center as it does in zinc dependent alkyl transfer enzymes such as the DNA repair protein, Ada, from *Escherichia coli.*

The role of zinc metalloproteins in enzymatic alkyl group transfer is an emerging area of bioinorganic chemistry.[1] Examples of such enzymes include the DNA repair protein Ada from *E. coli*, the cobalamin dependent and independent methionine synthases, farnasyl transferase, and others.[2–6] In all of these proteins the zinc ion is in a thiol rich coordination environment with multiple cysteine residues as ligands. Zinc in the Ada protein, for example, is surrounded by four such cysteine residues (designated a CCCC site) although systems in which one or two of the cysteine residues have been replaced by histidines (CCHC and CCHH) are also known.[2–6] Interestingly, these same motifs also characterize the non-enzymatic zinc-finger proteins.[1]

A major question, which has been addressed by several model compound studies, has been: what is the role of the zinc in modulating the reactivity of cysteine residues toward methyl group transfer? In seminal mechanistic work, Wilker and Lippard have shown that in reactions of $Zn(SPh)_4^{2-}$ and its derivatives with trimethylphosphate as the methyl donor, methyl group transfer from the model substrate did not require the presence of zinc.[7] Thiolate anions were actually more readily alkylated although no transfer was seen when thiols were the acceptors. Methylation also occurred in the presence of $Zn(SPh)_4^{2-}$ but since all of its reactivity could be attributed to dissociated thiolate anion, doubts existed regarding the reactivity of a true zinc bound thiolate. More recently however, both we and Vahrenkamp and associates, have presented evidence for a nondissociative mechanism in the alkylation of [(L)ZnSR] (where L is a trispyrazolylborate or other scorpionate ligand) complexes by a variety of methylating agents.[8,9] Evidence has been accumulating that zinc-bound thiolates are in fact the active nucleophiles in the enzymatic reactions as well.[10]

One aspect of the enzymatic reactions not mimicked in any model system studied thus far is the fact that in many of the former, the thioether produced in the alkyl transfer remains coordinated to the zinc. Thioether coordination has been unequivocally demonstrated for the Ada protein and spectroscopic evidence consistent with this has been presented in several other cases as well.[11–13] In all of the model studies conducted to date the thioether has never been found in the zinc coordination sphere after alkylation.[7–9] This observation has prompted Vahrenkamp to propose that the apparently very poor donor capabilities of the thioether group toward zinc may contribute significantly to the overall reactivity in these systems.[9] Using a new N_2S heteroscorpionate ligand that is isostructural and isoelectronic with the well known N_3 trispyrazolylborates, we report here a system where a zinc-bound thiolate appears to be the active nucleophile[8b] and the thioether resulting from methyl group transfer reaction remains in the coordination sphere of the zinc in the absence of superior anionic ligands.

The ligand L3SH is prepared in reasonable yield using the same approach used to prepare previous members of this family.[8a] Thus bis(3,5-dimethylpyrazolyl)ketone reacts with 2-methyldithioisobutyraldehyde as a melt at 80 °C in the presence of $CoCl_2$ as a catalyst. The thio-protected intermediate, L3SSMe, was cleanly reduced with $LiAlH_4$ to yield the desired product. Pseudotetrahedral zinc complexes such as [(L3S)ZnI] **1**, or [(L3S)ZnOAc] **2**, were readily prepared by either direct reaction of deprotonated $(L3S)^-$ (methoxide ion) with the appropriate zinc salt or protonation of the $[(L3S)ZnCH_3]$ derivative with HX. Methylation of the coordinated thiolate in these complexes was achieved using methyl iodide, trimethyloxonium tetrafloroborate, or *p*-nitrobenzene sulfonic acid methyl ester as methyl donors.

Reaction of **1** with an equivalent of methyl iodide in dichloromethane yields the complex $[(L3SCH_3)ZnI_2]$, **3**, where the thioether is uncoordinated as has been previously found in related systems. Reasoning that the neutral thioether could not compete with the anionic iodide ion released in the methylation reaction, we removed one iodide by treatment of **3** with an equivalent of $AgBF_4$. Isolation of the product after filtration of precipitated AgI yielded the pseudotetrahedral complex **4**, $[(L3SCH_3)ZnI][BF_4]$ where the thioether is now bound to the zinc (Fig. 1).† These transformations are summarized in Scheme 1. The same reaction sequence starting with **2** leads to $[(L3SCH_3)ZnOAc][BF_4]$ which in the solid state dimerizes to the acetato bridged complex **5**, $[(L3SCH_3)Zn(\mu\text{-}OAc)_2(\mu\text{-}OH)Zn(L3SCH_3)][BF_4]$ containing octahedral zinc with coordinated thioethers (Fig. 2).† These reactions show clearly,

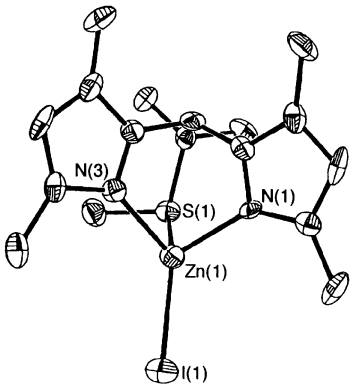

Fig. 1 Thermal ellipsoid diagram of $[(L3SCH_3)ZnI]^+$. The ellipsoids are drawn at the 30% probability level and hydrogens are removed for clarity. Selected bond distances (Å) and angles (°): Zn(1)–N(1) = 2.03(2); Zn(1)–N(3) = 2.03(2); Zn(1)–S(1) = 2.388(6); Zn(1)–I(1) = 2.471(3); N(1)–Zn(1)–N(3) = 94.2(7); N(1)–Zn(1)–S(1) = 90.6(5); N(1)–Zn(1)–I(1) = 122.4(5); N(3)–Zn(1)–S(1) = 89.9(5); N(3)–Zn(1)–I(1) = 124.3(5); S(1)–Zn(1)–I(1) = 125.8(2).

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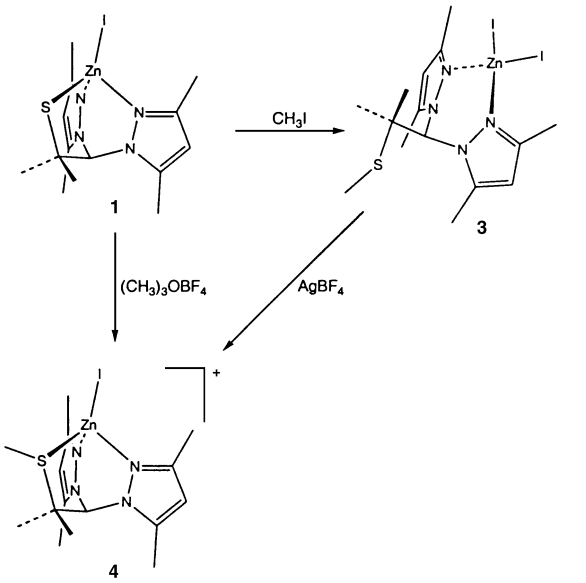

Scheme 1

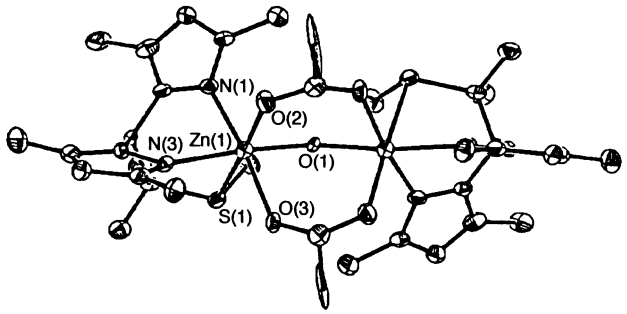

Fig. 2 Thermal ellipsoid diagram of $[(L3SCH_3)Zn(\mu\text{-}OAc)_2(\mu\text{-}OH)Zn(L3SCH_3)]^+$. The ellipsoids are drawn at the 30% probability level and hydrogens are removed for clarity. Selected bond distances (Å) and angles (°): Zn(1)–N(1) = 2.174(9); Zn(1)–N(3) = 2.118(9); Zn(1)–S(1) = 2.619(3); Zn(1)–O(1) = 1.979(4); Zn(1)–O(2) = 2.157(7); Zn(1)–O(3) = 2.078(8); N(1)–Zn(1)–N(3) = 85.1(3); N(1)–Zn(1)–O(1) = 96.0(3); N(1)–Zn(1)–O(2) = 173.5(3); N(1)–Zn(1)–O(3) = 93.5(3); N(1)–Zn(1)–S(1) = 82.6(2); N(3)–Zn(1)–O(1) = 173.0(3); N(3)–Zn(1)–O(2) = 90.0(3); N(3)–Zn(1)–O(3) = 92.0(3); N(3)–Zn(1)–S(1) = 87.9(2); O(1)–Zn(1)–O(2) = 88.3(3); O(1)–Zn(1)–O(3) = 94.9(3); O(1)–Zn(1)–S(1) = 85.3(2); O(2)–Zn(1)–O(3) = 91.0(3); O(2)–Zn(1)–S(1) = 92.9(2); O(3)–Zn(1)–S(1) = 176.2(2).

that in the absence of superior anionic ligands such as iodide, neutral thioethers can bind to a zinc center in either a tetrahedral or octahedral geometry.

Although coordinated thioether complexes could be prepared by removal of iodide by treatment with silver salts, the question remained, would a thioether formed by methylation of a zinc-bound thiolate remain coordinated without the addition of outside intervention? Trimethyloxonium tetrafloroborate and *p*-nitrobenzenesulfonic acid methyl ester are methyl donors used extensively to modify cysteine residues in proteins and are expected to produce only the weakly coordinating CH_3OCH_3 or *p*-nitrobenzene sulfonate as byproducts. Reaction of [(L3S)ZnX] where X = I^- or OAc^- with either of the above gave the expected $[(L3SCH_3)ZnX]^+$ directly as determined by NMR and electrospray-MS.‡ Thus the system reported herein mimics the known chemistry of the relevant zinc enzymes such as Ada.

Although the chelate effect is clearly important helping the thioethers produced here remain coordinated, it is not decisive. Thus while complexes of zinc with coordinated thioethers incorporated into anionic ligands are known,[14–16] numerous neutral chelates containing thioethers have been examined and invariably the thioethers are uncoordinated.[17–21] Thus we can envision zinc enzymes of this type being divided into two groups: the first where the sulfur to be methylated is part of the protein backbone *i.e.* one of the cysteine donors in the zinc coordination sphere as in the Ada protein. Under these conditions the resulting thioether can be expected to remain coordinated to the zinc due the macromolecular chelate effect. In the case where the thiol to be methylated represents an exogenous substrate, as in the cobalamin independent methionine synthases, the resulting neutral monodentate coordinated thioether is expected to be easily displaced by other ligands such as water (hydroxide). Such a process would yield free product and zinc enzyme with an open coordination site ready to repeat the catalytic cycle.

Notes and references

† *Crystal data* for **4**: $C_{15}H_{24}BF_4IN_4SZn$, M = 571.52, a = 9.754(2), b = 19.761(4), c = 22.977(4) Å, V = 4429(2) Å^3, orthorhombic, space group *Pbca*, Z = 8, T = 293(2) K, final $R1$ = 0.0795, $wR2$ = 0.1888, GOF (on F^2) = 1.047. For **5**: $C_{17}H_{27}B_{0.5}F_2N_4O_{2.5}SZn$, M = 468.26, a = b = 17.674(5), c = 15.531(3) Å, β = 120°, V = 4202(2) Å^3, trigonal, space group $P3_221$, Z = 6, T = 198(2) K, final $R1$ = 0.0799, $wR2$ = 0.2317, GOF (on F^2) = 1.067. CCDC 182/1726. See http://www.rsc.org/suppdata/cc/b0/b004338i/ for crystallographic files in .cif format

‡ In the case where X = OAc with *p*-nitrobenzenesulfonate as a counterion we can isolate solid monomeric **6** whose dimerization to $[(L3SCH_3)Zn(\mu\text{-}OAc)_2(\mu\text{-}OH)Zn(L3SCH_3)]^+$ is greatly suppressed *vis á vis* that of the BF_4^- salt.

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