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Control of Thiolate Nucleophilicity and Specificity in Zinc Metalloproteins by Hydrogen Bonding: Lessons from Model Compound Studies

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Thiolate coordination is an integral part of the coordination sphere of many of the hundreds of zinc metalloproteins known.[1] These sulfur-rich zinc centers fulfill one of two basic functional roles, either as structural elements, as for example in the zinc fingers and some metalloregulatory proteins,[2,3] or as a reactive entity for alkyl-group transfer as seen in the methionine synthesizing enzymes, MetE and MetH, of *Escherichia coli* or in the DNA repair protein Ada.[4] However, an as yet unanswered enigma is how two systems with essentially identical structure and ligation (compare for example the zinc fingers of the GATA family and the DNA repair protein Ada which both share the same pseudotetrahedral structure and CCCC ligation around the zinc) can fulfill such widely divergent roles. In the latter case the zinc thiolate functions as a reactive nucleophile that removes a methyl group from the phosphotriester backbone of alkylation-damaged DNA, while in the former case any such reactivity would be detrimental to its purely structural role. An additional question requiring an answer is how, in a thiol-rich construct such as that found in the Ada protein, is one specific zinc thiolate bond made to be reactive (cys 38 in this particular case) despite the similar steric accessibility of several such bonds.[5] Since it is well-known that the zinc-thiolate centers in the zinc fingers are involved in extensive N−H···S hydrogen bond networks with backbone amides, it has been proposed that such interactions might be a controlling factor in regulating reactivity and specificity of such bonds.[6] Although a statistical analysis of many Zn−S proteins pointed to a strong correlation between electrostatic screening of the core (predominantly by H-bonding interactions) and reactivity,[7] no direct experimental confirmation of this notion or determination of its magnitude has been reported. We show here, using model compounds designed to mimic the pseudotetrahedral binding site of the relevant zinc metalloproteins, that H-bonding can indeed control reactivity and that even a single such bond is sufficient to achieve specificity of reaction with a powerful, and hence inherently indiscriminate, electrophile. These results have a direct bearing on our understanding of these important metalloproteins.

We have previously used the reaction of LZnSPh complexes with CH_3I to model the reaction of Zn−S bonds with an electrophile in solution.[8] The ligand L in these studies is one of a series of N_2X tripodal scorpionates designated Tp, L1O, L4O, or L3S containing aromatic nitrogen, phenolate oxygen, carboxylate oxygen, or thiolate sulfur donors, respectively, in the "X" position.[9,10] To mimic the presence of an amide hydrogen-to-sulfur H-bond we have utilized *o-N*-Ac-thiophenol in place of thiophenol as the exogenous forth ligand. *o-N*-Ac-thiophenol and its derivatives are known to possess an internal H-bond between the amide and the thiolate sulfur, and extensive studies by Nakamura et al. have demonstrated the profound effects such bonds can have on redox potentials and other thermodynamic properties in models for Fe−S and molybdopterin proteins.[11−13]

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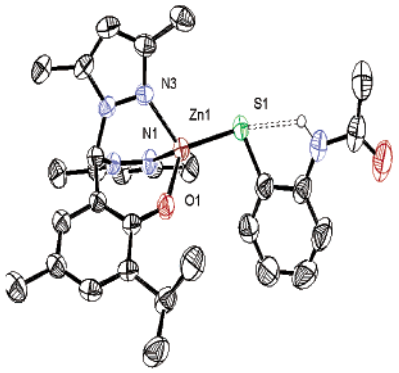

Figure 1. ORTEP diagram of **1** showing the internal H-bond.

Table 1. Pseudo First Order Rate Constants (s^{-1}) Determined by ^1H NMR for Reaction of Compounds **1** and **2** and Their Non-H-bonded Analogues with Methyl Iodide at 25 °C

cmpd	chloroform av rate	acetonitrile av rate
(L1O)ZnSPh	$3.53(5) \times 10^{-4}$	$1.42(3) \times 10^{-3}$
(L1O)ZnNAcSPh	$1.7(2) \times 10^{-6}$	$3.4(9) \times 10^{-5}$
(L4O)ZnSPh	$2.8(2) \times 10^{-6}$	$1.4(4) \times 10^{-5}$
(L4O)ZnNAcSPh	$4.1(4) \times 10^{-7}$	$5.9(2) \times 10^{-6}$

The desired complexes were readily prepared by borohydride reduction of the disulfide of *o-N*-acetylthiophenol followed by in situ reaction with [$LZnCH_3$] in THF. We have isolated and completely characterized the [LZn(*o-N*-Ac-thiophenol)] complexes for the L1O, **1**, and L4O, **2**, scorpionate ligands. As can be seen in the crystal structure of the L1O complex (Figure 1), an internal H-bond of the same type and nearly the same geometrical parameters as those described by Nakamura is present.[11−13] To determine the effects that this internal H-bond has on the reactivity of the zinc thiolate we have compared the kinetics of reaction between **1** and its non-H-bond-containing analogue with CH_3I at 25 °C. The results (Table 1) show that the presence of a single H-bond is sufficient to reduce the reactivity of the Zn−S bond toward the electrophile by up to 2 orders of magnitude. Thus, we were greatly surprised, when repeating this experiment using the L4O complex **2** and its non-H-bonding analogue, that the effect was now greatly reduced. A solution to this puzzle became evident however from an examination of the crystal structure of **2** (Figure 2), which clearly revealed that the H-bond formed in this case was with the carboxylate oxygen of the scorpionate ligand and *not* the thiolate sulfur. This provides a definitive and sensitive test to show that it is the H-bond itself rather than any peripheral electronic or steric effect of the *N*-acetyl group that controls the reactivity of the thiolate.

If H-bonding can control the reactivity of the zinc thiolate with electrophiles, then it can in principle also provide a means to generate specificity. To test the degree of such specificity we

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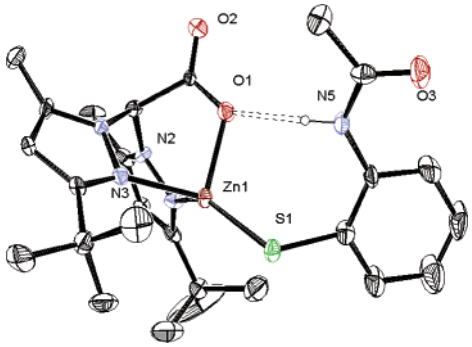

Figure 2. ORTEP diagram of **2** showing the internal H-bond.

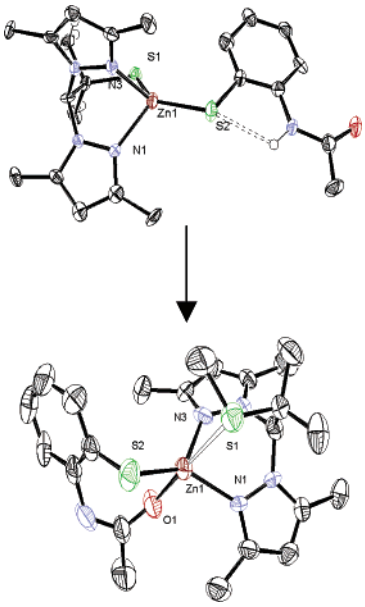

Figure 3. ORTEP diagram of **3** and **4**.

designed a model system, [L3SZn(*o*-*N*-Ac-thiophenol)], **3**, (Figure 3 upper) which contains two thiolates, one involved in H-bonding, and the other, not. We have already shown that in the absence of H-bonding effects the thiophenol and heteroscorpionate thiolate sulfurs react at similar rates (data not shown). To provide a strict test of the degree of selectivity we deliberately choose the strong and hence inherently indiscriminate alkylating agent, trimethyloxonium tetrafluoroborate. The result was in many ways remarkable with only the L3S thiolate sulfur being alkylated to give the monothioether product **4**. Even with a single H-bond the specificity was near 100% (we found no trace of the alternative methylated product). Interestingly the thioether in **4** appeared to be weakly bound to the Zn as indicated by its position and its long (2.77 Å) "bond" length. The acetylcarbonyl serves to provide the fourth strong ligand donor.

What insight into the functioning of thio-rich zinc cores in metalloproteins can be gleaned from this work? First, this work supports the notion that zinc-thiolate cores, which are predicted to be inherently reactive constructs, can be deactivated by the presence of H-bonds in complete accord with a recent detailed statistical analysis.[7] Thus, the unreactive CCCC cores found in the GATA zinc fingers are found to be more electrostatically screened than average, while the correspondingly reactive CCCC core of Ada is less screened than average.[7] Second, it suggests that the presence of H-bonds between the accessible but unreactive cys 69 or 42 and their absence at cys 38 could be the means by which the well-known regiospecifity of Ada is maintained. Surprisingly, the most recent NMR structure of an active fragment of the Ada protein, N-Ada10, is reported to contain no "characteristic" H-bonds to the ligand sulfur atoms.[14] However, the structural definition around the area in question may simply be insufficiently high to uniquely identify H-bonds from the large ensemble of structures derived from NMR data. In this regard it is notable that those amide protons on residues near the sulfur atoms exchange very slowly as expected for those engaged in H-bond interactions.[15]

In conclusion we have shown that even a single H-bond to a thiolate sulfur is sufficient to drastically reduce its nucleophilicity. Thus, nature can use this mechanism to modulate the reactivity of a zinc-thiolate construct so that it can be used for either structural or reactive purposes. In addition such H-bonding provides another means to generate the exquisite specificity seen in reactive biomolecules of this type.

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Supporting Information Available: X-ray crystallographic files (in CIF format) for **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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