Methylation of neutral pseudotetrahedral zinc thiolate complexes: model reactions for alkyl group transfer to sulfur by zinc-containing enzymes

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Abstract Eight scorpionate-zinc thiolate complexes, [(L1O)ZnSPh], [(L1O)ZnSPhF5], [(L1O)ZnSBz], [(L1O)Zn(SPh)2Me], [(L1O)Zn(SPh)4Me2], [(L1O)Zn(SPh)4NO3], [(Tp(SPh)2)ZnSPh], and [(L2S)ZnSPh], were reacted with methyl iodide in chloroform, liberating the corresponding methyl thioethers as determined by 1H NMR. Three of these complexes are new and their synthesis and structural characterization are reported here. Weak alkylating agents such as trimethyl phosphate failed to undergo methyl transfer to the zinc thiolates under these conditions. Analysis of kinetic data as a function of concentration, temperature, pKₐ of the exogenous thiolate, and donor atom of the tripodal ligand are consistent with a mechanism where the zinc-bound thiolate is the active nucleophile in an associative-type methyl transfer reaction. Our model studies also provide experimental evidence to support the hypothesis that some enzymes can use the charge of the metal coordination site to modulate catalytic activity.

Keywords Methylation · Zinc enzymes · Mechanism · Model complexes

Introduction

Zinc is the second most abundant transition metal in biology and constitutes the largest group of metalloproteins in nature. While the biological functions of zinc in metalloproteins varies considerably, the active sites exhibit a common structural motif [1]. Thus most active sites are composed of a pseudotetrahedral zinc center containing a combination of nitrogen, oxygen, and/or sulfur donors originating from histidine, tyrosine, aspartic or glutamic acids, and/or cysteine residues of the protein backbone and/or an exogenous water or hydroxide ion derived from the solvent. Although zinc enzymes have long been known to catalyze many hydrolytic transformations, recognition of their role in alkyl group transfer reactions has come only recently [2, 3]. Examples of such enzymes include the Ada protein of Escherichia coli, which is involved in DNA repair [4, 5, 6], the cobalamin-dependent and -independent methionine syntheses, farnesyltransferase, and others [7, 8, 9, 10]. In all of these proteins the zinc ion is in a pseudotetrahedral, thiol-rich coordination environment, but the role that the zinc plays in modulating the reactivity of these thiol ligands toward alkylation remains an open question.

The mechanisms of zinc metalloprotein-mediated alkyl group transfer to thiols has been studied both in model systems and directly in the enzymes. Although it is generally accepted that the alkyl group donor undergoes nucleophilic attack by a sulfur atom, i.e. an associative or Sₛ2 mechanism at the carbon residue [11], the nature of the attacking nucleophile remains unclear. Two types of nucleophiles can be envisioned: free thiolate anion produced by transient dissociation of the sulfur-containing ligand/substrate from the zinc center or an actual zinc-bound thiolate. Thus these reactions may also be considered as having associative or dissociative character at zinc. This question has been addressed in several model studies. In early
work, Wilker and Lippard [12] have shown that in reactions of Zn(SPh)\textsuperscript{2\textsuperscript{-}} and its derivatives with trimethyl phosphate as the methyl donor, the rate of the reaction can be attributed entirely to free thiolate anion, derived from dissociation of the parent complex, as being the reactive species. The observed low reactivities of \([\text{Zn}(S\text{-Cys})_2(\text{MeIm})]\)\textsuperscript{2\textsuperscript{-}} and \([\text{Zn}(S\text{-Cys})_2(\text{MeIm})]\)\textsuperscript{2\textsuperscript{-}} in this study are attributed to the decreased dissociation of the thiolate anion in the lower charged or neutral complexes. Likewise, the reduced activity of the Co(II) or Cd(II) analogs are considered due to decreased thiolate dissociation from these more thiophilic metal centers. Darenbourg and co-workers [13], using a solvated N\textsubscript{2}S\textsubscript{3} tetradentate ligand complex of zinc with methyl iodide as the alkylating agent, reached conclusions that were consistent with this result.

The biological implications of these studies are commonly interpreted to indicate that in the enzymatic systems the attacking nucleophile is a thiolate that has more or less dissociated from the zinc ion. However, recent studies using electrospray ionization mass spectrometry (ESI-MS), on both intact proteins and model peptides, show that in the CCCC. CCHC, and CCHH (C=cyteline, H=histidine) systems investigated, only two protons are lost for each Zn(II) ion chelated [14, 15]. Although these results cannot rule out that neutralization of the zinc coordination sphere occurs elsewhere in the protein, they do raise the distinct possibility that Zn(S-Cys)\textsubscript{2} sites in proteins are actually not dianionic [Zn(S-Cys)\textsubscript{2}]\textsuperscript{2\textsuperscript{-}} but rather neutral [Zn(S-Cys)\textsubscript{2}(SH-Cys)]. As such, they would be much less susceptible to thiolate anion dissociation than previously believed. These considerations illustrate the problems in extrapolating the mechanistic studies with the model complex [Zn(SPh)\textsubscript{2}]\textsuperscript{2\textsuperscript{-}} to enzyme systems where the overall charge of the zinc binding site may not be 2−. Indeed, more recent model studies by Vahrenkamp and co-workers [16] provided evidence for a mechanism that involved a zinc-bound thiolate rather than a free thiolate anion as the nucleophile in the reaction of neutral [Tp]ZnSR complexes with several methylating agents.

In an attempt to extend these studies and address the question of how the donor atoms in the coordination sphere of the zinc complex affect the reactivity of a bound thiol, we more closely examine the mechanism of the general reaction:

\[[\text{L}2\text{ZnSR}]+\text{MeI} \rightarrow [\text{L}2\text{ZnI}]+\text{MeSR}\] (1)

using a family of tridentate ligands (shown in Scheme 1) designed to systematically alter the nature of zinc thiolate complexes. The results of this work may aid in our understanding of the mechanism of alkyl group transfer in metalloenzymes.

Scheme 1

**Materials and methods**

**Synthesis**

All syntheses were carried out in air and the reagents and solvents purchased from commercial sources and used as received unless otherwise noted. Toluene and dichloromethane were distilled under argon over Na/benzophenone and CaH\textsubscript{2} respectively. The NMR solvents CDCl\textsubscript{3} and DMSO-d\textsubscript{6} were purchased from Aldrich and used as received. Potassium hydride(3,5-diphenyl-1-pyrazolyl)borate and its corresponding zinc complex [(Tp\textsuperscript{3\text{-Me}})Zn(SPh)] were prepared by literature methods [17]. The syntheses of the complexes [(L1O)ZnMe], [(L2S)ZnMe], [(L1O)ZnSPh\textsuperscript{3\text{-Me}}], [(L1O)ZnSbZ], [(L2S)ZnSPh\textsuperscript{3\text{-Me}}], and [(L2S)ZnSPh], in addition to the ligands [(3-tert-butyl-2-hydroxy-5-methylphenyl)bis(3,5-dimethylpyrazolyl)methane (L1OH) and [(3-tert-butyl-5-methyl-2-thiophenyl)bis(3,5-dimethylpyrazolyl)methane (L2SH), followed the reported procedures [18, 19].

\[[\text{L1O}]\text{ZnSPh}\textsuperscript{2\text{-Me}}\]

A solution of [(L1O)ZnMe] (0.35 g, 0.78 mmol) in CH\textsubscript{3}Cl\textsubscript{2} (20 mL) was treated with a CH\textsubscript{3}Cl\textsubscript{2} solution of 2,6-dimethylbenzenethiol (0.13 g, 0.94 mmol). The resulting solution was stirred for 1 h, dried under reduced pressure, and crystallized by layering a CH\textsubscript{3}Cl\textsubscript{2} solution of the complex with diisopropyl ether; yield: 0.24 g (54%). \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \(\delta\) 7.02 (d, 2H, J=8 Hz, S-ArH), 6.98 (d, 1H, J=2 Hz, ArH), 6.89 (1, 1H, J=8 Hz, S-ArH), 6.85 (s, 1H, CH\textsubscript{3}), 6.64 (d, 1H, J=2 Hz, ArH), 5.90 (s, 1H, PzH), 2.60 (s, 6H, Pz-C\textsubscript{3}H\textsubscript{3}), 2.44 (s, 6H, Pz-C\textsubscript{3}H\textsubscript{3}), 2.43 (s, 6H, S-Ar(C\textsubscript{6}H\textsubscript{4})), 2.16 (s, 3H, Ar(C\textsubscript{6}H\textsubscript{4})), 1.05 (s, 9H, -C(CH\textsubscript{3})\textsubscript{3}). \textsuperscript{13}C NMR (CDCl\textsubscript{3}) \(\delta\) 163.02, 150.42, 142.62, 141.33, 140.22, 138.49, 129.98, 128.80, 127.06, 123.25, 119.99, 119.75, 106.65, 73.39, 35.10, 29.04, 24.63, 20.49, 13.15, 11.72.

\[[\text{L1O}]\text{ZnSPh}\textsuperscript{2\text{-Me}}\]

A solution of [(L1O)ZnMe] (0.34 g, 0.76 mmol) in CH\textsubscript{3}Cl\textsubscript{2} (20 mL) was treated with a CH\textsubscript{3}Cl\textsubscript{2} solution of 2,4-dimethylbenzenethiol (0.13 g, 0.92 mmol). The resulting solution was stirred for 1 h, dried under reduced pressure, and crystallized by layering a CH\textsubscript{3}Cl\textsubscript{2} solution of the complex with diisopropyl ether; yield: 0.21 g (49%). \textsuperscript{1}H NMR (CDCl\textsubscript{3}) \(\delta\) 7.74 (d, 1H, J=8 Hz, S-ArH), 7.08 (d, 1H, J=2 Hz, ArH), 6.93 (1, 1H, S-ArH), 6.90 (s, 1H, -CH\textsubscript{3}), 6.70 (d, 1H, J=8 Hz, S-ArH), 6.69 (d, 1H, J=2 Hz, ArH), 5.88 (s, 1H, PzH), 2.49 (s, 3H, Ar-C\textsubscript{6}H\textsubscript{4}), 2.45 (s, 6H, Pz-\textsubscript{3}H\textsubscript{3}), 2.24 (s, 6H, Pz-CH\textsubscript{3}), 2.22 (s, 3H, S-Ar(C\textsubscript{6}H\textsubscript{4})), 1.39 (s, 9H, -C(CH\textsubscript{3})\textsubscript{3}). \textsuperscript{13}C NMR (CDCl\textsubscript{3}) \(\delta\) 150.39, 142.61, 140.27, 138.36, 135.94, 133.92, 132.27, 130.27, 130.14, 129.04, 126.46, 120.23, 119.94, 106.62, 73.49, 35.53, 29.50, 22.50, 20.88, 20.52, 12.98, 11.74.