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Modeling the Catalytic Site of Liver Alcohol Dehydrogenase: Synthesis and Structural Characterization of a [Bis(thioimidazolyl)(pyrazolyl)hydroborato]zinc Complex, [HB(tim^{Me})$_2$pz]ZnI

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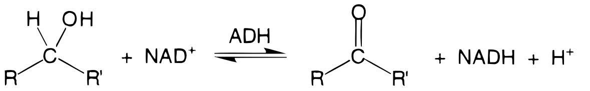
Alcohol dehydrogenases (ADH) are a class of zinc enzymes responsible for catalyzing the biological oxidation of primary and secondary alcohols *via* the formal transfer of a hydride anion to the oxidized form of nicotinamide adenine dinucleotide (NAD$^+$), coupled with the release of a proton (eq 1).[1,2] Of these enzymes, liver alcohol dehydrogenase (LADH) is the most widely studied, with the structures of several forms having been determined by X-ray diffraction.[1] These studies demonstrate that LADH consists of two similar subunits, each of which contains two zinc sites. However, only one site within each subunit is catalytically active, namely that in which the zinc is coordinated in a distorted tetrahedral manner to a histidine and two cysteine residues of a single polypeptide chain, with a water molecule occupying the fourth coordination site. The essential features of the catalytic cycle involve displacement of the water molecule by alcohol and subsequent deprotonation giving a zinc−alkoxide intermediate. Ensuing hydride transfer from the alkoxide to NAD$^+$ completes the dehydrogenation.[1]



(1)

The sulfur-rich composition of the active site of LADH is quite distinct from that of most other zinc enzymes, such as carbonic anhydrase and carboxypeptidase,[3,4] in which the zinc coordination environment consists solely of nitrogen and oxygen donors. Indeed, the distinctive coordination sphere about the catalytic zinc center in LADH has prompted the suggestion that it is critical for the effective function of the enzyme. The coordination environment of the catalytic site in LADH, however, is atypical and not well-precedented in zinc chemistry.[5] Therefore, in this paper, we describe an approach to model the active site of LADH with the synthesis of a polyfunctional tripodal ligand designed to emulate the means by which the protein binds the catalytically active zinc center.

With one histidine and two cysteine residues responsible for binding zinc at the active site of LADH, considerable attention has duly been given to the design of polyfunctional ligands that provide a [NSS] donor set capable of supporting a monomeric tetrahedral zinc center;[6−8] despite such endeavors, however, none of these studies have yielded structurally characterized mononuclear tetrahedral complexes that mimic the active site of LADH.[9] For example, due to the proclivity of thiolate groups to act as bridging ligands,[10] the bis(mercaptoalkyl)pyridine ligand [(C$_5$H$_3$N)(CH$_2$CPh$_2$SH)$_2$] yields a *dinuclear* zinc complex [{η^3-(C$_5$H$_3$N)(CH$_2$CPh$_2$S)$_2$}Zn]$_2$.[6ab,11,12] Related ligands, such as [(C$_5$H$_3$N)(CH$_2$SEt)$_2$], are likewise unsuitable because they bind in a meridional, “T-shaped”, fashion and thereby promote the formation of five-coordinate complexes, *e.g.* [(C$_5$H$_3$N)(CH$_2$SEt)$_2$]ZnBr$_2$.[13]

We recently utilized polyfunctional tripodal(pyrazolyl)hydroborato and imidazolylphosphine ligands for modeling the active sites of carbonic anhydrase[14,15] and thermolysin,[16] zinc enzymes that are closely related to LADH by virtue of a common tetrahedral geometry.[3] By analogy, we rationalized that such an approach could be extended to synthetic analogues of LADH by using a boron center to append a set of [NSS] donors. The use of a tetrahedral center as a point of attachment for the donor groups also serves to enforce a facial (rather than “T-shaped”) array of nitrogen and sulfur donors, thereby favoring a tetrahedral geometry at zinc. Indeed, a ligand comprising the requisite facial [NSS] donor array may be constructed by a sequence involving the initial synthesis of a [SS] donor followed by elaboration into a [NSS] donor (Scheme 1). Specifically, the reaction of LiBH$_4$ with 2 equivalents of

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[1] (a) Eklund, H.; Brändén, C.-I. In *Active Sites of Enzymes*; Jurnak, F. A., McPherson, A., Eds.; Biological Macromolecules and Assemblies, Vol. 3; Wiley: New York, 1987; Chapter 2. (b) *Zinc Enzymes*; Bertini, I., Luchinat, C., Maret, W., Zeppezauer M., Eds.; Progress in Inorganic Biochemistry and Biophysics, Vol. 1; Birkhäuser: Boston, MA, 1986; Chapters 28−35. (c) Pocker, Y. In *Metal Ions in Biological Systems*; Sigel, H., Sigel, A., Eds.; Dekker: New York, 1989; Vol. 25, pp 335−358.

[2] The oxidation of certain aldehydes is also catalyzed by LADH. See, for example: Olson, L. P.; Luo, J.; Almarsson, O.; Bruice, T. C. *Biochemistry* **1996**, *35*, 9782−9791.

[3] (a) Vallee, B. L.; Auld, D. S. *Acc. Chem. Res.* **1993**, *26*, 543−551. (b) Lipscomb, W. N.; Sträter, N. *Chem. Rev.* **1996**, *96*, 2375−2433.

[4] It should, however, be noted that spinach carbonic anhydrase[4a] and cytidine deaminase[4b,c] have been shown to bind zinc at their active sites *via* one histidine and two cysteine residues. (a) Bracey, M. H.; Christiansen, J.; Tovar, P.; Cramer, S. P.; Bartlett, S. G. *Biochemistry* **1994**, *33*, 13126−13131. (b) Betts, L.; Xiang, S.; Short, S. A.; Wolfenden, R.; Carter, C. W., Jr. *J. Mol. Biol.* **1994**, *235*, 635−656. (c) Xiang, S.; Short, S. A.; Wolfenden, R.; Carter, C. W., Jr. *Biochemistry* **1995**, *34*, 4516−4523.

[5] For example, only one compound containing a tetrahedral zinc center ligated by one nitrogen, one oxygen, and two sulfur donors is listed in the Cambridge Structural Database. See: McCleverty, J. A.; Morrison, N. J.; Spencer, N.; Ashworth, C. C.; Bailey, N. A.; Johnson, M. R.; Smith, J. M. A.; Tabbiner, B. A.; Taylor, C. R. *J. Chem. Soc., Dalton Trans.* **1980**, 1945−1957.

[6] (a) Kaptein, B.; Wang-Griffin, L.; Barf, G.; Kellog, R. M. *J. Chem. Soc., Chem. Commun.* **1987**, 1457−1459. (b) Kaptein, B.; Barf, G.; Kellogg, R. M.; Bolhuis, F. V. *J. Org. Chem.* **1990**, *55*, 1890−1901. (c) Koning, B.; Hulst, R.; Bouter, A.; Buter, J.; Meetsma, A.; Kellogg, R. M. *J. Chem. Soc., Chem. Commun.* **1997**, 1065−1066.

[7] Curtis, N. J.; Brown, R. S. *Can. J. Chem.* **1981**, *59*, 65−75.

[8] Brand, U.; Vahrenkamp, H. *Z. Anorg. Allg. Chem.* **1996**, *622*, 213−218.

[9] In view of the difficulty in synthesizing suitable tridentate [NSS] ligands, tetradentate [N$_2$S$_2$] ligands have also been invoked as models for the active site of LADH. See, for example: Anderson, O. P.; la Cour, A.; Findeisen, M.; Hennig, L.; Simonsen, O.; Taylor, L. F.; Toftlund, H. *J. Chem. Soc., Dalton Trans.* **1997**, 111−120.

[10] Dance, I. G. *Polyhedron* **1986**, *5*, 1037−1104.

[11] Similarly, {[S(C$_6$H$_4$)NHCH$_2$CH$_2$S]Zn}$_4$ has been structurally characterized as a tetramer. See ref 8.

[12] Several investigations concerned with systems in which the zinc coordination geometry bears little resemblance to the active site of LADH have also advanced our understanding of the mechanism of action of LADH. See, for example: (a) Shoner, S. C.; Humphreys, K. J.; Barnhart, D.; Kovacs, J. A. *Inorg. Chem.* **1995**, *34*, 5933−5934. (b) Kimura, E.; Shionoya, M.; Hoshino, A.; Ikeda, T.; Yamada, Y. *J. Am. Chem. Soc.* **1992**, *114*, 10134−10137. (c) Engbersen, J. F. J.; Koudijs, A.; van der Plas, H. C. *J. Org. Chem.* **1990**, *55*, 3647−3654.

[13] Texidor, F.; Escriche, L.; Casabó, J.; Molins, E.; Miravitlles, C. *Inorg. Chem.* **1986**, *25*, 4060−4062.

[14] Looney, A.; Han, R.; McNeill, K.; Parkin, G. *J. Am. Chem. Soc.* **1993**, *115*, 4690−4697.

[15] Kimblin, C.; Allen, W. E.; Parkin, G. *J. Chem. Soc., Chem. Commun.* **1995**, 1813−1815.

[16] Dowling, C.; Parkin, G. *Polyhedron* **1996**, *15*, 2463−2465.

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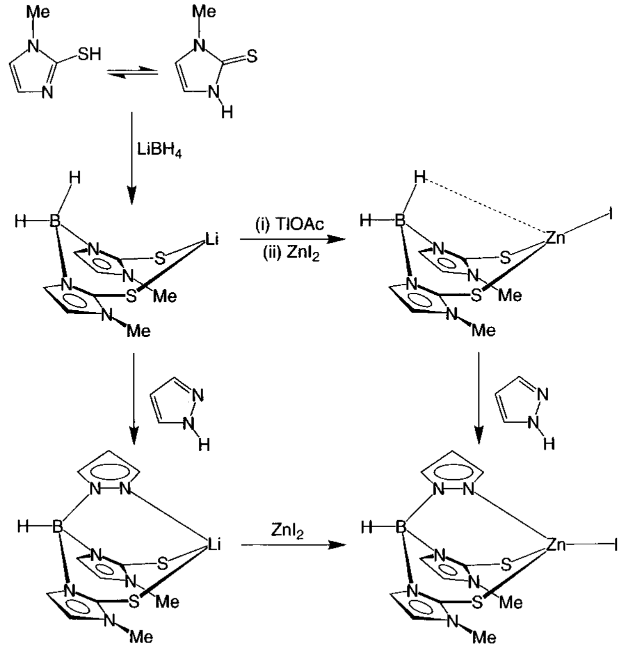
Scheme 1



methimazole (Htim^{Me})[17] yields Li[H_2B(tim^{Me})$_2$],[18] from which the desired [NSS] donor Li[HB(tim^{Me})$_2$pz] may be obtained by reaction with pyrazole. Subsequent transfer of the [NSS] ligand to zinc is readily achieved by treatment of Li[HB(tim^{Me})$_2$pz] with ZnI_2, thereby resulting in the formation of [HB(tim^{Me})$_2$pz]ZnI (Scheme 1). Alternatively, [HB(tim^{Me})$_2$pz]ZnI may be obtained *via* reaction of the three-coordinate zinc complex [H_2B(tim^{Me})$_2$]ZnI with pyrazole (Scheme 1).

The molecular structures of both [H_2B(tim^{Me})$_2$]ZnI[19] and [HB(tim^{Me})$_2$pz]ZnI[19] have been determined by X-ray diffraction, with the latter shown in Figure 1. Most importantly, the diffraction study demonstrates that [HB(tim^{Me})$_2$pz]ZnI does indeed exist as a mononuclear complex with a distorted tetrahedral coordination geometry about zinc (Table 1). Furthermore, the Zn−N and Zn−S bond lengths in [HB(tim^{Me})$_2$pz]ZnI are comparable to those within the enzyme, clearly supporting the notion that the [HB(tim^{Me})$_2$pz] ligand serves as a model for the groups binding zinc at the active site of LADH and its various derivatives. As an illustration, the coordination geometry about zinc in [HB(tim^{Me})$_2$pz]ZnI is compared with the active site of horse LADH-CNAD[20] in Table 1. In addition to LADH, the [HB(tim^{Me})$_2$pz] ligand also offers potential for investigating synthetic analogues of other zinc enzymes that utilize [NSS] coordination, *e.g.* spinach carbonic anhydrase and cytidine deaminase.[4]

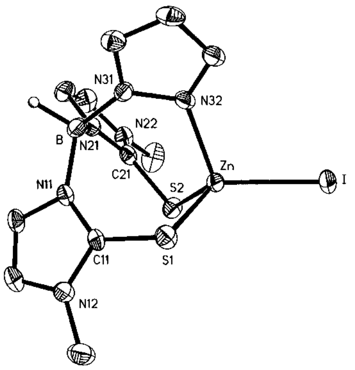


Figure 1. Molecular structure of [HB(tim^{Me})$_2$pz]ZnI.

Table 1. Comparison of the Zinc Coordination Environment in [HB(tim^{Me})$_2$pz]ZnI with That of the Active Site in LADH-CNAD

	[HB(tim^{Me})$_2$pz]ZnI[a]	LADH-CNAD[b]
d(Zn−S)/Å	2.320(2), 2.352(2)	2.3, 2.3
d(Zn−N)/Å	2.013(5)	2.0
d(Zn−X)/Å	2.5379(9)	2.2
S−Zn−S/deg	107.93(7)	125
S−Zn−N/deg	94.4(2), 109.2(2)	97, 106
S−Zn−X/deg	115.40(6), 117.82(5)	97, 98
N−Zn−X/deg	109.9(2)	136

[a] X = I. [b] X = $N5_N$ atom of the CNAD pyridine ring.

Finally, it is also of some interest to comment upon the fact that the [SS] donor ligand [H_2B(tim^{Me})$_2$] is capable of sustaining a planar three-coordinate zinc center in [H_2B(tim^{Me})$_2$]ZnI, a relatively uncommon coordination environment for zinc compared to four-coordination. The bonding at zinc is, however, supplemented by a secondary Zn···H−B interaction, with a zinc−hydride separation of 2.06(5) Å; such a value is at the long end of the range of Zn···H−B interactions listed in the Cambridge Structural database (1.78−1.98 Å)[21] but is significantly shorter than the sum of the van der Waals radii (2.59 Å).

In summary, the polyfunctional bis(thioimidazolyl)(pyrazolyl)hydroborato [NSS] donor ligand [HB(tim^{Me})$_2$pz]$^-$ has been prepared by the sequential reaction of $LiBH_4$ with methimazole and pyrazole. Importantly, the structural characterization of [HB(tim^{Me})$_2$pz]ZnI demonstrates that the ligand binds to zinc *via* its nitrogen and sulfur donors in a manner which resembles the active site of LADH and thereby provides a useful means for investigating fundamental aspects of the chemistry of the catalytic site of the enzyme in a well-defined synthetic analogue system.

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Supporting Information Available: ORTEP drawings, tables of analytical, spectroscopic, and crystallographic data, and text giving preparative details for [H_2B(tim^{Me})$_2$]ZnI and [HB(tim^{Me})$_2$pz]ZnI (19 pages). Ordering information is given on any current masthead page.

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(17) Methimazole = 2-mercapto-1-methylimidazole. In this paper we use the term "thioimidazolyl" and the abbreviation tim^{Me} to represent the [$MeC_3N_2H_2(S)$] fragment.

(18) Reglinski recently reported the synthesis of the tris(thioimidazolyl)hydroborato ligand by reaction of $NaBH_4$ with excess methimazole. See: Garner, M.; Reglinski, J.; Cassidy, I.; Spicer, M. D.; Kennedy, A. R. *J. Chem. Soc., Chem. Commun.* **1996**, 1975−1976.

(19) [H_2B(tim^{Me})$_2$]ZnI is monoclinic, $P2_1/n$ (No. 14), $a = 11.362(2)$ Å, $b = 9.937(2)$ Å, $c = 13.483(3)$ Å, $\beta = 110.31(2)°$, $V = 1427.6(5)$ Å3, $Z = 4$. [HB(tim^{Me})$_2$pz]ZnI is monoclinic, $P2_1/n$ (No. 14), $a = 11.833(2)$ Å, $b = 12.699(2)$ Å, $c = 12.353(2)$ Å, $\beta = 99.60(1)°$, $V = 1830.3(5)$ Å^3, $Z = 4$.

(20) CNAD is an isosteric C-glycosidic analogue of NADH containing a neutral pyridine ring which is a potent inhibitor of LADH. See: Li, H.; Hallows, W. H.; Punzi, J. S.; Pankiewicz, K. W.; Watanabe, K. A.; Goldstein, B. M. *Biochemistry* **1994**, *33*, 11734−11744.

(21) The mean value is 1.88[6] Å.