CHEMCOMM
Communication
www.rsc.org/chemcomm

The best structural model of ADH so far: a pyrazolylbis(thioimidazolyl)borate zinc ethanol complex

Jan Seebacher, Mouhai Shu and Heinrich Vahrenkamp*

Institut für Anorganische und Analytische Chemie der Universität Freiburg, Albertstr. 21, D-79104 Freiburg, Germany.

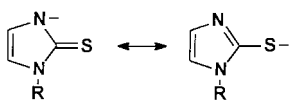
Received (in Cambridge, UK) 8th January 2001, Accepted 17th April 2001
First published as an Advance Article on the web 15th May 2001

The new *N,S,S* ligand $[HB(tim^{o\text{-}An})_2(pz^{Ph,Me})]^-$ (L^1) was combined with zinc perchlorate and ethanol to form the complex $[L^1{\cdot}Zn(ethanol)]ClO_4$ which is the first zinc complex in which the *N,S,S* ligation of zinc and the attachment of ethanol occurring in the active center of horse liver alcoholdehydrogenase (ADH) have been reproduced; the structural details of the $ZnNS_2O$ coordination in the complex and in DMSO-inhibited ADH are in reasonably good agreement.

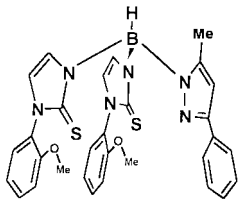
Both the function and the coordination environment of zinc in horse liver alcoholdehydrogenase, ADH,[1,2] are unusual. Zinc supports, but does not participate in, a redox reaction, *i.e.* the hydride transfer interconverting alcohol and aldehyde; and in the resting enzyme zinc is in an uncharged state, being bound to one histidine and two cysteinate residues. It can be assumed that the latter is the prerequisite for the former, making it possible that in the enzymatic environment the alcohol is bound to zinc as an alkoxide and that its ß-hydrogens are labilized for removal as H^- by NAD^+.

These unusual properties have made it impossible until now to build coordination compounds of zinc which correctly model the coordination environment of the metal[3–5] or which show a reasonable catalytic activity for hydride transfer to NAD^+/NADH systems.[6–8] Most of the *N,S,S* ligands which were designed and used for this purpose have too strong a tendency to form oligonuclear thiolate-bridged zinc complexes, and the fixation of the very labile Zn–OR function in mononuclear zinc alkoxides has required the use of coligands which suppress the desired catalytic activity of zinc. The closest approximations to structural models[9–11] were achieved by Parkin *et al.*[4] and ourselves.[5] Parkin's group prepared tetrahedral L·Zn–X complexes of a new *N,S,S* ligand L. We prepared tetrahedral $(RS)_2Zn(N,O)$ complexes using pyridine-derived alcohols or aldehydes as *N,O* ligands. A realistic functional modelling, *i.e.* by using NAD^+ derivatives as hydride acceptors, has been tried in only a few cases and with little success.[8,12,13]

The work described in this communication was triggered by a report by Reglinski *et al.*,[14] which describes the simple synthesis of tris(thioimidazolyl)borate ligands. In these ligands the thioimidazolyl sulfur donors can be considered 'tame' thiolate donors according to the following resonance formulation:



In agreement with this there is no sulfur bridging in metal complexes of thioimidazolylborate ligands and such ligands are quite reliable tetrahedral enforcers.[4,7,14–16] Furthermore, their synthesis allows the incorporation of pyrazolyl functions as mixed pyrazolylthioimidazolylborates with *N,S,S* or *N,N,S* donor sets.[4,15,16] Having realized this, it was a matter of varying the substituents on the pyrazole and thioimidazole groups of a pyrazolylbis(thioimidazolyl)borate[16] (*i.e.* a *N,S,S* ligand) in order to generate an appropriate electronic and geometrical environment for a ligated zinc ion to accommodate an alcoholic substrate in an ADH model complex.



Ligand L^1 was found to fulfil these conditions. Its constituent 1-(*o*-anisyl)-2-thioimidazole† is accessible from 2-methoxyaniline and KSCN by the established procedure.[17] The reaction between two equivalents of the thioimidazole and one equivalent each of 3-phenyl-5-methylpyrazole and KBH_4 in boiling toluene leads to very good yields of $K[L^1]$.† The reaction between $K[L^1]$ and $Zn(ClO_4)_2{\bullet}6H_2O$ in non-dehydrated ethanol produces the raw complex **1** in nearly quantitative yield.‡ Crystals of **1** for the structure determination were obtained by slow evaporation from ethanol.†

$$[HB(tim^{o\text{-}An})_2(pz^{Ph,Me})Zn(C_2H_5OH)]\,ClO_4{\bullet}C_2H_5OH$$

1

Complex **1** fulfils the conditions to be a close structural model of ADH. It bears a tridentate *N,S,S* ligand and an ethanol molecule comprising a $ZnNOS_2$ coordination pattern as in the enzyme and its pentafluorobenzyl alcohol-inhibited derivative.[2] The structure determination§ (see Fig. 1) has shown the complex to be tetrahedral to a good approximation. The alcohol ligand is embedded between the three aromatic substituents of

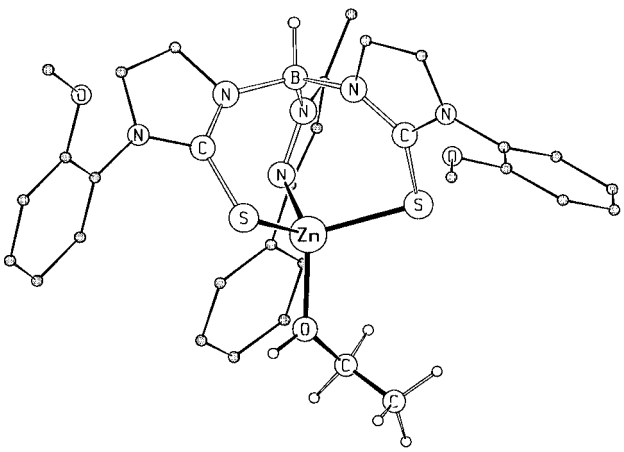


Fig. 1 Molecular structure of the cation of **1**. The OH hydrogen atom, which was not localized crystallographically, was placed on a reasonable position along the hydrogen bond to the attached second ethanol molecule.

 DOI: 10.1039/b100281n

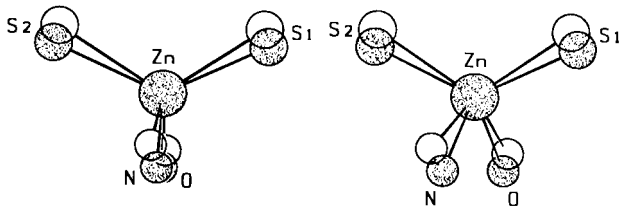


Fig. 2 Superpositions of the $ZnNOS_2$ cores of **1** (open circles) and DMSO-inhibited ADH[2] (filled circles). Left: ZnS_2 in the plane of the drawing, right: rotated by 20° about a vertical axis.

Table 1 Comparison of the $ZnNOS_2$ units in **1** and in DMSO-inhibited ADH[2]

	1	ADH•DMSO
Zn–N/Å	2.012(3)	2.02
Zn–O/Å	1.970(3)	2.13
Zn–S/Å	2.282(1), 2.314(1)	2.19, 2.25
N–Zn–O/°	116.8(1)	94.2
N–Zn–S/°	100.2(1), 111.7(1)	107.4, 113.1
O–Zn–S/°	105.4(1), 109.8(1)	102.5, 103.3
S–Zn–S/°	113.2(1)	129.4

L[1]. It is attached to a second ethanol molecule by a hydrogen bond, sharing this property with Parkin's tris(thioimidazolyl)borate–zinc–CH_3OH•CH_3OH complex.[7] It seems that the electronic 'saturation' of the zinc ion by the soft sulfur donors and the hydrophobicity of the aromatic environment provide the driving force for the preferred attachment of the less polar alcohol ligand, rather than the water or hydroxide ligands which are present in the reaction solution and which become bound to zinc in similar preparations of zinc complexes with tridentate *N,N,N* or *N,N,O* environments.[18]

The bond lengths and bond angles of **1** are very similar to those in Parkin's S_3Zn–CH_3OH complex.[7] Moreover they agree satisfactorily with those in the ADH structure of highest resolution (1.8 Å), *i.e.* that of the DMSO-inhibited enzyme.[2] Fig. 2 and Table 1 give the details for the $ZnNOS_2$ units. Both units can be described as close to tetrahedral, as can be seen in Fig. 2 by the perpendicular arrangements of the SZnS and NZnO planes. Almost all differences in bond lengths and bond angles are within an acceptable range. The major distortions lie in the enzyme's very small N–Zn–O and very large S–Zn–S angles and in the difference between the Zn–O('substrate') bond lengths.

In summary, a new thioimidazolylborate based *N,S,S* ligand has been prepared and used to obtain a zinc complex which binds ethanol on its fourth coordination site. This corresponds to the first step in the catalytic cycle of horse liver alcoholdehydrogenase (ADH), the incorporation of ethanol within the active center which contains *N,S,S* ligated zinc. The resulting complex **1** is the first model complex that reproduces both the *N,S,S* ligation and the alcohol binding.

This work was supported by the Deutsche Forschungsgemeinschaft.

Notes and references

† The new compounds were characterized by elemental analyses. Complex **1** shows the OH absorption in the IR spectrum as a broad band centered at 3150 cm^{-1} which is typical for O–H···O hydrogen bonding. ^1H NMR ($CDCl_3$): δ 1.18 (t, *J* 7.0 Hz, 6H, ethyl-CH_3), 2.50 (s, 3H, pz-CH_3), 3.61 (q, *J* 7.0 Hz, 2H, ethyl-CH_2), 3.62 (q, *J* 7.0 Hz, 2H, ethyl-CH_2), 3.82 (s, 6H, OCH_3), 6.40 (s, 1H, pz-CH), 6.94 (d, *J* 2.1 Hz, 2H, tim-CH), 7.02 (d, *J* 2.1 Hz, 2H, tim-CH), 7.0–7.6 (m, 13H, aromatic).

‡ **Caution**: perchlorate salts of metal–organic compounds should be handled with care and only in small amounts.

§ *Crystal data* for $C_{34}H_{42}BClN_6O_9S_2Zn$: M = 854.5, triclinic, space group $P\bar{1}$ (no. 2), a = 10.962(1), b = 14.131(2), c = 14.834(2) Å, α = 101.011(2), β = 109.568(2), γ = 94.234(2)°, V = 2101.6(4) Å^3, T = 200(2) K, Z = 2, λ(Mo-Kα) = 0.71073 Å, Bruker Smart CCD diffractometer. 19203 reflections were collected and subjected to an empirical absorption correction (SADABS). The final R index for [$I > 2\sigma(I)$] with 5057 reflections was R_1 = 0.061. CCDC 156428. See http://www.rsc.org/suppdata/cc/b1/b100281n/ for crystallographic data in .cif or other electronic format.

1 For the function of ADH, *cf.* H. Eklund, B. Nordström, E. Zeppezauer, G. Söderlund, I. Ohlsson, T. Boiwe, B. O. Söderberg, O. Tapia, C. I. Brändén and A. Akeson, *J. Mol. Biol.*, 1976, **102**, 27.
2 For recent structure determinations of ADH, *cf.* S. Ramaswamy, H. Eklund and B. V. Plapp, *Biochemistry*, 1994, **33**, 5230 ($C_6F_5CH_2OH$ bound to zinc); S. Al-Karadaghi, E. S. Cedergren-Zeppezauer, S. Hövmoller, K. Petratos, H. Terry and K. Wilson, *Acta Crystallogr., Sect. D.*, 1994, **50**, 793 (dimethyl sulfoxide bound to zinc).
3 For references on structural modelling see refs. 4 and 5.
4 C. Kimblin, T. Hascall and G. Parkin, *Inorg. Chem.*, 1997, **36**, 5680.
5 B. Müller, A. Schneider, M. Tesmer and H. Vahrenkamp, *Inorg. Chem.*, 1999, **3**, 1900.
6 For references on functional modelling, see refs. 7 and 8.
7 C. Kimblin, B. M. Bridgewater, D. G. Churchill and G. Parkin, *Chem. Commun.*, 1999, 2301.
8 R. Walz and H. Vahrenkamp, *Inorg. Chim. Acta*, 2001, **314**, 58.
9 *N,S,S* ligands with sulfur in the form of thioether functions, which can form L•Zn–X complexes, have been described by Riordan[10] and Berreau.[11]
10 S. J. Chiou, J. Innocent, C. G. Riordan, K. C. Lam, L. Liable-Sands and A. Rheingold, *Inorg. Chem.*, 2000, **39**, 4347.
11 L. M. Berreau, R. A. Allred, M. M. Makowska-Grzyska and A. Arif, *Chem. Commun.*, 2000, 1423.
12 B. Kaptein, L. Wang-Griffin, G. Barf and R. M. Kellogg, *J. Chem. Soc., Chem. Commun.*, 1987, 1457.
13 A. Shirra and C. J. Suckling, *Tetrahedron Lett.*, 1975, **38**, 3323.
14 M. Garner, J. Reglinski, I. Cassidy, M. D. Spicer and A. R. Kennedy, *Chem. Commun.*, 1996, 1975; J. Reglinski, M. Garner, I. D. Cassidy, P. A. Slavin, M. D. Spicer and D. R. Armstrong, *J. Chem. Soc., Dalton Trans.*, 1999, 2119; J. Reglinski, M. D. Spicer, M. Garner and A. R. Kennedy, *J. Am. Chem. Soc.*, 2000, **121**, 2317.
15 C. Kimblin, B. M. Bridgewater, T. Hascall and G. Parkin, *J. Chem. Soc., Dalton Trans.*, 2000, 1267; C. Kimblin, B. M. Bridgewater, D. G. Churchill, T. Hascall and G. Parkin, *Inorg. Chem.*, 2000, **39**, 4240.
16 R. Walz, M. Tesmer, B. Wu, M. Shu and H. Vahrenkamp, unpublished results.
17 J. Kister and G. Assef, *Can. J. Chem.*, 1979, **57**, 813.
18 H. Vahrenkamp, *Acc. Chem. Res.*, 1999, **32**, 589.