Metal Complexes that Model the Active Site of Liver Alcohol Dehydrogenase

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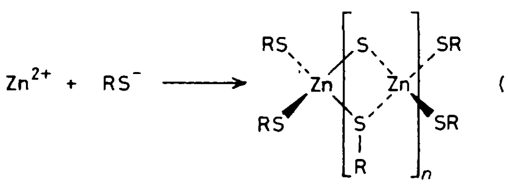
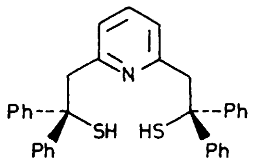
Two approaches to mimic the active site zinc atom of liver alcohol dehydrogenase are described.

The NAD$^+$/NADH-dependent enzyme, horse liver alcohol dehydrogenase (HLADH; E.C. 1.1.1.1), contains two types of zinc ion: one is involved at the active site, whereas the other seems to have exclusively a structural function. The zinc ion at the active site is tetrahedrally ligated by two thiolates from cysteine, and an imidazole from histidine. The fourth ligand position is occupied by solvent or substrate. The 'structural' zinc ion is ligated by four thiolates.[1]

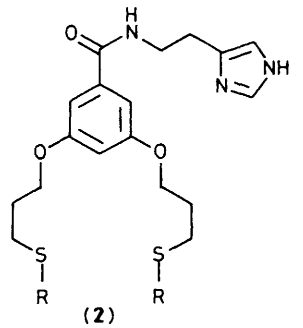
We have attempted to prepare complexes of Zn^{2+} and Co^{2+} (see later) that mimic this active site co-ordination. This work



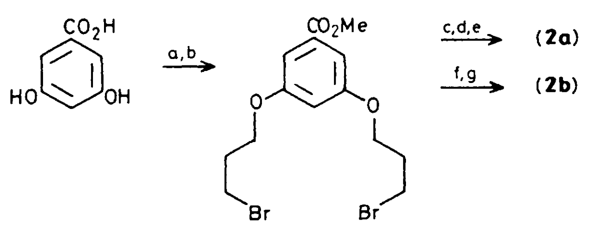
(i)



(1)



(2)
a; R = H
b; R = Me



Scheme 1. *Reagents:* a, MeOH/H$^+$ (93%); b, Br[CH$_2$]$_3$Br/Me$_2$CO/K$_2$CO$_3$ (85%); c, (H$_2$N)$_2$CS, then KOH (98%); d, MeOH/H$_2$SO$_4$ (100%); e, histamine/NH$_4$Cl (89%); f, MeSH/KOH/MeOH (60%); g, histamine/NH$_4$Cl (62%).

is motivated by the consideration that many studies of 1,4-dihydropyridines obtained synthetically as NADH mimics have depended on the use in non-aqueous solvents of stoicheiometric quantities of very hard electrophiles like Mg^{2+}.[2] The obtainment of catalytically active systems has remained elusive;[2e] we hoped a closer approach to the enzyme would provide the desired catalytic activity. Some catalytic activity has been observed recently for certain lanthanide complexes.[3]

The co-ordination found about the active site zinc ion is uncommon. The majority of known examples of zinc thiolates are oligomeric owing to the pronounced tendency of thiolate to act as a bridging ligand.[4]† This is represented schematically as reaction (i).

The ligands (**1**) and (**2a** and **b**) were prepared as models for the desired triligation. The former has been described recently by Holm *et al.*,[6] who used steric hindrance as an aid in stabilizing molybdenum complexes. The ligands (**2a** and **b**) were synthesized as shown in Scheme 1.‡ The introduction of histamine was eventually accomplished by use of a melt composed of the ester, histamine,[7] and ammonium chloride.

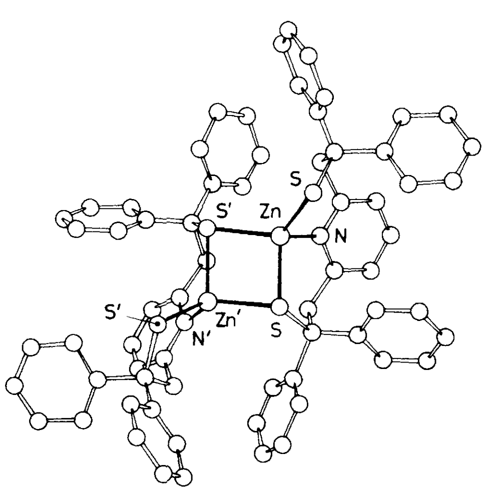


Figure 1. Computer drawing of crystal structure of Zn·(**1**) (hydrogen atoms omitted); selected bond distances and angles: Zn–S(endocyclic) = 2.425(4), Zn–S(exocyclic) = 2.268(4), Zn–S′ = 2.350(4), Zn–N = 2.081(11) Å; S$_{\text{endo}}$–Zn–S′ = 94.4(1), Zn–S–Zn′ = 83.0(1), S–Zn–S = 114.7(1), S$_{\text{exo}}$–Zn–S = 121.1(1), N–Zn–S$_{\text{exo}}$ = 103.6(3), N–Zn–S$_{\text{endo}}$ = 121.6(3)°; R = 0.057. The poor quality of the crystal leads to difficulties in refinement.

The Co^{2+} derivatives of HLADH, obtained by extraction of Zn^{2+} from the crystalline enzyme followed by replacement of Co^{2+}, are nearly as active as the native enzyme.[8—10] For this reason, attempts were made also to prepare the Co^{2+} complexes of (**1**) and (**2a** and **b**).

Despite the steric hindrance Zn·(**1**), which was obtained as crystals of rather poor quality on treatment of (**1**) with Zn(NO$_3$)$_2$ in acetonitrile, is dimeric (Figure 1).§ Two of the thiolates act as bridging ligands. Even in dilute solution Zn·(**1**) is dimeric, as seen from the non-equivalence of the ligand CH$_2$ signals in the ^{1}H n.m.r. spectrum, consistent with C_{2h} symmetry.

The Co^{2+} complex of (**1**) is barely soluble in acetonitrile and has not been obtained crystalline. The ligand (**2a**), which should wrap around the metal from three sides, was more promising. Titration of (**2a**)·HCl (50% EtOH/H$_2$O) revealed breaks at the anticipated pK_a values of 6.4 (imidazole) and 9.7 and 10.4 (two SH groups). Complexation of Zn(NO$_3$)$_2$ or

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† A monomeric complex of Zn^{2+} tetraligated with two 1-amino-2-methylpropane-2-thiolates as ligands has been described, however.[5] The steric hindrance about the ligands is apparently sufficient to prevent dimerization in this case.

‡ Consistent analyses and/or exact mass measurements were obtained for all new compounds. The structures are supported by spectroscopic data.

§ *Crystal data:* (C$_{33}$H$_{27}$NS$_2$Zn)$_2$ · CH$_3$CN, M = 1175.24, orthorhombic, a = 17.963(10), b = 27.433(3), c = 11.469(4) Å, D_c = 1.34 g cm^{-3}, U = 5651.7 Å^3, λ = 0.7107, μ(Mo-K_α) = 10.63 cm^{-1}, F(000) = 2440, space group $Aba2$, Z = 4 (No. 41). Nonius CAD4 diffractometer, interfaced to a PDP-11/23 computer; graphite-monochromated Mo-K_α radiation, ω–2θ scan, $1 \leq \theta \leq 25$; 2612 unique reflections, 1758 with $I \geq 3\,\sigma(I)$, 25 reflections with $8.7° \leq \theta \leq 17.6°$ used to refine cell parameters; crystal dimensions 0.4 × 0.45 × 0.35 mm. The heavy atoms Zn and S were found by direct methods. The other non-H atoms were revealed from succeeding difference maps. Full-matrix least-squares refinement of F converged to a final R = 0.057 (R_w = 0.076) (with unit weights), using anisotropic temperature factors. Attempts to locate the H atoms from a final difference map failed. No absorption corrections were applied. All computations were performed using CAD4SDP-programs (Enraf-Nonius-Frenz & Associates). Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

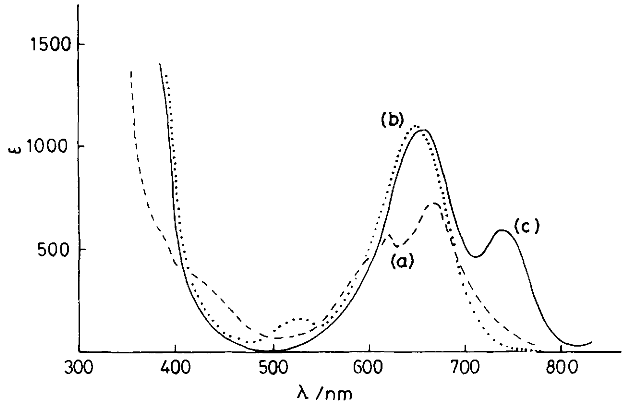
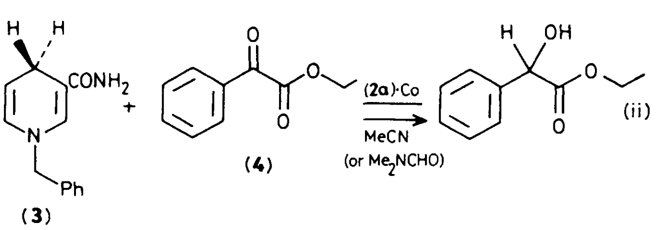


Figure 2. The visible spectrum (a) of Co·(**2a**) compared with those for horse liver alcohol dehydrogenase in H_2O (b) with catalytic Zn replaced by Co and (c) with structural Zn replaced by Co.



$Co(NO_3)_2$ with (**2a**)·HCl is accompanied by release of three H^+ (titration), indicative of a neutral complex. The Zn^{2+} complex is amorphous and insoluble, but the non-crystalline Co^{2+} complex is slightly soluble in dimethylformamide (*ca.* 8 × 10^{-4} M at 20 °C); the solubility in MeCN is even less ($<10^{-5}$ M at 70 °C). The visible spectra of (**2a**)·Co, 'active site' Co^{2+} in HLADH,[8,9a—c] and 'structural Co^{2+}' in HLADH[8,9a,b] are shown in Figure 2 (a—c, respectively). The long wavelength absorption of (**2a**)·Co agrees with that of Co^{2+} ligated in the active site of HLADH [Figure 2(b)],¶ but not with that of Co^{2+} ligated by four thiolates [Figure 2(c)], which has a band at 740 nm, characteristic of tetrathiolate-co-ordinated Co^{2+}.[9a]** The long-wavelength band, consisting of two maxima at 586 and 683 nm, shows a remarkable resemblance to recently published spectra of Co·HLADH enzyme in the presence of cofactor.[9c] The u.v. spectrum (not illustrated) of (**2b**)·$Co(NO_3)_2$, wherein the thiolates are alkylated, has a maximum in MeOH at 510 nm, close to that of Co^{2+}(imidazole) complexes.[12] The sulphides do not act well as ligands, consistent with previous observations.[2e]

In the test reaction illustrated [reaction (ii)], wherein (**4**) is reduced by (**3**) in the presence of (**2a**)·Co (70 °C in MeCN or Me_2NCHO), turnovers of 50—100 were obtained for conversions of about 10%.†† The relatively low solubility of the complex poses the greatest problem for obtaining high chemical conversions. Attempts to observe complexation of (**4**) (max. concn. 0.25 M) with (**2a**)·Co in Me_2NCHO or MeCN failed; no changes in the visible spectra were observed. The complexes derived from (**1**) are, in our hands, not catalytically active, although at 70 °C in Me_2NCHO or MeCN moderate concentrations of (**1**)·Zn and (**1**)·Co can be obtained.

In summary, we believe that a Co^{2+} model of active site metal in alcohol dehydrogenase has been prepared; it exhibits catalytic activity. $Mg(ClO_4)_2$ and $ZnCl_2$, on the other hand, are activators but not catalysts. Attempts to improve the system, including synthesis of a soluble and catalytically active monomeric triligated Zn^{2+} complex, are in progress.

The crystallographic determinations were carried out by Dr. F. van Bolhuis in this department.

Received, 9th February 1987; Com. 165

References

1 (a) C. I. Branden, H. Jornvall, H. Ekund, B. Furugren, in 'The Enzymes,' vol. XI, 3rd edn., ed. P. D. Boyer, Academic Press, New York, 1975, pp. 104—190; (b) H. Eklund, B. Nordstrom, E. Zeppezauer, G. Soderlund, I. Ohlsson, T. Boiwe, B-O. Soderberg, O. Tapia, C-I. Branden, and A. Akeson, *J. Mol. Biol.*, 1976, **102**, 27.

2 (a) See, for example, A. G. Talma, P. Jouin, J. G. de Vries, C. B. Troostwijk, G. H. Werumeus Buning, J. K. Waninge, J. Visscher, and R. M. Kellogg, *J. Am. Chem. Soc.*, 1985, **107**, 3981; (b) D. J. Creighton and D. S. Sigman, *ibid.*, 1971, **93**, 6314; (c) D. J. Creighton, J. Hajdu, and D. S. Sigman, *ibid.*, 1976, **98**, 4619; (d) K. Watanabe, R. Kawaguchi, and H. Kato, *Chem. Lett.*, 1978, 255; (e) N. J. Curtis and R. S. Brown, *Can. J. Chem.*, 1981, **59**, 65.

3 S. Zehani and G. Gelbard, *J. Chem. Soc., Chem. Commun.*, 1985, 1162.

4 (a) J. Boersma in 'Comprehensive Organometallic Chemistry,' ed. G. Wilkinson, Pergamon, Oxford, 1982, vol. 2, pp. 823—851; (b) R. H. Holm and M. J. O'Connor, *Prog. Inorg. Chem.*, 1971, **14**, 241.

5 B. Cohen, D. Mastropaolo, J. A. Potenza, and H. J. Schugar, *Acta Crystallogr., Sect. B.*, 1978, **34**, 2859.

6 (a) J. M. Berg and R. H. Holm, *J. Am. Chem. Soc.*, 1985, **107**, 917; (b) R. H. Holm and J. M. Berg, *Acc. Chem. Res.*, 1986, **19**, 363.

7 F. L. Pyman, *J. Chem. Soc.*, 1912, 543.

8 (a) M. Zeppezauer, I. Andersson, H. Dietrich, M. Gerber, W. Maret, G. Schneider, and H. Schneider-Bernlohr, *J. Mol. Catal.*, 1984, **23**, 377; (b) A. J. Sytkowski and B. L. Vallee, *Proc. Natl. Acad. Sci. USA*, 1976, **73**, 344.

9 (a) G. Schneider, H. Eklund, E. Cedergren-Zeppezauer, and M. Zeppezauer, *Proc. Natl. Acad. Sci. USA.*, 1983, **80**, 5289; (b) W. Maret, I. Andersson, H. Dietrich, H. Schneider-Bernlohr, R. Einarsson, and M. Zeppezauer, *Eur. J. Biochem.*, 1979, **98**, 501; (c) C. Sartorius, M. Gerber, M. Zeppezauer, and M. F. Dunn, *Biochemistry*, 1987, **26**, 871.

10 See also, for example: (a) M. W. Makinen, W. Maret, and M. B. Yim, *Proc. Natl. Acad. Sci. USA*, 1983, **80**, 2584; (b) M. W. Makinen and M. B. Yim, *ibid.*, 1981, **78**, 6221.

11 D. Mastropaolo, J. A. Thich, J. A. Potenza, and H. H. Schugar, *J. Am. Chem. Soc.*, 1977, **99**, 424.

12 J. Reedijk, *Recl. Trav. Chim. Pays-Bas.*, 1972, **91**, 507, and references therein.

¶ Also the spectrum of Co^{2+} ligated by two 1-amino-2-methylpropane-2-thiol shows a visible absorption band with $\lambda_{max.}$ 650 nm.

** Spectral data for Co^{2+} thiolate complexes are summarized in ref. 9b.

†† Reactions were carried out in MeCN at 70 °C; [1,4-dihydropyridine] and [(**4**)] 10^{-1} M; [catalyst] 10^{-4} M; the catalyst is not entirely in solution even at this concentration. In the absence of catalyst under otherwise identical conditions, 2—3% reduction occurs.