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Abstract

The coordination chemistry of the zinc ion in the active site of alcohol dehydrogenase has been studied by the ab initio Hartree-Fock method. Geometry optimisations were performed using analytical gradients and basis sets of double zeta quality. Correlation effects were included at the MP2 level. The active site was modelled by $Zn(HS)_2XL(H2O)_{0-2}$, where X denotes ammonia or imidazole and L denotes water, methanol, ethanol or the corresponding aldehydes or anions. It is shown that with uncharged L-ligands the four-coordinate complexes are about 20, 17 and 40 kJ/mole more stable than the corresponding three-, five- and six-coordinate complexes, respectively. If the L-ligand is negatively charged only the four-coordinate complexes are stable. These results suggest that the active-site zinc ion in alcohol dehydrogenase prefers a coordination number of four during the catalytic reaction, especially when the non-protein ligand is negatively charged. Ligand exchange at the zinc ion is likely to proceed by an associative mechanism with intermittent formation of a five-coordinate complex. The results lend no support to mechanistic proposals attributing an important catalytic role to a negatively charged five-coordinate hydroxide or alkoxide ligand.

Introduction

Alcohol dehydrogenase (EC 1.1.1.1) catalyses the reversible oxidation of primary and secondary alcohols using NAD^+ as coenzyme [1-3]. The active site of the enzyme contains a zinc ion that is essential for catalysis. Crystallographic studies [4-6] have shown that this zinc ion is bound by the enzyme through one histidine and two cysteine residues. In free enzyme, the catalytic zinc ion appears to be tetrahedrally coordinated with a water molecule (or hydroxide ion, depending on pH) as the fourth first-sphere ligand.

Kinetic pH-dependence studies of individual reaction steps in the mechanism of action of alcohol dehydrogenase led Kvassman and Pettersson [7] to propose that substrates are bound to catalytic zinc in the enzyme-coenzyme complexes with displacement of zinc-bound water. According to their mechanistic proposal, deprotonation of the enzyme-bound alcohol represents a catalytically crucial step facilitating subsequent hydride transfer from the substrate to NAD^+. Alternative proposals have been put forward, however, according to which five-coordinate intermediates may play an essential role during catalysis [8-14]. In particular, a five-coordinate hydroxide ion has been suggested to facilitate hydride transfer from zinc-bound alcohols to NAD^+.

Crystallographic studies of the enzyme and its binary or ternary complexes with coenzyme and different substrates have shown that the catalytic zinc ion as a rule exhibits four-coordination [4-6] and several spectroscopic investigations have provided evidence in the same direction [15-17]. On the other hand, there is also strong crystallographic and spectroscopic evidence showing that binding to zinc of certain bidentate inhibitors is five-coordinate [18-20]. Furthermore, spectroscopic studies of metal-substituted alcohol dehydrogenase have indicated that several binary and ternary complexes may be five-coordinate [11-13,20-22]. The kinetic evidence is also scattered and has been taken to favour four-coordination [3,23], as well as five-coordination [8-10], of zinc in the catalytically productive ternary-complexes.

The present investigation was undertaken to obtain mechanistically relevant information on the coordination number of the catalytic zinc ion in alcohol dehydrogenase by

a quantum chemical approach. Geometry optimisations have been performed on a model of the active site with a varied number of different non-protein ligands of biological interest. The results would seem to significantly advance our understanding of the factors governing the coordination structure of the catalytic metal site in free enzyme, as well as during catalysis, and hence provide several inferences of interest regarding the mechanism of action of alcohol dehydrogenase.

Methods

Zn(HS)$_2$XL(H$_2$O)$_{0-2}$, where X denotes NH$_3$ or imidazole and L denotes water, methanol, ethanol or the corresponding aldehydes or anions, was chosen as a model of the active site of alcohol dehydrogenase. The full geometry of these models were optimized until the change in energy and the coordinates were below 10^{-6} Hartree and 10^{-3} Bohr, respectively, using analytical gradient methods in the self-consistent field (SCF) Hartree-Fock approximation. Electron correlation effects were included through second order perturbation theory (Møller-Plesset, MP2), by single point calculations on the optimized structure. Thermodynamic corrections, i.e. the translational, rotational and vibrational (including zero-point energy) contributions to the Helmholtz free energy at 300 K and 101.3 kPa pressure, were calculated from the coordinates, atomic masses and vibrational frequencies of the systems, the latter uniformly scaled by a factor 0.9 [24]. All reported energies are total free energy (SCF + MP2 + thermodynamic corrections) unless otherwise stated. Several starting structures were tested to reduce the risk of being trapped in local minima and no symmetry restrictions were imposed. The Hessian matrix of each structure was examined to ensure that it represents a true potential minimum. The basis sets were of double zeta quality for all atoms (H: (31); C, N, O: (5111/31); S: (521111/4111); Zn: (62111111/51111/311)) [25]. All calculations were performed using the semi-direct program package TURBOMOLE [26] on an IBM RISC RS/6000 (530H) workstation.

Results and Discussion

Choice of the model system

The optimized geometry of Zn(imidazole)(SH)$_2$(H$_2$O) is shown in Figure 1a. The geometry of this complex is similar to those previously reported for other model systems of the active site of alcohol dehydrogenase examined with smaller basis sets and ammonia instead of imidazole [27-28]. The geometry is also very similar to the crystallographic structure of the active site of alcohol dehydrogenase [5], shown in Figure 1b, with a root-mean-square deviation between the two structures of 27 pm. The model system may, therefore, be expected to provide reliable inferences as to the coordination around the zinc ion. The similarity in geometry further indicates that the enzyme does not impose any major geometric constraints on the zinc ligands, which justifies the use of full geometry optimisations and indicates that to a first approximation no more enzyme residues need to be included in the model.

Fig 1c shows the optimized geometry of Zn(NH$_3$)(SH)$_2$(H$_2$O), where imidazole in the model above has been exchanged for ammonia. The charge distribution of these two complexes (according to a Mulliken population analysis) is similar, as is the geometry; the root-mean-square deviation is only 8.4 pm. To reduce computational costs, therefore, most calculations were performed using ammonia as a model for the histidinyl zinc ligand. Critical structures were examined also with imidazole.

The energy, coordination number and zinc-ligand distances of all examined complexes are recorded in Table I.

The coordination number of the model system

In order to obtain information on the relative stability of four- and five-coordinate complexes, a fifth ligand was added to the system in Fig. 1c. This ligand may be placed in the first- or second coordination sphere of the zinc ion. With uncharged ligands, there are several

stable structures (local minima) of each kind. Fig 2a shows the optimized geometry of the most stable five-coordinate structure of $Zn(NH_3)(SH)_2(H_2O)_2$, a quasi-trigonal bipyramidal structure with the two water molecules as axial ligands. Fig. 2b shows the most stable four-coordinate structure of the same complex, with one water ligand in the second coordination sphere of the zinc ion, hydrogen bonded to a sulphur ion and a hydrogen of the first-sphere water. Analogous structures were obtained with all combinations of uncharged water, alcohol and aldehyde ligands, as well as when ammonia was exchanged for imidazole.

In Table II, the energy of these four- and five-coordinate structures with different ligands are compared. It can be seen that the four-coordinate structures are 16-22 kJ/mole more stable than the corresponding five-coordinate structures (for $Zn(imidazole)(SH)_2(H_2O)_2$, the four-coordinate structure is 22.7 kJ/mole more stable than the five-coordinate one at the SCF level). With two different ligands (e.g. water and methanol) there are two possible four-coordinate complexes, depending on which ligand is in the first coordination sphere and which is in the second. It can be seen from Table II that with one alcohol and one water ligand, the structure with the alcohol in the first coordination sphere is most stable, while with aldehydes the structure with the aldehyde in the second sphere is much more stable. A stable planar three-coordinate structure of the complex in Fig. 2 with the two water ligands in the second coordination sphere of the zinc ion, was also examined. This structure, however, was appreciably less stable than the corresponding four- and five-coordinate complexes (43.0 and 27.1 kJ/mole).

For $Zn(NH_3)(SH)_2(H_2O)_3$, three different kinds of complexes were compared: five- and four- coordinate structures with one or two water molecules in the second coordination sphere and a six-coordinate octahedral structure. The most stable structure of each kind is shown in Fig. 3 and the relative stability of these complexes and their methanol or formaldehyde analogues are given in Table III. Again, the four-coordinate complexes, with both second-sphere ligands hydrogen bonded to a sulphur and a water or alcohol hydrogen, are about 40 and 25 kJ/mole more stable than the corresponding six- and five-coordinate

structures. No stable complexes with more than six first-sphere zinc ligands could be obtained.

When one of the water molecules in the five-coordinate complex in Fig. 2a was deprotonated, the second molecule was pushed into the second coordination sphere (Fig 4). As shown by the potential curve for the binding of a water molecule to $Zn(NH_3)(SH)_2(OH)^-$ in Fig 5, there is no local energy minimum representing a negatively charged five-coordinate complex. Similar results were obtained for every tested combination of five and six ligands of which one was a hydroxyl or alkoxide ion, as well as when ammonia was exchanged for imidazole. The only exception from this behaviour was observed for $Zn(NH_3)(SH)_2(OH)(CH_3OH)^-$ and its ethanol analogue. For these two species apparently stable, distorted (all zinc-ligand distances are longer than typical for a five-coordinate complex, especially one Zn-S distance) five-coordinate complexes were obtained (c.f. Table I). Yet, they were 77.1 kJ/mole less stable than the corresponding four-coordinate complexes with the alcohol in the second coordination sphere, and are therefore most certainly of minor significance.

Binding energy of the ligands

Tables IV and V give the binding energy of ligands in the first and second coordination sphere to the model of the active site at different coordination numbers. It can be seen that the binding energy for four-coordinate water and alcohol ligands in the first coordination sphere is about 45 kJ/mole, about 400 kJ/mole for hydroxyl ion and about 300 kJ/mole for alkoxide ions. The binding energy of uncharged ligands decreases with about 15 and 15 kJ/mole when the coordination number increases from four to five and from five to six. When ammonia is exchanged for imidazole the SCF binding energy is lowered by about 10 and 50 kJ/mole for uncharged and charged ligands, respectively (about 10%). Alcohols bind slightly stronger than water at the SCF level (5 kJ/mole) but the difference is cancelled by the thermodynamic corrections. The hydroxyl ion binds appreciably (about 100 kJ/mole) stronger than the alkoxide ions.

The binding energy of aldehydes is much lower than for the corresponding alcohols and also more strongly dependent on the size of the hydrocarbon side chain (acetaldehyde binds much stronger than formaldehyde, while ethanol binds with about the same strength as methanol). This is in accord with experimental observations that aldehydes bind much weaker to the zinc ion than the corresponding alcohols and that the binding is more dependent on the size of the side chain [1,3,29]. The binding energy of formaldehyde is only 10 kJ/mole, which indicates that formaldehyde hardly binds at all to alcohol dehydrogenase in water solution (the equilibrium binding constant in water should be about 10^{-8} M^{-1}). These observations provides a theoretical explanation for the experimental observation [1] that formaldehyde is a very poor substrate for alcohol dehydrogenase.

The binding energy of ligands in the second coordination sphere (Table V) is strongly dependent on the type and geometry of the hydrogen bonds formed. Hydrogen bonds to hydroxide or alkoxide ions are strongest and yield binding energies (50-75 kJ/mole) higher than those of four-coordinate first-sphere zinc ligands. The binding energy of second-sphere ligands involved in hydrogen bonds to a sulphide ion and a water hydrogen (35-45 kJ/mole) is similar to that of first-sphere zinc ligands. Second-sphere ligands with hydrogen bonds to water oxygen and ammonia hydrogen have binding energies (about 25 kJ/mole) similar to those of five-coordinate zinc ligands. Water and alcohols in the second coordination sphere bind with about the same strength, while aldehydes, which lack oxygen-bond hydrogens and therefore have to interact by the carbonyl oxygen and aliphatic hydrogens, have about 20-30 kJ/mole lower binding energies.

Zinc-ligand distances

The differences in binding energy of different ligands is paralleled by differences in the corresponding zinc-ligand distances (Table I). The Zn-O distance for alcohols are slightly (1 pm) shorter than for water, and appreciably shorter than for aldehydes (about 7 and 12 pm for formaldehyde and acetaldehyde, respectively). The hydroxide ion has shorter Zn-O distance

than the methoxide and ethoxide ion, but the difference less than 1 pm. For second sphere ligands, again the alcohols and water have almost the same Zn-O distance, while the bond length of aldehydes are about 40 pm longer. The Zn-O distance for anions increases 3 pm for each additional second-sphere zinc ligand, while for uncharged ligands, there are no clear correlation of the Zn-O distance and the coordination number of the zinc ion. The Zn-S distance increases 4, 7 and 12 pm when the number of first-sphere ligands are increased from three to six, while the Zn-N distance is almost constant at about 212-217 pm. Both the Zn-N and the Zn-S distance are strongly dependent on the charge and the type (water, alcohol or aldehyde) of the non-protein ligands. Model systems with imidazole have 4-8 pm shorter Zn-N distances, 2-6 pm longer Zn-S distances and 0-6 pm shorter Zn-O distances than the corresponding systems containing ammonia.

Comparison with experiments

The present results show that the model system prefers (by at least 16 kJ/mole) four-coordination for all tested combinations of up to six ligands. This is somewhat unexpected, considering that the zinc ion forms six-coordinate complexes with most ligands in aqueous solution [30]. The lower coordination number of the metal in the model system might to some extent reflect the bulkiness of the two sulphide ligands, but can primarily be attributed to the negative charges of these ligands which render the model system uncharged as a whole. This suggestion is in accord with previous reported experimental [30,31] and theoretical [32] evidence showing that the coordination number of zinc in hydroxide or halogenide solutions decreases from six to four as the net charge of the complex decreases from +2 to –2, as well as with the present observation that no stable five- or six-coordinate complexes exist when one of the non-protein ligands in the model system is negatively charged (yielding a net charge of -1).

Crystallographic studies of native alcohol dehydrogenase invariably have shown that the catalytic zinc ion is four-coordinate in free enzyme and in enzymic complexes with

monodentate substrates or inhibitors [4-6]. According to the present results, this preference for four-coordination does not result from steric restrictions imposed by the folding of the enzyme, but is primarily a consequence of the chemical properties of the protein ligands.

Spectroscopic studies of cadmium, copper, or cobalt substituted alcohol dehydrogenase in several instances have been taken to provide evidence for the presence of a five-coordinated catalytic metal site [18-20]. The present results indicate that, to the extent that five-coordinate complexes do form with metal-substituted enzyme, this is likely to reflect the disparity in coordination preferences of different metal ions and cannot be taken to suggest that the catalytic metal site in native enzyme is five-coordinate. A similar conclusion was recently drawn from a comparison between crystallographic and spectroscopic results on the coordination chemistry of the catalytic metal ion in carbonic anhydrase [33].

The present results were obtained from studies of small model systems in vacuum. It is likely that the surrounding enzyme and solvent have some influence on the coordination of the active-site zinc ion. Yet, the similarity of the four-coordinate model systems to the crystal structure of the active site indicates that the enzyme favours four-coordination, if anything. In future publications, the impact of the enzyme on the coordination of the zinc-ion will be more thoroughly studied by combined classical and quantum chemical geometry optimisations, and with molecular dynamics simulations using a force-field parametrization of the zinc ion and its ligands obtained from the present calculations.

Mechanistic implications

Several mechanistic proposals have been put forward according to which the catalytic action of alcohol dehydrogenase involves intermediate formation of negatively charged five-coordinate zinc complexes. Eklund & Brändén [4] and Merz et al. [14] suggested that the alcohol substrate is deprotonated by an internal proton transfer within a five-coordinate complex of the alcohol and a hydroxyl ion. Other authors have proposed that this complex

[8,10] or the resulting five-coordinate alkoxide-water complex [9], is the intermediate undergoing the catalytic hydride transfer. The ideas have also been advance that five-coordinate zinc complexes with an alkoxide ion and hydroxyl [9] or hydronium ion [13] are involved in the catalytic reaction mechanism.

The stability of the five-coordinate complexes postulated to form according to the above mechanistic proposals was examined by geometry optimisation using methanol as the alcohol ligand. The results (Table I) invariably showed that four-coordination is the only stable (in the first case, the most stable by about 77 kJ/mole) system configuration. This argues strongly against all of the above mechanistic proposals.

The present results are fully consistent, however, with the proposal of Kvassman and Pettersson [7] that alcohol dehydrogenase catalysis involves the formation of a four-coordinate active-site zinc complex where the alcohol substrate has displaced a zinc-bond water molecule. Such a ligand displacement can occur via intermediate three-coordination (dissociative mechanism) or five-coordination (associative mechanism) [34]. It is commonly assumed that the corresponding transitions states should resemble the three- and the five-coordinate species for the dissociative and the associative mechanisms, respectively [34]. More exactly, the energy of these two species should be a lower limit to the corresponding transition state energies. With this approximation, the calculated transition state energy for the exchange of a zinc-bond water molecule with another water molecule, or a methanol or formaldehyde molecule, initially present in the second coordination sphere of the metal ion, is 15.9, 17.5 or 21.9 kJ/mole, respectively, for the associative mechanism and 43.0, 43.2 or 35.2 kJ/mole, respectively, for the dissociative mechanism. These results strongly indicate that first sphere ligands are exchanged via a five-coordinate intermediate. Such a five-coordinate intermediate should be rather short-lived, however; at equilibrium, only about one active site out of 6000 is predicted to be five-coordinate. This conclusion is supported by kinetic measurements [7]

Acknowledgements

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Table I. Energies (Self Consistent Field and MP2), thermodynamic corrections (TDC), coordination numbers and the zinc-ligand distances of all optimized structures. A and B denote $Zn(H_2S)_2(NH_3)$ and $Zn(H_2S)_2(imidazole)$, respectively. A "+" in the formula indicates second sphere coordination. The Zn-O distances are ordered after the size. When ambiguity may arise, an [a] marks out the non-water ligands.

Complex	Energy (H)		TDC	Coord	Distance to Zn (pm)					
	SCF	SCF+MP2	(kJ/mole)	no.	N	S1	S2	O1	O2	O3
H_2O	−75.965370	−76.105120	8.16							
OH^-	−75.290344	−75.427460	−27.50							
CH_3OH	−114.967478	−115.203467	66.27							
CH_3O^-	−114.321033	−114.563914	31.34							
CH_3CH_2OH	−153.986222	−154.323220	131.83							
$CH_3CH_2O^-$	−153.345265	−153.691555	95.40							
CH_2O	−113.793902	−114.033405	11.17							
CH_3CH_2O	−152.819554	−153.160202	73.04							
A	−2629.982041	−2631.055505	40.10	3	212	228	228			
B	−2798.534621	−2800.015914	124.20	3	202	230	230			
$A(H_2O)$	−2705.984879	−2707.197040	93.75	4	212	233	233	211		
$A+(H_2O)$	−2705.972771	−2707.189253	92.45	3	207	229	231	369		
$B(H_2O)$	−2874.532927	−2876.153390	172.50	4	204	235	237	211		
$A(OH)^-$	−2705.441774	−2706.650303	62.36	4	218	243	243	187		
$B(OH)^-$	−2873.976467			4	210	246	247	187		
$A(CH_3OH)$	−2744.989145	−2746.297380	161.35	4	213	233	233	208		
$A+(CH_3OH)$	−2744.974700	−2746.287712	157.51	3	207	229	231	368		

A(CH3O)⁻	-2744.445435	-2745.751907	121.11	4	217	243	243	187	
A(CH3CH2OH)	-2784.007543	-2785.417111	226.24	4	213	235	235	208	
A(CH3CH2O)⁻	-2783.466060	-2784.875172	187.51	4	217	243	243	188	
A(CH2O)	-2743.804699	-2745.112300	102.32	4	211	232	235	216	
A+(CH2O)	-2743.795703	-2745.109129	97.42	3	207	229	230	407	
A(CH3CH2O)	-2782.835988	-2784.242939	165.91	4	211	234	234	212	
A(H2O)2	-2781.977110	-2783.330823	141.55	5	212	241	241	216	217
A(H2O)+(H2O)	-2781.984127	-2783.339457	148.34	4	212	234	238	204	369
A+(H2O)2	-2781.961057	-2783.320461	141.43	3	203	232	232	362	363
B(H2O)2	-2950.521465			5	207	247	247	210	211
B(H2O)+(H2O)	-2950.530125			4	206	237	240	204	374
A(OH)⁻+(H2O)	-2781.449373	-2782.804665	117.50	4	214	243	244	190	358
B(OH)⁻+(H2O)	-2949.981630			4	210	243	247	190	384
A(CH3OH)(OH)⁻	-2820.422826	-2821.873553	175.96	5	223	242	266	189	230
A(OH)⁻+(CH3OH)	-2820.451240	-2821.904741	180.77	4	214	242	244	191	359
A(CH3CH2OH)(OH)⁻	-2859.441758			5	222	242	265	189	231
A(OH)⁻+(CH3CH2OH)	-2859.470030			4	215	242	244	191	360
A(H2O)(CH3OH)	-2820.980628	-2822.431294	213.22	5	212	240	244	212[a]	218
A(H2O)+(CH3OH)	-2820.986327	-2822.437838	212.91	4	212	234	238	204	370
A(CH3OH)+(H2O)	-2820.986343	-2822.438199	242.62	4	213	234	239	203	369
A+(H2O)(CH3OH)	-2820.962895	-2822.418863	206.34	3	203	232	232	362	364[a]
A(CH3OH)2	-2859.983235			5	213	243	243	216	216
A(CH3O)⁻+(H2O)	-2820.449651	-2821.903215	178.85	4	214	242	243	191	355
A(OH)(MeO)²⁻	-2819.741307			4	348	257	263	190	191[a]
A(H2O)(CH3CH2OH)	-2859.998709	-2861.550902	278.59	5	212	240	244	215[a]	219
A(H2O)+(CH3CH2OH)	-2860.004974	-2861.557618	278.54	4	213	234	238	204	369
A(CH3CH2OH)+(H2O)	-2860.004521	-2861.558008	277.59	4	213	234	239	203	369
A(CH3CH2O)⁻+(H2O)	-2859.469062	-2861.025387	246.21	4	214	242	243	191	355

A(H2O)(CH2O)	-2819.796592	-2821.246793	152.40	5	211	236	240	219	233[a]	
A(H2O)+(CH2O)	-2819.804571	-2821.255803	154.16	4	213	234	236	205	411	
A(CH2O)+(H2O)	-2819.795728	-2821.246837	154.43	5	208	235	236	216	369	
A+(CH2O)(H2O)	-2819.783785	-2821.239537	146.63	3	204	231	232	363	378[a]	
A(H2O)(CH3CH2O)	-2858.826232	-2860.375323	215.44	5	211	236	241	221	227[a]	
A(H2O)+(CH3CH2O)	-2858.833702	-2860.384107	217.31	4	213	234	236	204	410	
A(CH3CH2O)+(H2O)	-2859.826726	-2860.377110	218.71	4	207	234	239	211	368	
A(H2O)3	-2857.965023	-2859.460733	197.59	6	217	253	257	213	217	217
A(H2O)2+(H2O)	-2857.973358	-2859.470847	197.84	5	212	243	247	209	217	382
A(H2O)+(H2O)2	-2857.976787	-2859.475117	195.60	4	214	237	238	199	364	372
A(OH)⁻+(H2O)2	-2857.444986	-2858.947625	177.55	4	212	242	242	193	330	331
A(H2O)2(CH3OH)	-2896.968277	-2898.560498	264.63	6	216	253	259	213[a]	214	217
A(H2O)2+(CH3OH)	-2896.975412	-2898.569250	262.58	6	212	243	247	208	219	383
A(H2O)(CH3OH)+ (H2O)	-2896.975664	-2898.569994	265.80	6	213	241	251	211	217[a]	385
A(H2O)+(CH3OH) (H2O)	-2896.981096	-2898.575755	265.04	4	212	238	238	199	366	366[a]
A(CH3OH)+(H2O)₂	-2896.976263	-2898.572805	267.26	4	209	238	238	203	367	370
A(CH3O)⁻+(H2O)2	-2896.443956	-2898.045254	238.62	4	212	242	242	194	329	329
A(H2O)2(CH3CH2OH)	-2935.986697	-2937.680843	330.29	6	216	253	258	213[a]	215	217
A(CH3CH2O)⁻+(H2O)2	-2935.462476	-2937.166917	306.48	4	212	242	242	193	329	329
A(H2O)2(CH2O)	-2895.785783	-2897.378081	208.34	6	215	253	255	213	214	225[a]
A(H2O)2+(CH2O)	-2895.793369	-2897.387284	203.56	5	212	243	244	210	221	429
A(H2O)(CH2O)+(H2O)	-2895.792885	-2897.387491	204.87	5	211	240	240	212	240[a]	388
A(H2O)+(CH2O)(H2O)	-2895.800369	-2897.395335	206.19	4	213	236	239	199	364	405[a]
A(CH2O)+(H2O)₂	-2895.783677	-2897.377908	206.01	4	204	236	239	214	364	369
A(H2O)2(CH3CH2O)	-2934.815438	-2936.506883	271.68	6	215	254	257	214	214	220[a]

Table II. The relative stability (kJ/mole) of different complexes with five ligands. The optimized energy of five-coordinate complexes are compared to the energy of the four-coordinate complexes with the ligand (L) or water (W) in the second coordination sphere of the zinc ion, i.e. $E(Zn(NH_3)(SH)_2L(H_2O)) - E(Zn(NH_3)(SH)_2(H_2O)+L)$ (L) or $E(Zn(NH_3)(SH)_2L(H_2O)) - E(Zn(NH_3)(SH)_2L+H_2O)$ (W), where "+" indicates second sphere coordination and L is the ligand. The SCF energy is given in parenthesis.

Type	Ligand				
	H_2O	CH_3OH	CH_3CH_2OH	CH_2O	CH_3CH_2O
W	15.9(18.4)	18.7(15.0)	19.7(15.3)	-1.9(-2.3)	1.4(1.3)
L	15.9(18.4)	17.5(15.0)	17.7(16.4)	21.9(20.9)	21.1(19.6)

Table III. The relative stability (kJ/mole) of different complexes with six ligands. The optimized energy of six-coordinate complexes are compared to the corresponding five- or four-coordinate systems with one or two ligands in the second coordination sphere. The energy differences (kJ/mole) are calculated as in Table 2. The SCF energy is given in parenthesis.

Coordination number	Type	Ligand		
		H_2O	CH_3OH	CH_2O
4	W	39.8(30.9)	29.7(21.0)	4.9(-5.5)
4	L	39.8(30.9)	37.2(33.7)	47.5(38.3)
5	W	26.3(21.9)	23.8(19.4)	28.2(18.6)
5	L	26.3(21.9)	25.0(18.7)	28.9(19.9)

Table IV. The binding energy (kJ/mole) of ligands in the first coordination sphere at different coordination numbers. The binding energy is $E(Zn(NH_3)(SH)_2(H_2O)_{0-2}) + E(L) - E(Zn(NH_3)(SH)_2(H_2O)_{0-2}L)$. The SCF energy is given in parenthesis.

Coord.	Ligands							
no.	H_2O	CH_3OH	CH_3CH_2OH	CH_2O	CH_3CH_2O	OH^-	CH_3O^-	$CH_3CH_2O^-$
4	44.4(98.4)	45.9(104.0)	46.5(103.1)	10.4(75.5)	18.8(86.0)	390.(445.)	298.(374.)	284.(364.)
5	29.9(70.5)	27.6(74.2)	27.4(72.5)	-4.5(46.8)	-1.1(52.9)			
6	11.5(59.2)	12.0(62.2)	13.7(61.3)	-19.3(38.8)	-15.4(45.0)			

Table V. The binding energy (kJ/mole) of ligands in the second coordination sphere at different coordination numbers. The binding energy is $E(L) + E(Zn(NH_3)(SH)_2(H_2O)_{0-2}) - E(Zn(NH_3)(SH)_2(H_2O)_{0-2}+L)$. The SCF energy is given in parenthesis.

Coord. no.	Ligands				
	H_2O	CH_3OH	CH_3CH_2OH	CH_2O	CH_3CH_2O
3	25.3(66.6)	24.3(66.1)		6.9(51.9)	
4	45.8(88.9)	45.1(89.2)	45.1(88.9)	17.3(67.7)	20.1(72.5)
5	37.8(81.1)	37.0(80.9)		9.7(58.7)	

Legends to the Figures

Figure 1. Optimized geometry of a. Zn(imidazole)(SH)$_2$(H$_2$O) and c. Zn(NH$_3$)(SH)$_2$(H$_2$O). 1b. shows the zinc ligands (including the CB atom) in the x-ray crystal structure of alcohol dehydrogenase in complex with NADH and dimethylsulfoxide [5].

Figure 2. Two optimized structures of the most stable five- (A) and four-coordinate (B) structures of Zn(NH$_3$)(SH)$_2$(H$_2$O)$_2$.

Figure 3. Optimized geometry of the most stable six- (A), five (B) and four-coordinate (C) structures of Zn(NH$_3$)(SH)$_2$(H$_2$O)$_3$.

Figure 4. Optimized geometry of Zn(NH$_3$)(SH)$_2$(OH)(H$_2$O)$^-$.

Figure 5. The binding energy of a water molecule to Zn(NH$_3$)(SH)$_2$(OH)$^-$ as a function of the Zn-OH$_2$ distance. At each fixed Zn-OH$_2$ distance, all other internal coordinates were optimized.