

# Molecular Dynamics Simulations of Alcohol Dehydrogenase With a Four- or Five-Coordinate Catalytic Zinc Ion

Ulf Ryde

*Department of Theoretical Chemistry, University of Lund, Chemical Center, S-221 00 Lund, Sweden*

**ABSTRACT** A detailed parameterization is presented of a zinc ion with one histidine and two cysteinate ligands, together with one or two water, hydroxide, aldehyde, alcohol, or alkoxide ligands. The parameterization is tailored for the active site of alcohol dehydrogenase and is obtained entirely from quantum chemical computations. The force-field reproduces excellently the geometry of quantum chemically optimized zinc complexes as well as the crystallographic geometry of the active site of alcohol dehydrogenase and small organic structures. The parameterization is used in molecular dynamics simulations and molecular mechanical energy minimizations of alcohol dehydrogenase with a four- or five-coordinate catalytic zinc ion. The active-site zinc ion seems to prefer four-coordination over five-coordination by at least 36 kJ/mol. The only stable binding site of a fifth ligand at the active-site zinc ion is opposite to the normal substrate site, in a narrow cavity behind the zinc ion. Only molecules of the size of water or smaller may occupy this site. There are large fluctuations in the geometry of the zinc coordination sphere. A four-coordinate water molecule alternates frequently (every 7 ps) between the substrate site and the fifth binding site and even two five-coordinate water molecules may interchange ligation sites without prior dissociation. Ligand exchange at the zinc ion probably proceeds by a dissociative mechanism. The results show that it is essential to allow for bond stretching degrees of freedom in molecular dynamics simulations to get a correct description of the dynamics of the metal coordination sphere; bond length constraints may restrict the accessible part of the phase space and therefore lead to qualitatively erroneous results.

© 1995 Wiley-Liss, Inc.

**Key words:** zinc parameterization, effective force-field, four-coordination, five-coordination, reaction mechanism, ligand exchange, bond length constraint, ligand dynamics

## INTRODUCTION

Alcohol dehydrogenase (EC 1.1.1.1) catalyzes the reversible oxidation of primary and secondary alcohols using  $\text{NAD}^+$  as the coenzyme.<sup>1,2</sup> The active site of the enzyme contains a zinc ion that is essential for catalysis. Crystallographic studies of the horse liver enzyme and its binary or ternary complexes with coenzyme and different substrates have shown that this zinc ion is bound to the enzyme through two cysteine and one histidine residue and that it, as a rule, is tetrahedrally four-coordinate with one water or substrate molecule (or the corresponding anions, depending on pH) as the fourth first-sphere ligand.<sup>2–5</sup>

Several mechanistic proposals have been put forward, however, according to which five-coordinate intermediates play an essential role during catalysis.<sup>6–13</sup> Crystallographic and spectroscopic data showing that binding to zinc of certain bidentate inhibitors is five-coordinate<sup>14,15</sup> have been taken as evidence in favor of these suggestions. Furthermore, spectroscopic studies of metal-substituted alcohol dehydrogenase have indicated that several binary and ternary complexes may be five-coordinate,<sup>6–8,15–20</sup> although other spectroscopic investigations contradict these results.<sup>21–25</sup> The kinetic evidence is also scattered and has been taken to favor four-coordination,<sup>1,26</sup> as well as five-coordination,<sup>6,7,9,11,27</sup> of zinc in the catalytically productive ternary complexes.

Recently, Ryde published an extensive series of quantum chemical geometry optimizations of models of the active site of alcohol dehydrogenase with a varying number of different nonprotein ligands.<sup>28</sup> These calculations showed that in vacuum, the zinc ion with ligands similar to those found in the enzyme prefers four-coordination over five-coordination by about 20 kJ/mol. The relevance of these results for the active-site zinc ion in alcohol dehydrogenase is unclear, however, since it is conceivable that five-

Received February 17, 1994; revision accepted October 6, 1994.

Address reprint requests to Ulf Ryde, Department of Theoretical Chemistry, University of Lund, Chemical Center, P.O.B. 124, S-221 00 Lund, Sweden.

coordinate zinc complexes may be stabilized by the enzyme. Therefore, some sort of calculations including the enzyme seemed to be necessary.

One obvious choice would be classical Monte Carlo, molecular dynamics, or molecular mechanics simulations. For such models, a force-field parameterization describing the potential around a zinc ion with a varying number of different ligands would be necessary. Existing parameterizations of the zinc ion,<sup>29–32</sup> however, either lack parameters for some of the zinc ligands found in alcohol dehydrogenase or contain unreliable data. Therefore, we decided to construct a new zinc parameterization. The parameterization is obtained entirely on the basis of quantum chemistry from the Hessian matrix of the optimized structures in ref. 28. The choice of the ligands and the procedure of the parameterization makes the potential an effective force-field for the zinc ion, tailored for the active site of alcohol dehydrogenase. The parameterization is used in molecular dynamics and molecular mechanics simulations of alcohol dehydrogenase with a varying number of nonprotein ligands in order to obtain information on the relative stability of four- and five-coordinate complexes, and on the dynamics of the ligands around the zinc ion.

## METHODS

### Quantum Chemical Computations

$\text{Zn}(\text{HS})_2\text{XL}(\text{H}_2\text{O})_{0-1}$ , where X denotes  $\text{NH}_3$  or imidazole and L denotes a nonprotein ligand, was chosen as a model of the active site of alcohol dehydrogenase. The full geometry of these models was optimized until the change in energy and the coordinates were below  $10^{-6}$  Hartrees and  $10^{-3}$  Bohr or radians, respectively, using analytical gradient methods at the Hartree–Fock level. No symmetry restrictions were imposed. If not otherwise stated, the results refer to computations with basis sets of double- $\zeta$  quality for all atoms [H, (31); C, N, O, (5111/31); S, P, (52111/4111); Zn, (6211111/5111/311)].<sup>33,34</sup> In some cases more extensive basis sets were used; for zinc the double- $\zeta$  basis (6211111/33111/311) of Ahlrichs et al.<sup>35</sup> was used, enhanced with *p*, *d*, and *f* functions with exponents 0.162, 0.132, and 0.390, respectively. For all other atoms, the 6-31+G\* series of basis sets were employed.<sup>36</sup> Partial charges were estimated by standard Mulliken analysis. All quantum chemical calculations were performed on an IBM RISC RS/6000 workstation using the semidirect program package TURBOMOLE.<sup>37</sup>

### Molecular Dynamics and Molecular Mechanical Simulations

Molecular dynamics simulations were performed by the program MUMOD.<sup>38</sup> The energy function in this program is given in Eq. (1):

$$\begin{aligned} & \sum_{\text{bonds}} A_i(r_i - r_{i0})^2 + \sum_{\text{angles}} B_i(\alpha_i - \alpha_{i0})^2 \\ & + \sum_{\text{dihedrals}} \sum_{j=1}^3 C_{ij}[\cos(j\phi_i) + 1] \\ & + \sum_{\text{nonbonded } i < j} \left( \frac{D_{ij}}{r_{ij}^6} + \frac{E_{ij}}{r_{ij}^{12}} + \frac{q_i q_j}{4\pi\epsilon_0\epsilon r_{ij}} \right). \quad (1) \end{aligned}$$

The first three terms represent the energies of bond stretching, angle bending, and dihedral torsions, where  $r_i$ ,  $\alpha_i$ , and  $\phi_i$  are the actual bond lengths, angles, and dihedral angles and  $r_{i0}$  and  $\alpha_{i0}$  are the corresponding equilibrium values. The fourth term represents the nonbonded interactions, consisting of a Lennard–Jones 6–12 term and a Coulomb term, where  $r_{ij}$  is the distance between atom *i* and *j*. The force field does not contain any specific terms for hydrogen bonds or improper dihedral angles.

MUMOD integrates the Newton equations of motion using a double time-step Gear predictor–corrector algorithm of order 4. The forces are divided into a slow (the nonbonded and the dihedral terms) and a fast component; the slow forces are updated every five time steps.<sup>38</sup> The time step was 0.05–0.2 fs. If not otherwise stated, no cutoff was used for the nonbonded interactions. The neighbor list was updated every 40 time steps and the temperature was scaled toward 300 K every 250 time steps.

For molecular mechanics simulations, MUMOD was extended with a procedure for energy minimization by a conjugate gradient method using Polak–Ribieres algorithm and line search according to Fletcher.<sup>39</sup> The energy of the structures was minimized until the norm of the gradient was below 0.01 kJ/mol/Å. No cutoff was used for the nonbonded interactions.

Energies reported are the total potential energy, excluding interactions between fixed atoms. In the molecular dynamics simulations the energy was sampled every time step after 20 ps equilibration (totally  $2-8 \cdot 10^6$  configurations). To compare the energy of a four- and five-coordinate zinc ion in alcohol dehydrogenase, we used the fact that the difference in energy of  $\text{Zn}(\text{HS})_2(\text{imidazole})(\text{H}_2\text{O})_2$  with a five- and a four-coordinate zinc ion is accurately known from quantum chemical computations<sup>28</sup>: 25.2 kJ/mol (including thermodynamic corrections). The energy of these two structures was minimized by molecular mechanics (with the same charges and atom types as in the simulations) and the resulting energy difference was subtracted from the quantum chemical energy difference. The result, –105.1 kJ/mol, was added to all energy differences.

### Parameterization of the Zinc Ion

The parameterization of the zinc ion is based on 14 models of the active site of alcohol dehydrogenase that have been geometry optimized by quantum

**TABLE I. Energy and Zinc-Ligand Distances of the Quantum Chemically Geometry Optimized Structures<sup>28</sup> Used for Force-Field Parameterization of the Zinc Ion\***

Complex	Energy (H)	Distance to Zn (pm)				
		N	S1	S2	O1	O2
A(H <sub>2</sub> O)	-2705.984879	212	233	233	211	
B(H <sub>2</sub> O)	-2874.532927	204	235	237	211	
A(OH) <sup>-</sup>	-2705.441774	218	243	243	187	
A(CH <sub>3</sub> OH)	-2744.989145	213	233	233	208	
A(CH <sub>3</sub> O) <sup>-</sup>	-2744.445435	217	243	243	187	
A(CH <sub>2</sub> O)	-2743.804699	211	232	235	216	
Zn(CH <sub>3</sub> S) <sub>2</sub> (NH <sub>3</sub> )(H <sub>2</sub> O)	-2783.986830	213	232	232	212	
A(H <sub>2</sub> O) <sub>2</sub>	-2781.977110	212	241	241	216	217
B(H <sub>2</sub> O) <sub>2</sub>	-2950.521465	207	247	247	210	211
A(H <sub>2</sub> O) + (H <sub>2</sub> O)	-2781.984127	212	234	238	204	369
A(OH) <sup>-</sup> + (H <sub>2</sub> O)	-2781.449373	214	243	244	190	358
A(H <sub>2</sub> O)(CH <sub>3</sub> OH)	-2820.980628	212	240	244	212 <sup>a</sup>	218
A(CH <sub>3</sub> O) <sup>-</sup> + (H <sub>2</sub> O)	-2820.449651	214	242	243	191	355
A(H <sub>2</sub> O)(CH <sub>2</sub> O)	-2819.796592	211	236	240	219	233 <sup>a</sup>

\*A and B denote Zn(HS)<sub>2</sub>(NH<sub>3</sub>) and Zn(HS)<sub>2</sub>(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 nonwater ligand.

chemistry.<sup>28</sup> The structures are listed and shortly characterized in Table I. The cysteine ligands are modeled as HS<sup>-</sup> or CH<sub>3</sub>S<sup>-</sup>. As shown in Figure 1 and Table I HS<sup>-</sup> gives virtually the same result as CH<sub>3</sub>S<sup>-</sup>; the root-mean-square deviation of the two structures in Figure 1 is only 0.015 Å and the Zn-S bond lengths and force constants differ by less than 0.6%. The histidine ligand is modeled by either imidazole or ammonia. As can be seen in Table I, these two ligands give slightly different results. Therefore, all parameters involving histidine are derived from the imidazole structures.

Quadratic force-constants were obtained from the diagonal elements of the analytically calculated quantum chemical Hessian, projected into a space of internal coordinates. Several different sets of internal coordinates were tested to ensure that the Cartesian-to-internal transformation does not distort the values. The force-constants were uniformly scaled by a factor 0.81.<sup>36</sup>

For bond lengths and angles these quadratic force-constants represent  $A_i$  and  $B_i$  in Eq. (1) and could be used directly. For dihedral angles, the periodicity of the potential was determined from the values of dihedral angles found in the structures and the  $C_{ij}$  constants in Eq. (1) were obtained by multiplying the quadratic force-constants by an appropriate Taylor coefficient (2, 1/2, and 2/9 for 360°, 180°, and 120° periodicity, respectively). The equilibrium values of the bond lengths and angles were calculated by averaging the values observed in any of the structures in ref. no. 28 (about 20 four-coordinate and 30 five-coordinate structures). The van der Waals parameters for the zinc ion were taken from the AMBER force field:  $\sigma = 1.70$  Å and  $\epsilon = 0.0523$  kJ/mol.<sup>40</sup>

In addition to zinc, five new atom types had to be added to the potential library of MUMOD, in order to describe a negatively charged cysteine residue, NADH, and hydroxide and alkoxide ions; S<sup>-</sup>, negatively charged sulfur; P, phosphorus; OS, ester oxygen; OH<sup>-</sup>, oxygen in the hydroxide ion; and OC<sup>-</sup>, alkoxide oxygen. The force-field parameters of P and OS were taken from the AMBER<sup>40</sup> and XPLOR<sup>41</sup> force fields. The parameters of the negatively charged atom types were obtained from quantum chemical computations on HX<sup>-</sup>, CH<sub>3</sub>X<sup>-</sup>, and CH<sub>3</sub>CH<sub>2</sub>X<sup>-</sup>, X=S or O, using the 6-31+G\*\* basis sets. These latter parameters are listed in Table II.

### The Protein

Throughout, the coordinates of horse liver alcohol dehydrogenase in complex with NADH and dimethyl sulfoxide at 1.8 Å resolution (*R*-factor=0.172)<sup>4</sup> were used. This is at present the most accurate structure of alcohol dehydrogenase. The enzyme is in the closed conformation that is the catalytically interesting conformation and also the one to which most reports of a five-coordinate zinc ion refer. Both subunits of the enzyme were included in the simulations, as well as the coordinates of 509 crystal water molecules; totally 12941 atoms. The dimethyl sulfoxide molecule at the active site was substituted by a water molecule. All Asp and Glu residues were treated as anions and all Lys and Arg residues as cations. The protonation state of the His residues was determined by examination of the hydrogen bonding structure; His-34, 67, 138, and 139 were found to have a proton on ND1, His-105 on NE2, while His-51 and 348 have protons on both nitrogens. The six cysteine zinc ligands, Cys-46, 97, 100,

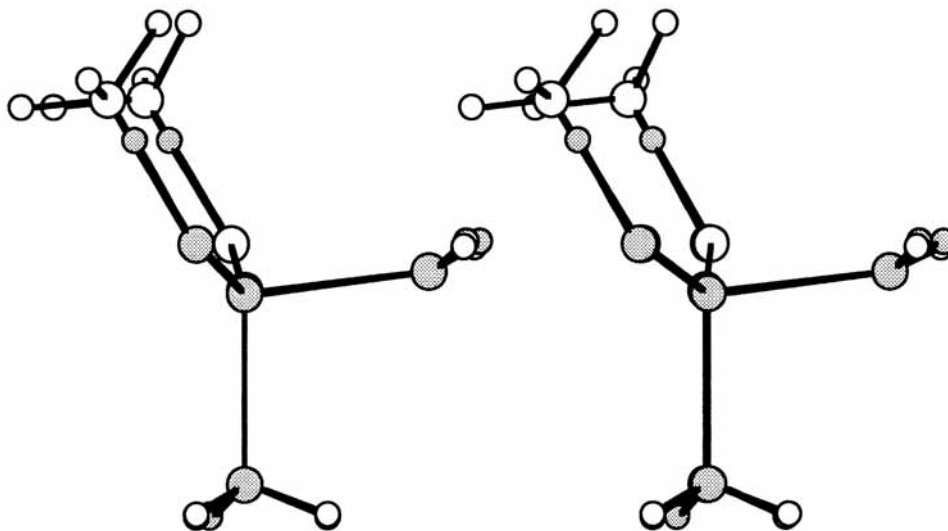


Fig. 1. Difference in the quantum chemical geometries of  $\text{Zn}(\text{CH}_3\text{S})_2(\text{NH}_3)\text{H}_2\text{O}$  and  $\text{Zn}(\text{HS})_2(\text{NH}_3)\text{H}_2\text{O}$  (shaded). The root-mean-square deviation is 0.015 Å.

**TABLE II. Quantum Chemically Calculated Force-Field Parameters for the New Atom Types OH<sup>-</sup>, OC<sup>-</sup>, and S<sup>-</sup>\***

a. van der Waals parameters,  $\sigma_i$  and  $\epsilon_i$ , which are related to  $D_{ij}$  and  $E_{ij}$  in Eq. (1):

$$D_{ij} = 4\epsilon_{ij}\sigma_{ij}^6; E_{ij} = 4\epsilon_{ij}\sigma_{ij}^{12}; \epsilon_{ij} = \sqrt{\epsilon_i\epsilon_j}; \sigma_{ij} = \frac{\sigma_i + \sigma_j}{2}.$$

Atom type	$\sigma$ (Å)	$\epsilon$ (kJ/mol)
OH <sup>-</sup>	3.17	0.639
OC <sup>-</sup>	2.94	1.021
S <sup>-</sup>	4.97	0.998

b. Bond parameters; equilibrium distance  $A_i$  and quadratic force constant  $r_{i0}$  in Eq. (1).

Atom types	$A_i$ (Å)	$r_{i0}$ (kJ/mol/Å <sup>2</sup> )
OH <sup>-</sup> HO	0.946	2267
OC <sup>-</sup> C	1.335	1788
S <sup>-</sup> H	1.337	1096
S <sup>-</sup> C	1.835	848

c. Angle parameters: equilibrium angle  $B_i$  and quadratic force constant  $\alpha_{i0}$  in Eq. (1).

Atom types	$B_i$ (°)	$\alpha_{i0}$ (kJ/mol/radian <sup>2</sup> )
HC C OC <sup>-</sup>	114.6	528
C C OC <sup>-</sup>	113.9	600
HC C S <sup>-</sup>	109.8	358
C C S <sup>-</sup>	114.0	494
P OS P	123.6	543

d. Dihedral parameters:  $C_{in}$  in Eq. (1);  $C_{ij} = 0$  for  $j \neq n$ .

Atom types	$n$	$C_{in}$ (kJ/mol)
OC <sup>-</sup> C C HC	3	8.15
S <sup>-</sup> C C HC	3	7.32

\*The MUMOD atom types are described in the note to Table IV.

103, 111, and 174, were assumed to be negatively charged, the other eight cysteine residues uncharged. This charge assignment gave a total charge of +4 for the dimer (34 Asp, 42 Glu, 12 Cys, 2 NADH, 2 carboxy terminals, 60 Lys, 24 Arg, 4 His<sup>+</sup>, 4 Zn, and 2 amino terminals).

Partial charges of the amino acids were taken from the MUMOD program library. The charges of the adenine, ribose, and phosphate moieties of NADH and of histidine protonated on NE2 or on both NE2 and ND1 were taken from the AMBER library.<sup>40</sup> Partial charges of cysteinate (CA, 0.028; HA, 0.017; CB, -0.416; HB, 0.028; SG, -0.603) and of the nicotinamide moiety of NADH (C1'N, 0.264; N1N, -0.707; C2N, 0.347; H2N, 0.187; C3N, -0.340; C7N, 0.634; O7N, -0.529; N7N, -0.732; H7N, 0.280; C4N, -0.177; H4N, 0.146; C5N, -0.217; H5N, 0.116; C6N, 0.221; H6N, 0.143) were obtained from a Mulliken analysis of the quantum chemically optimized structures of  $\text{CH}_3\text{CH}_2\text{SH}$  and  $\text{NC}_5\text{H}_6\text{CONH}_2$ .

The charge on the zinc ion and its ligands were determined by quantum chemical Mulliken analysis on the systems in Table III and in ref. 28. The zinc charge varied by only  $\pm 0.1$  e for four- and five-coordinate systems with different ligands but the same basis sets. In order to facilitate comparisons, we therefore decided to use the same zinc charge in all simulations, +0.488, which was obtained for the four-coordinate system with the largest basis set in Table III. Since the charges on the oxygen and imidazole ligands do not change significantly on coordination to a zinc ion, the residual formal zinc charge (1.512) was distributed uniformly on the SG and CB atoms of the two cysteine ligands; the resulting charges were -0.225 and -0.038, respectively.

TABLE III. The Dependence of the Geometry on the Basis Sets\*

Complex	Basis (Zn/other)	Energy (H)	Distance to Zn				
			N	S1	S2	O1	O2
A(H <sub>2</sub> O)	d $\zeta$ /d $\zeta$	-2705.984879	212	233	233	211	
A(H <sub>2</sub> O)	d $\zeta$ /6-31G	-2706.140377	212	234	234	210	
A(H <sub>2</sub> O)	d $\zeta$ + p/6-31G**	-2706.279881	218	230	230	226	
A(H <sub>2</sub> O)	d $\zeta$ + p/6-31(+)G**	-2706.284772	218	230	230	225	
A(H <sub>2</sub> O)	d $\zeta$ + p/6-31 + G**	-2706.289841	218	230	230	228	
A(H <sub>2</sub> O)	d $\zeta$ + pdf/6-31 + G**	-2706.300360	218	229	229	231	
A(OH) <sup>-</sup>	d $\zeta$ /d $\zeta$	-2705.441774	218	243	243	187	
A(OH) <sup>-</sup>	d $\zeta$ + p/6-31 + G**	-2705.743774	228	240	240	193	
A(OH) <sup>-</sup>	d $\zeta$ + pdf/6-31 + G**	-2705.753362	227	240	240	192	
A(H <sub>2</sub> O) <sub>2</sub>	d $\zeta$ /d $\zeta$	-2781.977110	212	242	242	217	218
A(H <sub>2</sub> O) <sub>2</sub>	d $\zeta$ + p/6-31G*	-2782.271259	214	234	234	238	246
A(H <sub>2</sub> O) <sub>2</sub>	d $\zeta$ + p/6-31 + G**	-2782.332593	215	233	233	242	252
A(H <sub>2</sub> O) <sub>2</sub>	d $\zeta$ + pdf/6-31 + G**	-2782.343160	214	231	231	227	327

\*Energies, basis sets, and the zinc-ligand distances of different structures, obtained by quantum chemical geometry optimizations. A denotes Zn(HS)<sub>2</sub>(NH<sub>3</sub>). The basis sets are described in Methods.

The positions of the hydrogen atoms were determined with standard algorithms (program MUMIN). Since some positions are not fully determined by these methods, and also in order to determine the solvent water structure around the enzyme, 1.8 ps molecular dynamics simulation at 0 K was performed. In this simulation, a water bath of 1719 water molecules was included and all heavy atoms were kept fixed except the oxygen atoms of the solvent water molecules. After this simulation all uncharged amino acids and water molecules with a distance larger than 3.0 Å from the large system (see below) were removed, while charged residues were substituted by an integer charge at the position of the NZ, CZ, CG, CD, SG, CE1, ZN, and both P atoms for Lys, Arg, Asp, Glu, Cys<sup>-</sup>, His<sup>+</sup>, ZN, and NADH, respectively. These integer charges were scaled by a dielectric constant  $\epsilon = 4.0$ , while  $\epsilon = 1.0$  in the rest of the system. The remaining 2813 atoms were subjected to two further molecular dynamics simulations, 0.5 ps at 300 K and 2.5 ps at 0 K, followed by an energy minimization to a gradient norm below 0.01 kJ/mol/Å. In these calculations, periodic boundary conditions with a nonbonded cutoff of 10 Å was used and the heavy nonsolvent atoms were kept fixed. The resulting coordinates were used as input in all the simulations.

Two different systems were studied. One system, termed the *small* system, comprised all residues of the enzyme within 3.0 Å from the catalytic zinc ion (in the A subunit of the enzyme) and its ligands (including CB of the protein ligands). These are Cys-46, Ser-48, Asp-49, Gly-66, His-67, Glu-68, Phe-93, Phe-140, Leu-141, Gly-173, Cys-174, Gly-175, Ile-318, Arg-369, H<sub>2</sub>O158, and the nicotinamide moiety of NADH. In addition, all charged residues outside this radius were represented by scaled ( $\epsilon = 4.0$ ) integer charges. Totally, the small system contained 400 atoms. The positions of all atoms except the zinc ion and its ligands (including CB) were kept fixed.

The other system, termed *large*, comprised all residues within 3.0 Å from any atom in the small system (except the integer charges), i.e., residues 43–53, 57, 59, 63–64, 66–69, 90, 92–95, 109–110, 115, 116, 139–142, 146, 170–176, 178–179, 202–203, 292, 294, 318–321, 345–348, 359, 368–370, crystalwaters number 5, 8, 21, 35, 55, 58–59, 158–161, 167, 172, the nicotinamide, N-ribose, and pyrophosphate moiety of NADH, and residues 309 and 310 from the B subunit of the protein; 176 scaled ( $\epsilon = 4.0$ ) integer charges were also included, leading to a total number of 1224 atoms. The atoms of the small system (not the integer charges) were free to move, while the other atoms were kept fixed.

## RESULTS AND DISCUSSION

### Basis Set Dependence of the Geometry of the Zinc Coordination Sphere

In order to study the impact of the quality of the basis set on the geometry of the zinc coordination sphere, geometry optimizations were performed on Zn(NH<sub>3</sub>)(SH)<sub>2</sub>X, X = H<sub>2</sub>O, OH<sup>-</sup>, and (H<sub>2</sub>O)<sub>2</sub>, with increasing quality of the basis sets. The results, collected in Table III, show that the zinc-ligand distances are strongly dependent on the quality of the basis sets. As the basis sets are extended by one set of polarizing functions and two sets of diffuse functions, the Zn–N distance increases by 0.03–0.09 Å, the Zn–S distances decrease by 0.03–0.09 Å, the Zn–OH distance increases by 0.05 Å, while the Zn–OH<sub>2</sub> distance increases by as much as 0.20–0.34 Å. The angles and the dihedrals around the zinc ion also change, but to a much smaller extent.

The greatest changes are found for the Zn(NH<sub>3</sub>)(SH)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub> system. As the quality of the basis sets is increased, the lengths of the two Zn–O bonds become increasingly unequal. This is due to weakening of one Zn–O bond and strengthening of an internal hydrogen bond from an ammonia hydrogen to one of the water oxygens. With the largest

**TABLE IV. Parameterization of  $\text{Zn}^{2+}$** 

a. Bond parameters; equilibrium distance  $A_i$  and quadratic force constant  $r_{i0}$  in Eq. (1). The equilibrium bond distances are recalculated to fit the results with the most extended basis set; the original values are given in parentheses.

		Zn4		Zn5	
Atom types		$A_i$ (Å)	$r_{i0}$ (kJ/mol/Å <sup>2</sup> )	$A_i$ (Å)	$r_{i0}$ (kJ/mol/Å <sup>2</sup> )
Zn	N	2.181 (2.120)	262	2.150 (2.120)	262
Zn	Na	2.101 (2.040)	358	2.070 (2.069)	327
Zn	O=C	2.359 (2.160)	189	2.583 (2.330)	100
Zn	OH	2.279 (2.080)	243	2.388 (2.135)	201
Zn	OH <sub>2</sub>	2.309 (2.110)	204	2.418 (2.165)	161
Zn	OH <sup>-</sup>	1.923 (1.871)	690		
Zn	OC <sup>-</sup>	1.926 (1.874)	662		
Zn	S <sup>-</sup>	2.293 (2.343)	342	2.326 (2.427)	270

b. Angle parameters: equilibrium angle  $\alpha_{i0}$  and quadratic force constant  $B_i$  in Eq. (1).

			Zn4		Zn5	
Atom types			$\alpha_{i0}$ (°)	$B_i$ (kJ/mol/radian <sup>2</sup> )	$\alpha_{i0}$ (°)	$B_i$ (kJ/mol/radian <sup>2</sup> )
HO	N	Zn	109.0	126.5	109.0	126.5
Ca	Na	Zn	123.6	186.3	126.1	186.3
C	O=C	Zn	131.2	83.4	131.2	100.4
C	OH	Zn	133.4	119.9	133.4	119.9
HO	OH	Zn	110.3	120.7	110.3	120.7
H <sub>2</sub> O	OH <sub>2</sub>	Zn	119.0	73.4	119.0	81.3
HO	OH <sup>-</sup>	Zn	135.1	49.5		
C	OC <sup>-</sup>	Zn	141.4	42.0		
C	S <sup>-</sup>	Zn	111.6	79.1	111.1	69.4
HO	S <sup>-</sup>	Zn	109.9	78.6	109.4	68.8
N	Zn	O=C	90.9	95.6	83.6	221.9
N	Zn	OH	98.0	95.6	91.4	221.9
N	Zn	OH <sub>2</sub>	97.0	95.6	89.2	221.9
N	Zn	OH <sup>-</sup>	83.5	95.6		
N	Zn	OC <sup>-</sup>	86.5	95.6		
N	Zn	S <sup>-</sup>	103.0	127.2	103.0	127.2
Na	Zn	O=C	97.9	67.2	94.6	135.3
Na	Zn	OH	105.0	67.2	102.4	135.3
Na	Zn	OH <sub>2</sub>	104.0	67.2	100.2	135.3
Na	Zn	OH <sup>-</sup>	95.0	67.2		
Na	Zn	OC <sup>-</sup>	98.0	67.2		
Na	Zn	S <sup>-</sup>	103.0	127.2	103.0	127.2
O=C	Zn	OH <sub>2</sub>			172.3	37.3
O=C	Zn	S <sup>-</sup>	104.4	129.6	93.6	129.6
OH	Zn	OH <sub>2</sub>			173.9	37.3
OH	Zn	S <sup>-</sup>	99.7	129.6	92.0	129.6
OH <sub>2</sub>	Zn	OH <sub>2</sub>			168.5	37.3
OH <sub>2</sub>	Zn	S <sup>-</sup>	96.1	129.6	88.8	129.6
OH <sup>-</sup>	Zn	S <sup>-</sup>	120.7	129.6		
OC <sup>-</sup>	Zn	S <sup>-</sup>	120.3	129.6		
S <sup>-</sup>	Zn	S <sup>-</sup>	146.5	207.4	151.8	69.1

*Continued on overleaf*

TABLE IV. Parameterization of  $\text{Zn}^{2+}$  (Continued)c. Dihedral parameters:  $C_{in}$  in Eq. (1).  $C_{ij} = 0$  for  $j \neq n$ . <sup>a</sup>Indicates that an  $n = 6$  term would be more appropriate.

Atom types				$n$	Zn4 $C_{in}$ (kJ/mol)	Zn5 $C_{in}$ (kJ/mol)
HC	C	OC <sup>-</sup>	Zn	3	0.27	0.46
C	C	OC <sup>-</sup>	Zn	3	0.27	0.46
HC	C	S <sup>-</sup>	Zn	3	0.76	0.76
C	C	S <sup>-</sup>	Zn	3	0.76	0.76
HC	Ca	Na	Zn	1	-196.8	-195.2
C	Ca	Na	Zn	1	73.8	85.8
Na	Ca	Na	Zn	1	80.0	88.5
HO	N	Zn	O=C	3	-1.03	0.00
HO	N	Zn	OH	3 <sup>a</sup>	-0.75	0.00
HO	N	Zn	OH2	3 <sup>a</sup>	-0.19	-0.13
HO	N	Zn	OH <sup>-</sup>	3	-5.45	
HO	N	Zn	OC <sup>-</sup>	3	-4.02	
HO	N	Zn	S <sup>-</sup>	3	-0.79	-0.42
Ca	Na	Zn	O=C	2	11.8	7.82
Ca	Na	Zn	OH	2	11.8	7.82
Ca	Na	Zn	OH2	2	11.8	7.82
Ca	Na	Zn	OH <sup>-</sup>	2	-11.8	
Ca	Na	Zn	OC <sup>-</sup>	2	-11.8	
Ca	Na	Zn	S <sup>-</sup>	2	-11.8	-7.82
Zn	O=C	C	HC	2	-7.61	-7.61
Zn	O=C	C	C	2	-7.61	-7.61
C	O=C	Zn	N	3	-3.22	-3.52
C	O=C	Zn	Na	3	-3.22	-3.52
C	O=C	Zn	OH2	3		-3.52
C	O=C	Zn	S <sup>-</sup>	3	-3.22	-3.52
Zn	OH	C	HC	3	-2.24	-2.68
Zn	OH	C	C	3	-2.24	-2.68
HO	OH	Zn	N	2	15.5	21.6
HO	OH	Zn	Na	2	15.5	21.6
HO	OH	Zn	OH2	2		-21.6
HO	OH	Zn	S <sup>-</sup>	2	-15.6	-21.6
C	OH	Zn	N	2	15.5	21.6
C	OH	Zn	Na	2	15.5	21.6
C	OH	Zn	OH2	2		-21.6
C	OH	Zn	S <sup>-</sup>	2	-15.6	-21.6
H2O	OH2	Zn	N	2	7.0	9.1
H2O	OH2	Zn	Na	2	5.6	9.6
H2O	OH2	Zn	O=C	2		10.1
H2O	OH2	Zn	OH	2		-10.1
H2O	OH2	Zn	OH2	2		8.7
H2O	OH2	Zn	S <sup>-</sup>	2	-6.2	-9.2
HO	OH <sup>-</sup>	Zn	N	2	-1.47	
HO	OH <sup>-</sup>	Zn	Na	2	-1.47	
HO	OH <sup>-</sup>	Zn	S <sup>-</sup>	2	1.47	
C	OC <sup>-</sup>	Zn	N	2	-1.34	
C	OC <sup>-</sup>	Zn	Na	2	-1.34	
C	OC <sup>-</sup>	Zn	S <sup>-</sup>	2	1.34	
HO	S <sup>-</sup>	Zn	N	3 <sup>a</sup>	-0.13	-0.16
HO	S <sup>-</sup>	Zn	Na	3 <sup>a</sup>	-0.13	-0.12
HO	S <sup>-</sup>	Zn	O=C	3	-0.14	-0.18
HO	S <sup>-</sup>	Zn	OH	3 <sup>a</sup>	-0.13	-0.21
HO	S <sup>-</sup>	Zn	OH2	2 <sup>a</sup>	0.22	0.30
HO	S <sup>-</sup>	Zn	OH <sup>-</sup>	3	0.12	
HO	S <sup>-</sup>	Zn	OC <sup>-</sup>	3	0.12	
HO	S <sup>-</sup>	Zn	S <sup>-</sup>	2	-0.28	-0.39
C	S <sup>-</sup>	Zn	N	3 <sup>a</sup>	-0.14	-0.18
C	S <sup>-</sup>	Zn	Na	3 <sup>a</sup>	-0.14	-0.08
C	S <sup>-</sup>	Zn	O=C	3	-0.12	-0.18
C	S <sup>-</sup>	Zn	OH	3 <sup>a</sup>	-0.11	-0.21
C	S <sup>-</sup>	Zn	OH2	3 <sup>a</sup>	-0.11	-0.14
C	S <sup>-</sup>	Zn	OH <sup>-</sup>	3	-0.12	
C	S <sup>-</sup>	Zn	OC <sup>-</sup>	3	-0.12	
C	S <sup>-</sup>	Zn	S <sup>-</sup>	2	-0.14	-0.18

The relevant MUMOD atom types are HO, polar hydrogen; HC, nonpolar hydrogen; H2O, water hydrogen; C, sp3 hybridized carbon; Ca, sp2 hybridized carbon; N, amine nitrogen; Na, aromatic nitrogen (in histidine); O=C, carbonyl oxygen; OH, hydroxyl oxygen; OH2, water oxygen; OH<sup>-</sup>, hydroxide oxygen; OC<sup>-</sup>, alkoxide oxygen; S<sup>-</sup>, negatively charged sulfur (in cysteine); Zn4, four-coordinate zinc ion; Zn5, five-coordinate zinc ion.

basis set, this hydrogen bond becomes more favorable than zinc coordination, and the complex reorganizes to a four-coordinate structure with one water molecule in the second coordination sphere of the zinc ion. Apparently, the stability of five-coordinate complexes decreases as the quality of the basis set is increased. Therefore, the relative stability of five-coordinate complexes is overestimated in this work as well as in our earlier studies.<sup>28</sup>

In summary, split-valence + polarization (double- $\zeta$  + a p-function on Zn) seems to be an appropriate level to describe the zinc coordination sphere, except perhaps the Zn–O bond. Unfortunately, it was not possible to use such large basis sets in the parameterization. Instead, the zinc–ligand equilibrium bond distances in the zinc parameterization were rescaled to reproduce the values of the most extended basis set.

### Parameterization of the Zinc Ion

The force-field parameters for a four- and five-coordinate zinc ion are listed in Table IV. The bond lengths are scaled to reproduce the values of the most extended basis set; the original values are given in parentheses. Several dihedral angles, especially some X–Y–S–H and X–Y–O–H dihedrals, apparently showed a 60° periodicity. Since such a potential is not allowed in the present version of MUMOD, a 120° term with a decreased  $C_{i3}$  constant was used instead. Affected angles are marked with the letter *a* in Table IV.

Another problem was encountered for the five-coordinate zinc ion. There exist several different local minima of the quasi trigonal-bipyramidal five-coordinate structures, characterized by different ligands in the axial positions. Each structure has different sets of equilibrium parameters and force-constants and strictly a separate parameterization should be performed at each minimum. The present parameterization is obtained from the global minimum with the two oxygen atoms in the axial positions and is strictly valid only near this structure. As will be seen below and as is also confirmed by combined quantum chemical and molecular mechanical geometry optimizations,<sup>42</sup> this is the optimal structure also in the enzyme.

Many force-constant parameters depend on all the ligands around the zinc ion (i.e., also on atoms not explicitly involved in the parameter). The Zn–S distance, for example, changes from 2.32 to 2.43 Å as an aldehyde ligand (which binds weakly) is replaced by a hydroxide ion (which binds strongly). The corresponding force constant, which is almost perfectly linearly dependent on the bond length, changes accordingly, leading to an uncertainty in the equilibrium bond distances and the bonding force constants of about 0.05 Å and 50 kJ/mol/Å<sup>2</sup> in complexes involving the strongest or weakest ligands. Similarly, bond angle parameters of complexes involving

alkoxide and hydroxide ligands are very different from those of complexes with uncharged ligands, due to the electrostatic repulsion between the oxygen and the negatively charged sulfide ions. For example, the S–Zn–S angle is 141–162° in uncharged complexes, but only 114–116° in negatively charged complexes.

These problems could, of course, be solved by introducing a special zinc type for every set of zinc ligands. Yet, this would lead to an unfortunately large number of atom types. A more interesting possibility would be to include the electrostatic 1,3-interactions in the potential energy term. Then the bonded parameters of all atoms would need to be recalculated, but the result would probably be much more accurate without any need of new atom types. In the present parameterization no attempt was made to correct these problems (as in all common force fields); the present parameters are most accurate for an uncharged zinc coordination sphere with water or alcohol ligands.

The van der Waals parameters [ $D_{ij}$  and  $E_{ij}$  coefficients in Eq. (1)] of the zinc ion provided the largest problems. An attempt was made to estimate these parameters from quantum chemical interaction energies. This turned out to be impossible due to the inappropriateness of the nonbonding potential. The fourth term in Eq. (1) may be appropriate for interactions of atoms with low charges, but for highly charged atoms such as a zinc ion, significant terms of any negative power of the interatomic distance can be expected. At least four  $r^{-k}$  terms (including the Coulomb term) turned out to be necessary to achieve an acceptable fit to the calculated interaction potential. Yet, the van der Waals parameters of the zinc ion should be of little significance in the current parameterization since the first sphere ligands are described by bonded parameters. Therefore, the van der Waals parameters of the zinc ion were simply taken from literature.

The first simulations of alcohol dehydrogenase gave an eight-coordinate zinc ion with four nonbonded water molecules at unphysically short Zn–O distances (about 1.5 Å). Therefore, the Zn–OH<sub>2</sub> van der Waals parameters were recalculated so that the molecular mechanically minimized structure reproduces the quantum chemically optimized Zn–O distance and the change in energy when a water molecule is added to Zn(NH<sub>3</sub>)(SH)<sub>2</sub>(H<sub>2</sub>O) (2.364 Å and –70.52 kJ/mol<sup>28</sup>). This gave  $D = -1477 \text{ kJ } \text{\AA}^6 \text{ mol}^{-1}$  and  $B = 473500 \text{ kJ } \text{\AA}^{12} \text{ mol}^{-1}$ .

### Performance of the Parameterization

The quality of the parameterization of the zinc ion was tested by comparing the geometry of 34 small model systems optimized by molecular mechanics using the current zinc parameterization with results obtained quantum mechanically.<sup>28</sup> Of these structures 14 were used in the parameterization of the



TABLE V. Test of the Parameterization\*

Group	With 1,4-interactions	Used in the parameterization	No.	Bonds (Å)	Angles (°)	Dihedrals (°)	Distances (Å)
All	Yes	Yes	14	0.078	8.9	25.0	0.76
All	Yes	No	20	0.057	8.1	29.6	0.79
<b>All</b>	<b>Yes</b>	<b>All</b>	<b>34</b>	<b>0.066</b>	<b>8.5</b>	<b>27.7</b>	<b>0.78</b>
4 coord	No	Yes	7	0.040	5.0	12.8	0.51
4 coord	No	No	4	0.041	4.8	12.2	0.49
4 coord	No	All	11	0.040	4.9	12.6	0.50
5 coord	No	Yes	4	0.072	4.6	8.3	0.34
5 coord	No	No	2	0.058	4.0	8.3	0.37
5 coord	No	All	6	0.067	4.4	8.3	0.35
Second	No	Yes	3	0.046	6.2	18.0	0.47
Second	No	No	14	0.043	4.3	11.2	0.44
Second	No	All	17	0.044	4.6	12.4	0.44
All	No	Yes	14	0.050	5.1	12.6	0.45
All	No	No	20	0.044	4.7	11.0	0.44
<b>All</b>	<b>No</b>	<b>All</b>	<b>34</b>	<b>0.047</b>	<b>4.7</b>	<b>11.7</b>	<b>0.45</b>

\*All bond lengths, bond angles, dihedral angles, and interatomic distances are compared for 34 zinc complexes, geometry optimized quantum mechanically and molecular mechanically (with the new zinc parameterization and charges obtained from a quantum chemical Mulliken analysis). The complexes are divided into three groups, viz. four-coordinate systems, five-coordinate systems, and systems with ligands in the second coordination sphere. Of the complexes 14 were used in the construction of the parameterization of the zinc ion (listed in Table I). The comparison is done with, as well as without, the nonbonded 1,4-interactions included.

zinc ion (Table I). The charges of the atoms in the molecular mechanical optimizations were obtained by Mulliken analysis from the quantum chemical computations. The differences in bond lengths, bond angles, dihedral angles, and interatomic distances are presented in Table V.

If 1,4-interactions (i.e., interactions involving atoms separated by three bonds) but not 1,3-interactions are included in the nonbonded term in Eq. (1) (default in MUMOD), the result is moderately impressing. This is due to deficiencies in the treatment of the nonbonded interactions. In the model systems there are many potential hydrogen bonds between the zinc ligands, i.e., between atoms separated by only three bonds (e.g., H–N–Zn–O). If the attractive 1,4-interaction (hydrogen-acceptor) is included in the potential, but the repulsive 1,3-interaction is excluded, the hydrogen bonds become unphysically short, leading to a highly distorted geometry, as can be seen in Figure 2.

If also the 1,4-interactions are excluded, the performance of the parameterization is greatly improved (rows 4–11 in Table V and Fig. 3); the errors in the internal coordinates are almost halved. The hydrogen bonds are much better described, even if 1,5-interactions (repulsion between hydrogen atoms) lead to slightly increased zinc ligand distances. Yet, in simulations with more realistic ligands, which have a lower number of hydrogen atoms, this effect is negligible. Therefore, in the following, 1,4-interactions are excluded from the potential. In most other force-fields, 1,4-interactions are either totally excluded, or damped by a factor of about 4.

There is no significant difference in the perfor-

mance of the zinc parameterization between the structures used in the parameterization and the other structures. In fact, the structures not included perform a little bit better. This indicates that the number of structures included in the parameterization was large enough and that the parameterization is well transferable to other systems. Further, there is no significant difference in the quality of the parameterization between the five-coordinate and the four-coordinate zinc ion. From Table V, it may seem that the four-coordinate systems have slightly better bond distances but worse angles and dihedral angles, but this is an artifact. The difference in bond lengths (and also the rather poor performance of the bond lengths on the whole) is due to the recalibration of the bond lengths. As discussed above, the change is larger for five-coordinate complexes. The worse angles and dihedrals in the four-coordinate systems, on the other hand, are explained by the fact that complexes with negatively charged oxygen-ligands can only be four-coordinate. As discussed above, these systems have distorted bond angles around the zinc ion.

The Zn–N and Zn–S equilibrium bond distances are in excellent conformity with the distances observed in small organic crystals<sup>31</sup> (Zn–N 1.97–2.21 and 2.00–2.39 Å, Zn–S 2.25–2.50 and 2.36–2.51 Å, for four- and five-coordinate structures, respectively). The Zn–O distances, on the other hand, differ significantly (1.91–2.04 and 1.93–2.39 Å, for four- and five-coordinate structures). This can most probably be attributed to the fact that the crystal data do not discriminate between charged and uncharged ligands and that in alcohol dehydrogenase

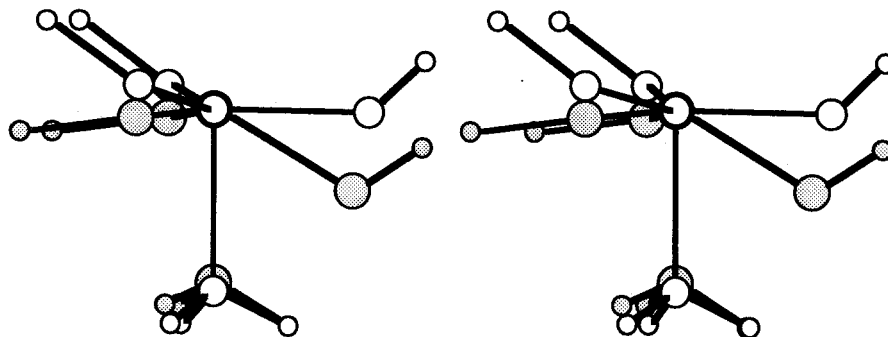


Fig. 2. The influence of 1,4-interactions on the zinc geometry.  $\text{Zn}(\text{HS})_2(\text{NH}_3)\text{OH}^-$  was minimized by molecular mechanics using the zinc parameterization, with (shaded atoms) and without 1,4-interactions in the nonbonded potential.

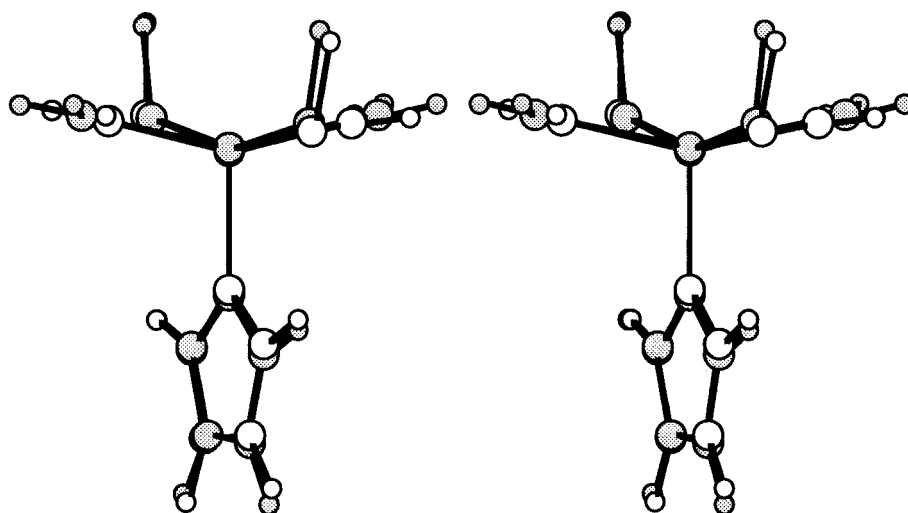


Fig. 3. Performance of the parameterization.  $\text{Zn}(\text{HS})_2(\text{imidazole})(\text{H}_2\text{O})_2$  was optimized by quantum mechanics and by molecular mechanics (shaded) using the zinc parameterization. The root-mean-square deviation is 0.23 Å.

the two negatively charged sulfide ligands make the bond lengths of the other zinc ligands longer than normal. Compared to the parameterization of Merz et al.,<sup>32</sup> the equilibrium values (except the Zn–O distances) are similar, but our force constants are usually slightly larger.

### Simulations of Alcohol Dehydrogenase

Molecular dynamics simulations were performed on seven models of alcohol dehydrogenase, differing in the size of the system (large or small), the coordination number of the zinc ion (four or five), and the nonprotein zinc ligands (water, methanol, formaldehyde, or hydroxide). In addition, at least three molecular mechanical optimizations were performed on each system, differing in the starting structure (the initial structure, the structure at the end of the molecular dynamics simulation, and the structure

with the lowest energy in the molecular dynamics simulation). The results of the simulations are collected in Table VI and are viewed in Figures 4 and 5.

In the molecular dynamics simulations of a four-coordinate zinc ion, the zinc-bond water molecule alternates frequently between two different coordination sites at the zinc ion (changing sites every 7 ps, cf. Fig. 6a). One site is the normal substrate site at the bottom of the substrate cleft, where the molecule may make a hydrogen bond to OG of Ser-48 (Fig. 4a). The other site (Fig. 4b; termed the *alternative site* below) is on the opposite side of the zinc ion, in a narrow cavity buried behind the zinc ion. In this site the molecule may interact with OD of Asp-49, OE of Glu-68, or a crystal water molecule (which is hydrogen bonded to Asp-49). It is usually very close to the walls of the cavity, especially Cys-46 and Glu-68.

**TABLE VI. Results of the Molecular Dynamics Simulations (MD) and the Best (Lowest Energy) Molecular Mechanical Energy Minimizations (MM)\***

System	CN	Ligand	Method	Energy (kJ/mol)	Distance to Zn				
					S46	S174	N67	O1	O2
Small	4	H <sub>2</sub> O	MD						
			100 ps	1561.0	229	225	214	224	
			SD	135	26	26	24	37	
			min.	1013.2	155	164	144	116	
			max.	1859.4	293	307	286	343	
Small	4	OH <sup>-</sup>	MM	-52.7	223	218	211	226	
			MD						
			100 ps	1665.8	224	222	215	192	
			SD	79	20	20	20	16	
			min.	1492.9	164	163	153	143	
Small	4	CH <sub>3</sub> OH	max.	1998.5	287	283	280	232	
			MD						
			100 ps	1462.9	228	219	214	233	
			SD	68	19	20	19	25	
			min.	1254.1	171	161	161	154	
Small	4	CH <sub>2</sub> O	max.	1785.1	277	278	270	308	
			MD						
			100 ps	1445.0	225	219	215	242	
			SD	84	22	19	22	31	
			min.	1230.1	165	170	144	160	
Small	5	(H <sub>2</sub> O) <sub>2</sub>	max.	1761.7	286	273	288	334	
			MD						
			100 ps	1733.7	238	226	208	256	223
			SD	125	23	25	23	35	33
			min.	1435.6	166	150	148	154	125
Large	4	H <sub>2</sub> O	max.	2322.6	317	295	276	353	313
			MM	186.8	226	222	207	243	212
			MD						
			40 ps	346.8	231	224	210	229	
			SD	61	15	14	15	22	
Large	5	(H <sub>2</sub> O) <sub>2</sub>	min.	234.5	192	184	180	179	
			max.	616.1	265	263	241	277	
			MM	-4308.8	225	224	210	226	
			MD						
			40 ps	497.5	234	229	207	250	238
Large	5	(H <sub>2</sub> O) <sub>2</sub>	SD	63	16	16	13	19	20
			min.	385.4	191	187	175	194	197
			max.	726.4	276	266	254	294	300
			MM	-4167.5	224	228	208	244	238

\*The system (large or small), the coordination number (CN), the nonprotein zinc–ligand(s), the energy, and the zinc–ligand distances are listed. For the molecular dynamics simulations, the standard deviation and the minimum and maximum values (after 20 ps equilibration) are also given. O1 is the ligand in the substrate site, O2 the ligand in the alternative site.

In the simulations of a five-coordinate zinc ion with two water ligands, the water molecules occupy both water sites (Fig. 5). The two Zn–O distances are very dissimilar. The small size of the cavity at the alternative water sites forces the Zn–O distance to be short, 2.12–2.38 Å, while the Zn–O distance of the water molecule in the normal substrate binding site is longer: 2.43–2.56 Å. The two water molecules stay in their sites most of the simulation. Yet, they interchange sites twice during a 100 ps simulation, showing that such interchange in fact is possible without dissociation of any of the water molecules.

Since the cavity around the alternative coordination site is quite narrow, it was of great interest to deduce whether other molecules than water may occupy this site. Therefore simulations were performed on the small system with three other ligands of interest for the reaction mechanism of alcohol dehydrogenase, namely, methanol, formaldehyde, and hydroxide ion. Figure 6 shows the distance between the oxygen atom of these ligands and the CD atom of Glu-68. The latter atom is located almost opposite to the substrate site and was kept fixed during the calculation. Therefore, a distance of 3.3–4.0 Å is indic-

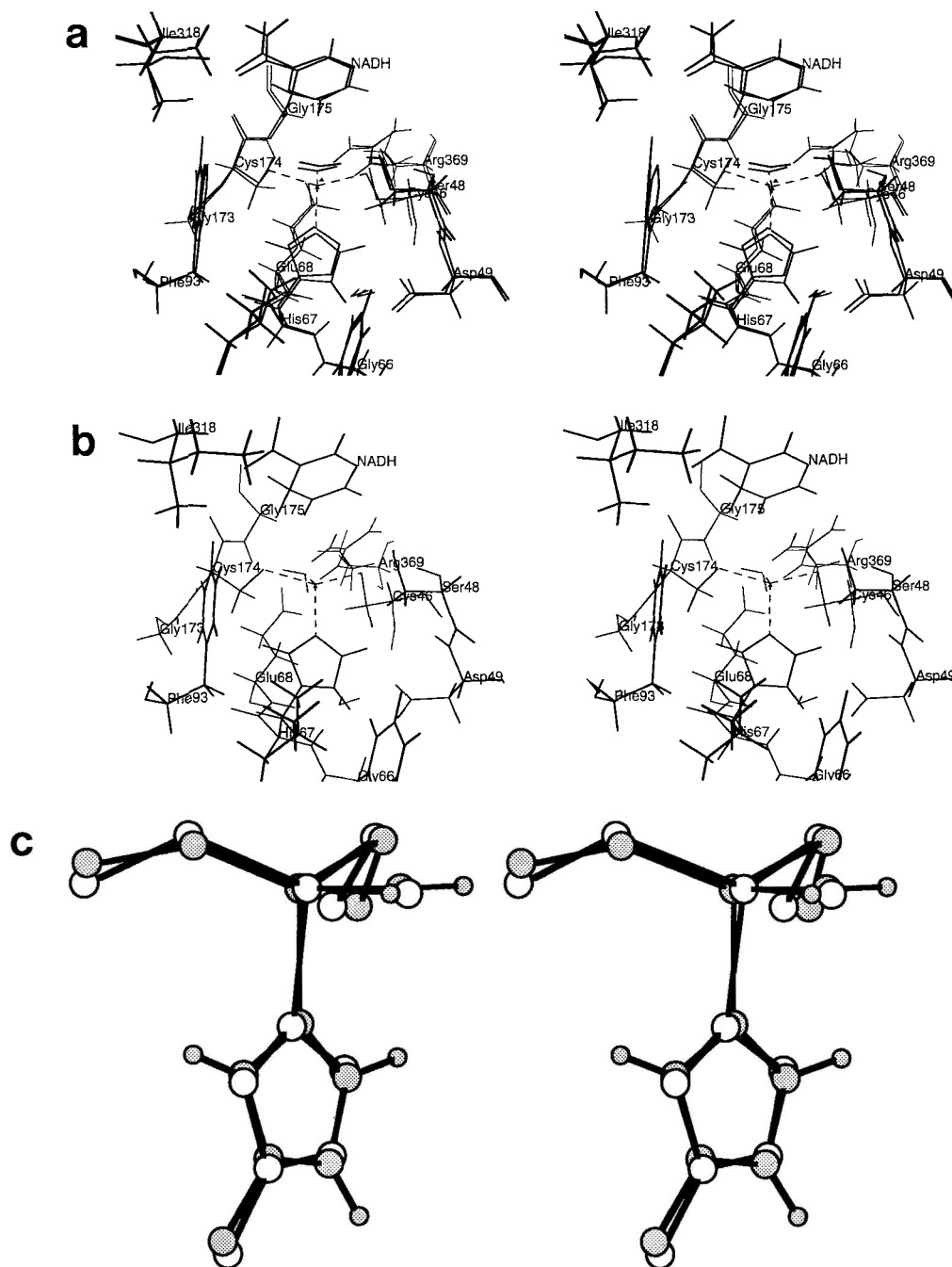


Fig. 4. The energy minimized structure of alcohol dehydrogenase (large system) with a four-coordinate catalytic zinc ion, compared to the crystal structure<sup>4</sup> (without hydrogen atoms). In (a) the water molecule occupies the substrate site, in (b) the alternative site. Only amino acids in the small system are shown. (c) A detail of the zinc coordination sphere in (a). The root-mean-square deviation is 0.16 Å.

ative for coordination at the alternative site, while 6.1–7.1 Å is typical for coordination at the substrate site. With these criteria, it can be seen from Figure 6 that water and the hydroxide ion occupy the alternative site 15 times and once, respectively, during a 100 ps simulation, while methanol and formaldehyde never diffuse into that site during the simula-

tions. Apparently, water is the largest molecule that may occupy the alternative site.

The hydroxide ion is smaller than the water molecule, but has nevertheless much lower occupancy at the alternative site than the water molecule. This is probably due to the negatively charged thiolate groups of the two cysteine ligands, which impede

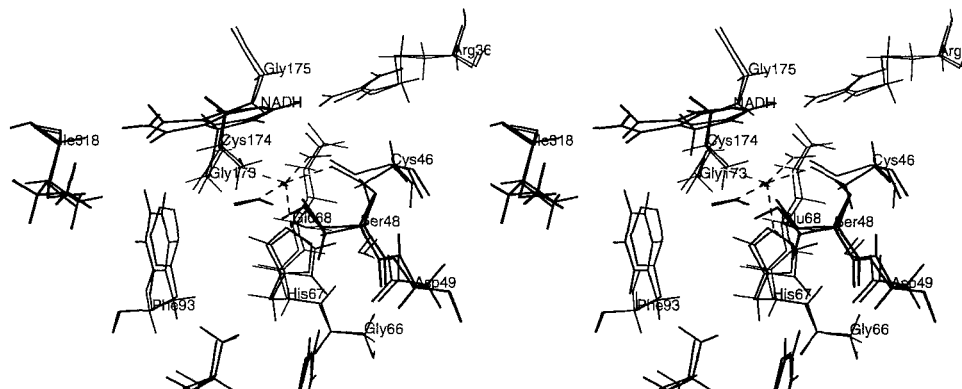


Fig. 5. The energy minimized structure of alcohol dehydrogenase (large system) with a five-coordinate catalytic zinc ion, compared to the crystal structure<sup>4</sup> (without hydrogen atoms). Only amino acids in the small system are shown.

diffusion of the hydroxide ion to the alternative site of the zinc ion. Furthermore, the carboxyl groups of Asp-48 and Glu-68 prevent the hydroxide ion from staying in the alternative site; no potential minimum for the hydroxide ion at the alternative site could be found by molecular mechanical energy minimisations.

### Zinc-Ligand Distances

The average zinc-ligand distances in the molecular dynamics simulations of alcohol dehydrogenase are in excellent agreement with the crystal structure of the enzyme.<sup>4</sup> The Zn-N and Zn-S distances are 2.10–2.15 and 2.19–2.31 Å in the simulations, compared to 2.05–2.14 and 2.21–2.34 in the crystal structure (the two subunits). The Zn-O bond length does not show the same agreement. Yet, this is probably due to the fact that the fourth zinc ligand in the crystal structure is dimethyl sulfoxide (DMSO) and not water. Quantum chemical geometry optimizations of  $\text{Zn}(\text{NH}_3)(\text{SH})_2(\text{DMSO})$  in Table VII show that DMSO binds more strongly to the zinc ion than water; the Zn-O distance is 2.12 Å for DMSO compared to 2.26 Å for  $\text{H}_2\text{O}$  with similar basis sets (Table III). If the difference, 0.14 Å, is added to the Zn-O crystallographic distance, we get 2.29–2.33 Å, which is in reasonable agreement with 2.24–2.29 Å observed in the molecular dynamics simulations of a four-coordinate zinc water molecule. The Zn-ligand distances in the molecular dynamics simulations are usually about 0.05 Å longer than in the molecular mechanical optimizations, as a result of the kinetic energy at 300 K.

In all simulations (except the energy minimizations of the large system with a five-coordinate zinc ion) the Zn-S bond of Cys-46 is longer than the one of Cys-174 while in the crystal structure the state of affairs is the opposite. This discrepancy can probably be attributed to the uncertainty in the crystal coordinates; in the recent 1.9 Å resolution structures

of the free form of liver alcohol dehydrogenase and the 2.1 Å resolution structure of the copper substituted enzyme in complex with NADH and dimethyl sulfoxide,<sup>43,44</sup> the trend is the same as in the calculations.

The molecular dynamics simulations show large fluctuations in the Zn-ligand distances. The Zn-O distance varies  $\pm 45\%$  of the equilibrium distance with a standard deviation of about 0.4 Å, while the Zn-S and Zn-N distances vary  $\pm 35\%$  with a standard deviation of about 0.27 Å. As a comparison the O-H distance of the zinc-bound water molecule varies  $\pm 28\%$  with a standard deviation of 0.09 Å. This reflects the lower force constants of the zinc-ligand bonds, which are intermediate between a normal bond and a hydrogen bond. These large fluctuations in the zinc-ligand distances indicate that ligand exchange at the zinc ion probably proceeds by a dissociative mechanism (old ligand dissociates before the new ligand associates); even in a 100 ps simulation performed with a bonding potential that is unphysically steep at large bond lengths, the maximum Zn-OH<sub>2</sub> distance is 3.4 Å, a normal distance for a second sphere zinc ligand.<sup>28</sup>

### The Inappropriateness of Bond Stretching Constraints

Molecular dynamics simulations are usually performed with bond lengths constrained to the equilibrium value using the SHAKE algorithm.<sup>45</sup> This approximation is based on the assumption that the frequencies of the bond stretching vibrations and the other vibrations are well separated, and it leads to appreciable savings in computer time by allowing a larger time-step. The present results show, however, that such a constraint may restrict the accessible parts of the phase space. For a zinc-bound water molecule to interchange between the two possible ligand sites, concerted changes in the zinc-ligand bond lengths and angles are necessary. If the bond

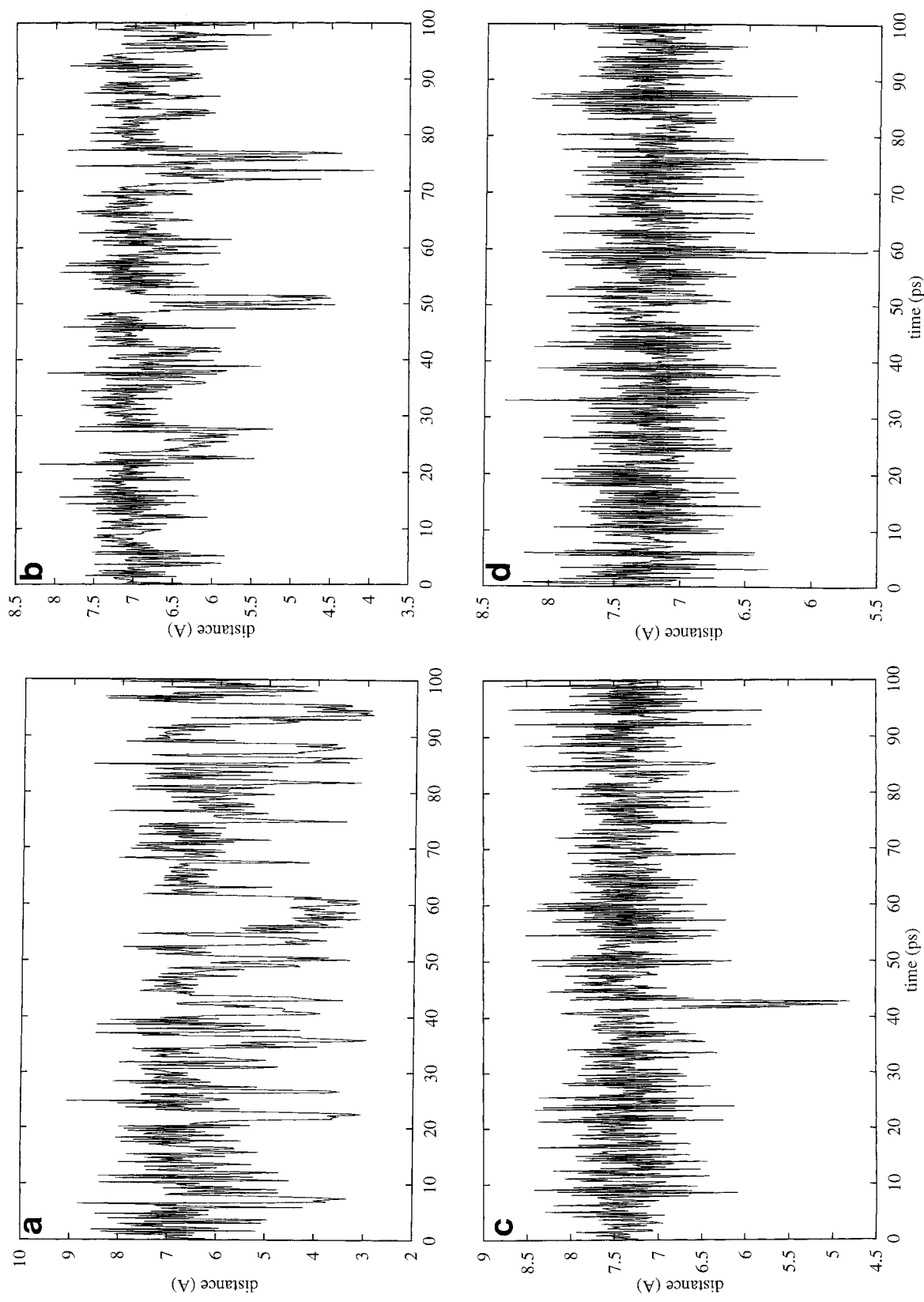


Fig. 6. The fluctuation of the distance between the CD atom of Glu-68 and the oxygen atom of (a) water, (b) hydroxide ion, (c) formaldehyde, and (d) methanol during 100 psec molecular dynamics simulations of the small system of alcohol dehydrogenase. Distances shorter than about 4 Å are indicative for coordination in the alternative zinc site.

TABLE VII. Quantum Chemically Optimized Structures Involving Dimethyl Sulfoxide (DMSO)\*

Complex	Basis (Zn/other)	Energy (H)	Distance to Zn			
			N	S <sub>1</sub>	S <sub>2</sub>	O <sub>1</sub>
A(DMSO)	d $\zeta$ /6-31G(*)	-3181.649259	212	235	238	203
A(DMSO)	d $\zeta$ + p/6-31G*	-3181.793933	218	234	235	212

\*Energies, basis sets, and zinc-ligand distances of the structures are listed. A denotes Zn(HS)<sub>2</sub>(NH<sub>3</sub>). The basis sets are described in the Methods.

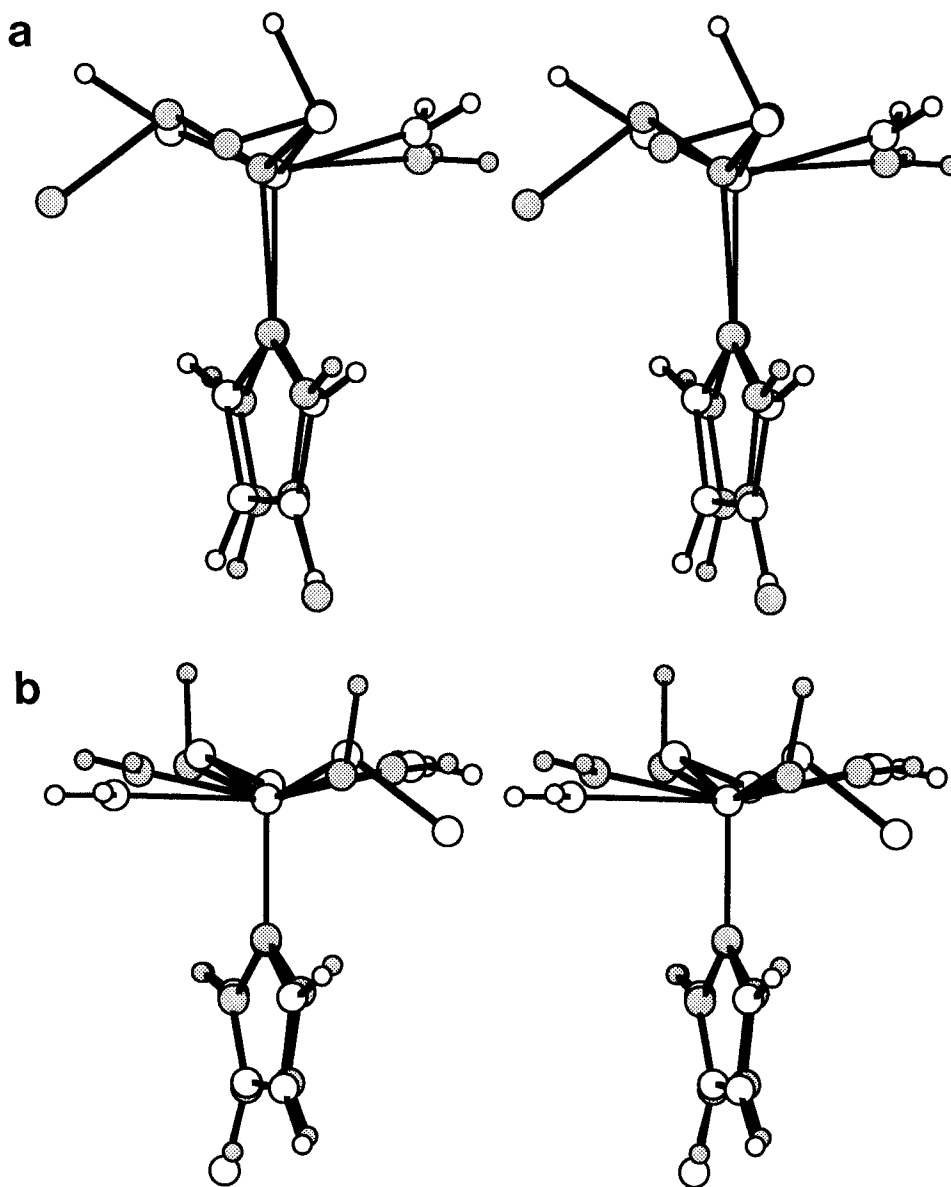


Fig. 7. The geometry of (a) Zn(HS)<sub>2</sub>(imidazole)(H<sub>2</sub>O) and (b) Zn(HS)<sub>2</sub>(imidazole)(H<sub>2</sub>O)<sub>2</sub> optimized by quantum mechanics compared to the corresponding energy minimized structures of the active site of alcohol dehydrogenase (large system; shaded). The root-mean-square deviation is 0.20 and 0.27 Å, respectively.

lengths were constrained, such site-interchange is impossible,<sup>46</sup> and qualitatively incorrect results would be obtained.

The conclusion must therefore be that bond length constraints cannot be used unless it is explicitly shown that the vibrational frequencies are sepa-

rated, and that the accessible phase space is not changed by such constraints. It seems that a double time-step algorithm such as the one used in MUMOD<sup>38</sup> is preferable over constrained dynamic algorithms for the integration of Newton's equations of macromolecular systems.

### The Relative Stability of Four- and Five-Coordinate Zinc Complexes

The present calculations may be used to compare the stability of four- and five-coordinate active-site zinc ions in alcohol dehydrogenase. In the large system, the four-coordinate structure is more stable than the five-coordinate one by 46 kJ/mol in the molecular dynamics simulations and 36 kJ/mol in the molecular mechanics simulations. In the small system, the differences are 67 kJ/mol for the molecular dynamics simulations and 133 kJ/mol for the molecular mechanics simulations, again in favor of the four-coordinate zinc ion. These differences seem mainly to originate from the zinc coordination sphere (the enzyme outside the active site contributes by about 5 kJ/mol). As shown in Figure 7, the five-coordinate structure differs more from the ab initio vacuum structure than the four-coordinate one; the root-mean-square deviations are 0.20 and 0.27 Å, respectively. In particular, the bond between the zinc ion and Cys-46 seems to be strained in the five-coordinate structure.

It must thus be concluded that a four-coordinate zinc ion is more stable than a five-coordinate one. This confirms quantum chemical vacuum calculations<sup>28</sup> and shows that four-coordination of the catalytic zinc ion in alcohol dehydrogenase is preferred not only by the chemical properties of the zinc ion and its ligands, but also by the folding of the enzyme at the active site. This conclusion has strong implications on the reaction mechanism of alcohol dehydrogenase. Several mechanisms have been proposed that involve five-coordinate structures as significant intermediates.<sup>6-13</sup> The present calculations do not support such proposals; since a 46 kJ/mol energy difference corresponds to an equilibrium constant of  $1 \times 10^8$ , five-coordinate structures should be very rare. Furthermore, the dynamic data indicate that even the exchange of zinc ligands proceeds via a three-coordinate (rather than a five-coordinate) intermediate.

Alcohol dehydrogenase is known to assume two different main conformations, an open and a closed one.<sup>1-3</sup> The present calculations are based on a crystal structure of the closed conformation. It cannot be ruled out that all experimental reports of five-coordination refer to another conformation of the protein, and that the barrier between these two forms is too high or the interchange time is too long, to be bridged in the simulations. Yet, all experimental evidence (crystallographic and spectroscopic) agrees on that the free enzyme in the open conformation is

four-coordinate.<sup>2,3,10,16,19-22,24</sup> Further, it is widely assumed that the dehydration of the active site that occurs when the enzyme closes is important for the catalytic mechanisms.<sup>1,2</sup> Thus, if five-coordinate structures obtain in other conformations, the kinetic significance of that is unclear.

### ACKNOWLEDGMENTS

This investigation has been supported by a grant from the Swedish Natural Science Research Council (NFR) and by IBM Sweden under a joint study contract. The protein coordinates were courteously provided by Eila Cedergren-Zeppezauer. The author is indebted to her and to Björn O. Roos, Gunnar Karlström, and Gösta Pettersson for fruitful discussions.

### REFERENCES

- Pettersson, G. Liver alcohol dehydrogenase. *CRC Crit. Rev. Biochem.* 21:349-389, 1987.
- Eklund, H., Brändén, C. I. In: "Zinc Enzymes." Spiro, T. G., ed. New York: John Wiley, 1983: 124.
- Eklund, H., Samama, J.-P., Wallén, L., Brändén, C. I. Structure of a triclinic ternary complex of horse-liver alcohol dehydrogenase at 2.9 Å resolution. *J. Mol. Biol.* 146: 561-587, 1981.
- Al-Karadaghi, S., Cedergren-Zeppezauer, E. S., Petrantos, K., Hovmöller, S., Terry, H., Dauter, Z., Wilson, K. S. Refined crystal structure of liver alcohol dehydrogenase-NADH complex at 1.8 Å resolution. *Acta Cryst. D*, in press.
- Brändén, C. I., Jörnvall, H., Eklund, H., Furugren, B. Alcohol dehydrogenases. *Enzymes* 11:103-190, 1975.
- Dworschack, R. T., Plapp, B. V. pH, isotope, and substituent effects on the interconversion of aromatic substrates catalyzed by hydroxybutyrimidylated liver alcohol dehydrogenase. *Biochemistry* 16:2716-2725, 1977.
- Schmidt, J., Chen, J., DeTraglia, M., Minkel, D., McFarland, J. T. Solvent deuterium isotope effect on the liver alcohol dehydrogenase reaction. *J. Am. Chem. Soc.* 101: 3634-3640, 1979.
- Dunn, M. F., Dietrich, H., MacGibbon, A. K. H., Koerber, S. C., Zeppezauer, M. Investigation of intermediates and transition states in the catalytic mechanisms of active site substituted cobalt(II), nickel(II), zinc(II), and cadmium(II) horse liver alcohol dehydrogenase. *Biochemistry* 21:354-363, 1982.
- Makinen, M. W., Maret, W., Yim, M. B. Neutral metal-bound water is the base catalyst in liver alcohol dehydrogenase. *Proc. Natl. Acad. Sci. U.S.A.* 80:2584-2588, 1983.
- Makinen, M. W., Wells, G. B. Application of EPR saturation methods to paramagnetic metal ions in proteins. *Met. Ions Biol. Syst.* 22:129-206, 1987.
- Dutler, H., Ambar, A. Ligand sphere transitions: A new concept in zinc-enzyme catalysis. In: "The Coordination Chemistry of Metalloenzymes." Bertini, I., Drago, R. S., Luchinat, C., eds. London: Reidel, 1983: 135-145.
- Dutler, H., Ambar, A., Donatsch, J. Function of zinc in liver alcohol dehydrogenase. In: "Zinc Enzymes." Bertini, I., Luchinat, C., Maret, W., Zeppezauer, M., eds. Stuttgart: Birkhauser, 1986: 471-483.
- Merz, K. M., Hoffmann, R., Dewar, M. J. S. Mode of action of carbonic anhydrase. *J. Am. Chem. Soc.* 111:5636-5649, 1989.
- Boiwe, T., Brändén, C. I. X-ray investigation of the binding of 1,10-phenanthroline and imidazole to horse-liver alcohol dehydrogenase. *Eur. J. Biochem.* 77:173-179, 1977.
- Sartorius, C., Dunn, M. F., Zeppezauer, M. The binding of 1,10-phenanthroline to specifically active-site cobalt(II)-substituted horse-liver alcohol dehydrogenase. *Eur. J. Biochem.* 177:493-499, 1988.
- Makinen, M. W., Yim, M. B. Coordination environment of the active-site metal ion of liver alcohol dehydrogenase. *Proc. Natl. Acad. Sci. U.S.A.* 78:6221-6225, 1981.
- Maret, W., Zeppezauer, M., Desideri, A., Morpurgo, L.,



- Rotilio, G. Ligand binding to the blue copper center of horse liver alcohol dehydrogenase. *FEBS Lett.* 136:72–74, 1981.
18. Andersson, I., Maret, W., Zeppezauer, M., Brown, R. D., Koenig, S. H. Metal ion substitution at the catalytic site of horse-liver alcohol dehydrogenase; Results from solvent magnetic relaxation studies. 1. Copper(II) and cobalt(II) ions. *Biochemistry* 20:3424–3432, 1981.
  19. Andersson, I., Bauer, R., Demeter, I. Structural information concerning the catalytic metal site in horse liver alcohol dehydrogenase, obtained by perturbed angular correlation spectroscopy on  $^{111}\text{Cd}$ . *Inorg. Chim. Acta* 67:53–59, 1982.
  20. Bauer, R., Adolph, H. W., Andersson, I., Danielsen, E., Formicka, G., Zeppezauer, M. Coordination geometry for cadmium in the catalytic zinc site of horse liver alcohol dehydrogenase: studies by PAC spectroscopy. *Eur. Biophys. J.* 20:215–221, 1991.
  21. Bertini, I., Gerber, M., Lanini, G., Maret, W., Rawer, S., Zeppezauer, M.  $^1\text{H}$  NMR investigation of the active site of cobalt(II)-substituted liver alcohol dehydrogenase. *J. Am. Chem. Soc.* 106:1826–1830, 1984.
  22. Maret, W., Zeppezauer, M., Sanders-Loehr, J., Loehr, T. M. Resonance Raman spectra of copper(II)-substituted liver alcohol dehydrogenase: A type I copper analogue. *Biochemistry* 22:3202–3206, 1983.
  23. Maret, W., Shiemke, A. K., Wheeler, W. D., Loehr, T. M., Sanders-Loehr, J. Resonance Raman spectroscopy of blue copper proteins: Ligand and coenzyme effects in copper(II)-substituted liver alcohol dehydrogenase. *J. Am. Chem. Soc.* 108:6351–6359, 1986.
  24. Maret, W., Zeppezauer, M. Influence of anions and pH on the conformational change of horse liver alcohol dehydrogenase induced by binding of oxidized nicotinamide adenine dinucleotide: Binding of chloride to the catalytic metal ion. *Biochemistry* 25:1584–1588, 1986.
  25. Corwin, D. T., Fikar, R., Koch, S. A. Four- and five-coordinate cobalt(II) thiolate complexes: Models for the catalytic site of alcohol dehydrogenase. *Inorg. Chem.* 26:3079–3080, 1987.
  26. Pocker, Y., Raymond, K. W., Thompson, W. H. Liver alcohol dehydrogenase: Kinetic and mechanistic studies. In: "Zinc Enzymes." Bertini, I., Luchinat, C., Maret, W., Zeppezauer, M., eds. Stuttgart: Birkhauser, 1986: 435–449.
  27. Maret, W., Makinen, M. W. *J. Biol. Chem.* 266:20636–20644, 1991.
  28. Ryde, U. The coordination chemistry of the catalytic zinc ion in alcohol dehydrogenase studied by ab initio quantum chemical calculations. *Int. J. Quant. Chem.* 52:1229–1243, 1994.
  29. Clementi, E., Corongiu, G., Jönsson, B., Romano, S. Monte Carlo simulations of water clusters around  $\text{Zn}^{++}$  and a linear  $\text{Zn}^{++}\cdot\text{CO}_2$  complex. *J. Chem. Phys.* 72:260–264, 1980.
  30. Vedani, A., Dobler, M., Dunitz, J. D. An empirical potential function for metal centers: application to molecular mechanics calculations on metalloproteins. *J. Comp. Chem.* 7:701–710, 1986.
  31. Vedani, A., Huhta, D. W. A new force field for modelling metalloproteins. *J. Am. Chem. Soc.* 112:4759–4767, 1990.
  32. Hoops, S. C., Anderson, K. W., Merz, K. M. Force field design for metalloproteins. *J. Am. Chem. Soc.* 113:8262–8270, 1991.
  33. Huzinaga, S. Gaussian-type functions for polyatomic systems. I. *J. Chem. Phys.* 42:1293–1302, 1965.
  34. Wachters, A. J. H. Gaussian basis set for molecular wave functions containing third-row atoms. *J. Chem. Phys.* 52:1033, 1970.
  35. Schäfer, A., Horn, H., Ahlrichs, R. Fully optimized contracted Gaussian basis sets for atoms Li to Kr. *J. Chem. Phys.* 97:2571–2577, 1992.
  36. Hehre, W. J., Radom, L., Schleyer, P. v. R., Pople, J. A. "Ab Initio Molecular Orbital Theory." New York: Wiley-Interscience, 1986.
  37. Ahlrichs, R., Bär, M., Häser, M., Horn, H., Kölmel, C. Electronic structure calculations on workstation computers: The program system Turbomole. *Chem. Phys. Lett.* 162:165–169, 1989.
  38. Teleman, O., Jönsson, B. Vectorizing a general purpose molecular dynamics simulation program. *J. Comp. Chem.* 7:58–66, 1986.
  39. Fletcher, R. "Practical Methods of Optimization." Chichester: John Wiley, 1987: 33–39, 80–87.
  40. Weiner, S. J., Kollman, P. A., Nguyen, D. T., Case, D. A. An all atom force field for simulations of proteins and nucleic acids. *J. Comp. Chem.* 7:230–252, 1986.
  41. Bio-Xplor, Version 1.0, New York: Biostructure Inc., 1991.
  42. Ryde, U. The coordination of the catalytic zinc ion in alcohol dehydrogenase studied by combined quantum chemical and molecular mechanical calculation. *J. Comp. Chem.*, submitted.
  43. Al-Karadaghi, S., Cedergren-Zeppezauer, E. S., Dauter, Z., Wilson, K. S. Refined crystal structure of Cu-substituted alcohol dehydrogenase at 2.1 Å resolution. *Acta Crystallogr. D.*, in press.
  44. Al-Karadaghi, S., Cedergren-Zeppezauer, E. S., Dauter, Z., Lamzin, V., Wilson, K. S. Manuscript in preparation.
  45. van Gunsteren, W. F., Berendsen, H. J. C. Algorithms for macromolecular dynamics and constraint dynamics. *Mol. Phys.* 34:1311–1327, 1977.
  46. Ryde, U. Unpublished results, 1993.