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Coordination geometry for cadmium in the catalytic zinc site of horse liver alcohol dehydrogenase: studies by PAC spectroscopy*

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Abstract. Active site substituted Cd(II) horse liver alcohol dehydrogenase has been studied by Perturbed Angular Correlation of Gamma rays Spectroscopy during turnover conditions for benzaldehyde and 4-trans-(N,N-dimethylamino)cinnamaldehyde. The ternary complex between alcohol dehydrogenase NAD^+ and Cl^-, and the binary complex between alcohol dehydrogenase and orthophenanthroline have also been studied. The Nuclear Quadrupole Interaction parameters have been interpreted in terms of different coordination geometries for Cd(II) in the catalytic zinc site of the enzyme. Calculation of the nuclear quadrupole interaction for cadmium in the catalytic site of the enzyme with and without coenzyme, based upon the four coordinated geometries determined from X-ray diffraction, agrees with the experimentally determined values. The ternary complexes between enzyme, NAD^+ and either Cl^- or trifluoroethanol and the binary complex between enzyme and orthophenanthroline have almost identical spectral parameters which are not consistent with a four coordinated geometry, but are consistent with a five coordinated geometry. The non-protein ligands for the ternary complex with trifluoroethanol are suggested to be an alkoxide group and a water molecule. The Nuclear Quadrupole Interaction parameters for the productive ternary complex between enzyme, NADH and an aldehyde is consistent with the four coordinated geometry predicted from X-ray diffraction data having the carbonyl group of the aldehyde substituting the water molecule as ligand to the metal.

Key words: Perturbed angular correlation – Cadmium – Catalytic site – Alcohol dehydrogenase – Substrate

Introduction

The zinc in the catalytic site in horse liver alcohol dehydrogenase (LADH) can be replaced by cadmium (Andersson et al. 1982). The coordination geometry for cadmium in the catalytic site of LADH (Cd_2Zn_2LADH) is similar to the coordination geometry for zinc, except for the longer cadmium-ligand bond lengths relative to the zinc ligand bond lengths (Schneider et al. 1985). Furthermore, the cadmium derivative is catalytically active. Cd-substituted LADH derivatives have previously been studied by ^{113}Cd NMR (Bobsein and Myers 1980, 1981) and by ^{111}Cd Perturbed Angular Correlation of Gamma rays (PAC) (Andersson et al. 1982).

The involvement of the active site metal ion in catalysis has been studied for the Zn(II), Co(II), Ni(II) and Cd(II) derivatives by following the spectral changes of the absorption spectra for the aldehyde, 4-trans-(N,N-dimethylamino)cinnamaldehyde (DACA), during catalysis and by following the spectral changes of the visible absorption of the Co(II) enzyme (Dunn et al. 1982; Sartorious et al. 1987).

For ^{111}Cd PAC it has been shown that calculations of the nuclear quadrupole interaction (NQI) via the Angular Overlap Model (AOM), based upon semi-empirical ligand parameters and knowledge about the metal geometry, can predict the NQI parameters derived from PAC measurements (Bauer et al. 1988). The semi-empirical ligand parameters, called partial NQI, have been obtained for a series of biologically relevant ligands such as, for example, H_2O and imidazole (Bauer et al. 1988). If the geometry if known it is possible to obtain knowledge

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Abbreviations: LADH, Horse liver alcohol dehydrogenase; H_4Zn_2LADH, derivative of LADH free of zinc in the catalytic site; $^{111}Cd Zn_2$LADH, derivative of LADH with ^{111}Cd (carrier free) in the catalytic site; Cd_2Zn_2LADH, derivative of LADH with 2 mole of Cd(II) per mole LADH in the catalytic site; PAC, perturbed angular correlation of gamma rays; NQI, Nuclear quadrupole interaction; AOM, Angular overlap model; trifluoroethanol, 2,2,2-trifluoroethanol; DACA, trans-4-(N,N-dimethylamino)cinnamaldehyde; NAD^+ and NADH, oxidized and reduced nicotinamide adenine dinucleotide; $NADH_2$, reduced 1,4,5,6-tetrahydronicotinamide adenine dinucleotide

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about the types of ligands. Alternatively, if the geometry is unknown but the ligands are known it is possible to obtain information about the coordination geometry. An NQI calculated from the coordination geometry, derived from X-ray diffraction data on bovine Cu_2Zn_2 superoxide dismutase (with Zn(II) substituted by Cd(II)), agreed with the NQI determined from PAC experiments.

In the present work we have performed PAC measurements on $^{111}CdZn_2$LADH during catalysis of aldehyde reduction. The results are analysed within the AOM and conclusions about the coordination geometries for the Cd(II) ion are drawn. The possible relevance to the mechanism of the enzyme is discussed.

Experimental

Horse liver alcohol dehydrogenase (EC 1.1.1.1) and coenzymes were purchased from Boehringer/Mannheim/FRG and the enzyme was recrystallized prior to use. All other reagents were the purest available commercially. LADH depleted of zinc in the catalytic site (H_4Zn_2LADH) was prepared in the crystalline state as described earlier (Maret et al. 1979). H_4Zn_2LADH was dissolved shortly before (within 1 h) addition of ^{111}Cd (carrier free). All manipulations with H_4Zn_2LADH were done under nitrogen or argon atmosphere. All glassware was cleaned by soaking in 1:1 nitric and sulfuric acids, followed by rinsing in metal-free distilled water. Enzymatic activity was measured by the method of Dalziel (1957).

The ^{111}Cd isotope was produced by bombardment of metallic Pd (98% enriched ^{108}Pd) with 21 MeV α particles using the Niels Bohr Institutes' Tandem accelerator and cyclotron. The ^{111}Cd isotope exists in its 497 keV excited state with a $T_{1/2}$ of 49 min. The ^{111}Cd was isolated from ^{108}Pd as described elsewhere (Bauer et al. 1991).

$^{111}CdZn_2$LADH was prepared by adding either 0.1 mg H_4Zn_2LADH (for substrate experiments) or 1 mg H_4Zn_2LADH to 200 µl ^{111}Cd (1–10 picomole) in 0.1 *M* HEPES, pH 7. After 5 min this solution was passed over a Sephadex G25 column, equilibrated with 50 m*M* MES, pH 6.0. Judged from the overlap between the ^{111}Cd radioactive counts and protein absorption at 280 nm more than 80% of the added Cd was bound to H_4Zn_2LADH. This separation procedure was used throughout. For a PAC experiment, 850 µl of ^{111}Cd(II) Zn_2LADH from the column were transferred to a solution of 3.1 g sucrose dissolved in 2 ml of the desired buffer. All PAC experiments were carried out at 4 °C. The pH of each sample was measured at room temperature, after the experiment. For PAC experiments, performed during turnover of the substrate, the substrate and coenzyme concentrations were chosen to be equal and the substrate concentrations were 5 and 20 times greater than the corresponding K_d values for DACA and benzaldehyde, respectively (Dunn et al. 1982; Kvassman and Pettersson 1980).

Cadmium-D-penicillamine crystals were prepared as described by Freeman et al. (1976). ^{111}Cd was added to Cd(II)Cl_2 before addition of D-penicillamine. The resulting randomly oriented crystals, containing more than 80% of ^{111}Cd, were used for PAC experiments. The crystals were proven to consist of the same type of crystals as used in the three dimensional structure determination by carrying out X-ray diffraction measurements on the polycrystalline PAC sample after the PAC experiment.

The diffractograms were taken on the powdered sample with the X-ray spectrometer at the Institute of Chemistry at the Agricultural University of Denmark.

PAC setup

PAC spectra were recorded with a four-detector fast-slow coincidence spectrometer. The theory and technique of PAC and its application to the studies of metalloenzymes have been described elsewhere (Bauer 1985). The coincident counting rate $W(\theta, t)$ was measured at 180° and 90° as a function of the delay time t between the emission of the two γ rays. θ is the angle between the two γ rays. From the experimental ratio between the coincident rates at 180° and 90°, the NQI parameters ω_0 and η were derived (Bauer 1985). A least χ^2 procedure was then applied to this experimental ratio compared to the following theoretical function

$$\frac{W(180°, t)}{W(90°, t)} = b\left(\frac{1 + A_2 G_2(t, \omega_0, \eta, \delta)}{1 - \frac{1}{2} A_2 G_2(t, \omega_0, \eta, \delta)}\right) \quad (1)$$

where A_2 is a constant dependent upon the properties of the emitted γ rays and detectors and source geometries and b is baseline shift. $G_2(t, \omega_0, \eta, \delta)$ represents the effect of the NQI on the PAC spectrum (Bauer 1985). δ represents a relative Gaussian spread of ω_0 values. No effect of rotational diffusion could be detected, owing to the high viscosity of the solution and the high molar weight for alcohol dehydrogenase. The function $G_2(t, \omega_0, \eta, \delta)$ was corrected for the finite time resolution of the spectrometer, which was equal to 3 ns. The following parameters A_2, b, ω_0, η and δ were least square fitted for all PAC spectra included in this work, except for penicillamine where no frequency distribution could be observed. The χ^2 minimum values for the baseline shift b deviated by less than 2% from 1.

AOM analysis of the NQI parameters

The interaction observed in ^{111}Cd PAC spectroscopy is the hyperfine interaction between the nuclear quadrupole moment and the electric field gradient at the center of the nucleus. This represents the nuclear quadrupole interaction (NQI). For spin 5/2, ω_0 is defined as

$$\omega_0 = \left|\frac{12\pi e Q V_{zz}}{40 h}\right| \quad (2)$$

where e is the unit charge, Q the nuclear quadrupole moment of the 5/2 state of ^{111}Cd, V_{zz} the electric field gradient at the ^{111}Cd nucleus and h is Planck's constant.

Theoretical calculation of the NQI parameters ω_0 and η for cadmium(II) complexes can be performed within the Angular Overlap Model (AOM) (Bauer et al. 1988). The

NQI, which is a tensor, can according to Bauer et al. (1988) be written as

$$\omega_{a,b} = \sum_i \omega_i \left(\tfrac{3}{2}\alpha_i \beta_i - \tfrac{1}{2}\delta_{a,b}\right) \quad (3)$$

where $a, b = x, y$ or z are Cartesian coordinates, $\alpha_i, \beta_i = x_i$, y_i or z_i are directional cosines from the z-axis of the ith ligand to the x, y or z direction and ω_i are a partial NQI parameter for the ith ligand. Thus the ligands affect the NQI tensor in (3) by the set of values for ω_i, x_i, y_i and z_i which characterize the ligand geometry. A coordinate system, where the tensor in (3) is diagonal, can be chosen and in this coordinate system we get the two NQI parameters as

$$\omega_0 = \omega_{zz}$$

$$\eta = \left|\frac{\omega_{xx} - \omega_{yy}}{\omega_{xx} + \omega_{yy}}\right| \quad (4)$$

These two NQI parameters ω_0 and η are those derived from the least χ^2 fitting to the PAC spectra using Eq. (1). The NQI resulting from a hypothetical complex with only one ligand with a partial NQI parameter equal to ω_i can be derived from (3) in the coordinate system with the metal-ligand direction as z-axis. The resulting NQI parameters are: $\omega_0 = \omega_i$ and $\eta = 0$.

A critical assumption for the application of the AOM is the additivity of the effect from each ligand expressed as the summation over i in the tensor stated in (3). The validity of this assumption is verified for a set of Cd(II) complexes in Bauer et al. (1988). This means that the NQI can be calculated from the partial NQI parameters for the ligands involved, irrespective of the coordination geometry. It follows from (3) that the NQI is sensitive both to the type of ligand and to its angular position in the Cd(II) complex. For NQI calculations for $^{111}CdZn_2LADH$ the coordination geometries derived from X-ray diffraction data were used (Eklund et al. 1986). However, in some cases a water molecule coordinated to cadmium(II) was positioned opposite *his*67. Furthermore, angular deviations from the geometry from X-ray diffraction data, for *cys*174 and the solvent site, were also tried. As the standard deviations in angles are stated as 5° (Schneider et al. 1985), a variation of up to 10° in the position of *cys*174 and in the position of the solvent ligand site were considered acceptable.

Results

PAC measurements have been performed on ^{111}Cd Zn_2LADH in the presence of NADH plus DACA, NADH plus benzaldehyde, $NADH_2$ plus DACA and orthophenanthroline. In addition a PAC experiment on Cd(II)-D-penicillamine was carried out. The experimental conditions and corresponding NQI parameters are described in Table 1. Spectra from $^{111}CdZn_2LADH$ in the presence of either NADH alone or NADH plus DACA are shown in Fig. 1 and spectra from $^{111}CdZn_2LADH$ in the presence of either orthophenanthroline or NAD^+ plus Cl^- ions are shown in Fig. 2.

In the case of the ternary complex with either aldehydes or Cl^- ions a Gaussian frequency distribution of $4.0 \pm 0.5\%$ was derived. For the binary complex between $^{111}CdZn_2LADH$ and either orthophenanthroline or coenzyme, a $9 \pm 1\%$ frequency distribution was needed to fit the PAC spectra. This is the major cause of the damping of oscillations observed in the PAC spectra for the binary complexes (Figs. 1 and 2, upper panels).

The NQI parameters for the ternary chloride complex are virtually identical to those of the ternary complex with NAD^+ and trifluoroethanol (Andersson et al. 1982). The NQI parameters in the presence of orthophenanthroline are also close to the NQI parameters for the ternary trifluoroethanol complex.

For the PAC experiment performed during turnover of DACA, the absorption at 398 nm was measured immediately before and immediately after the PAC experiment. The result was, that less than 55% of DACA were converted during collection of PAC data. For the PAC experiment performed during active turnover of benzaldehyde the absorption at 340 nm, equivalent to the peak absorption of NADH, was measured before and after the PAC experiment. According to these measurements, less than 70% of NADH was converted during PAC data collection.

Table 1. Experimental conditions and NQI parameters

Coenzyme, substrate, inhibitor	pH	Buffer (50 m*M*)	ω_0 (Mrad/s)	η
80 μ*M* NADH, 80 μ*M* DACA, –	6.1	MES	348±13	0.72±0.06
80 μ*M* NADH, 80 μ*M* DACA, –	9.4	Glycine	356±10	0.72±0.06
80 μ*M* $NADH_2$, 80 μm DACA, –	6.2	MES	369±17	0.64±0.07
10 m*M* NADH, 10 m*M* benzaldehyde, –	6.3	MES	395±17	0.62±0.08
10 m*M* NADH, 10 m*M* benzaldehyde, –	8.3	TES	398±20	0.63±0.07
10 m*M* NADH, 10 m*M* benzaldehyde, –	9.3	Glycine	407±10	0.59±0.07
–, –, 10 m*M* orthophenanthroline	8.0	TES	118± 3	0.60±0.04
2 m*M* NAD^+, –, 1 *M* Cl^-	6.3	MES	138± 3	0.91±0.04

Average NQI parameters for ternary NADH-aldehyde-$^{111}CdZn_2LADH$ complexes			NQI parameters for Cd-D-penicillamine	
Substrate	ω_0 (Mrad/s)	η	ω_0 (Mrad/s)	η
DACA	354±5	0.71±0.03	289±3	0.38±0.02
Benzaldehyde	403±8	0.62±0.05		

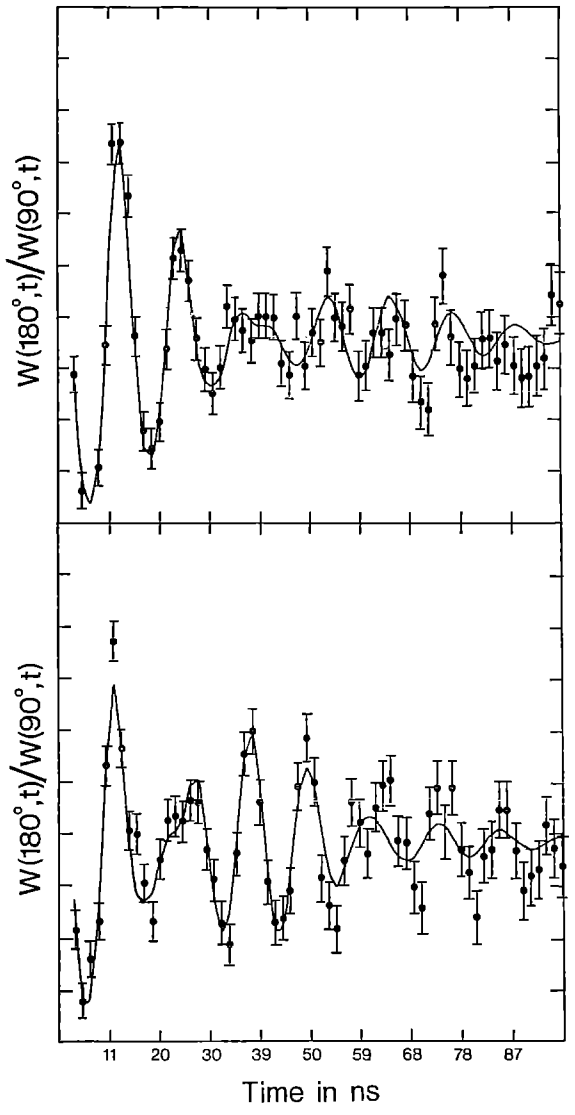

Fig. 1. PAC spectra from $^{111}CdZn_2LADH$ plus coenzyme (average of three spectra, upper panel) and $^{111}CdZn_2LADH$ plus NADH and DACA (average of three spectra, lower panel). The full drawn lines are least square fits to (1). The corresponding NQI parameters are listed in Tables 1 and 5

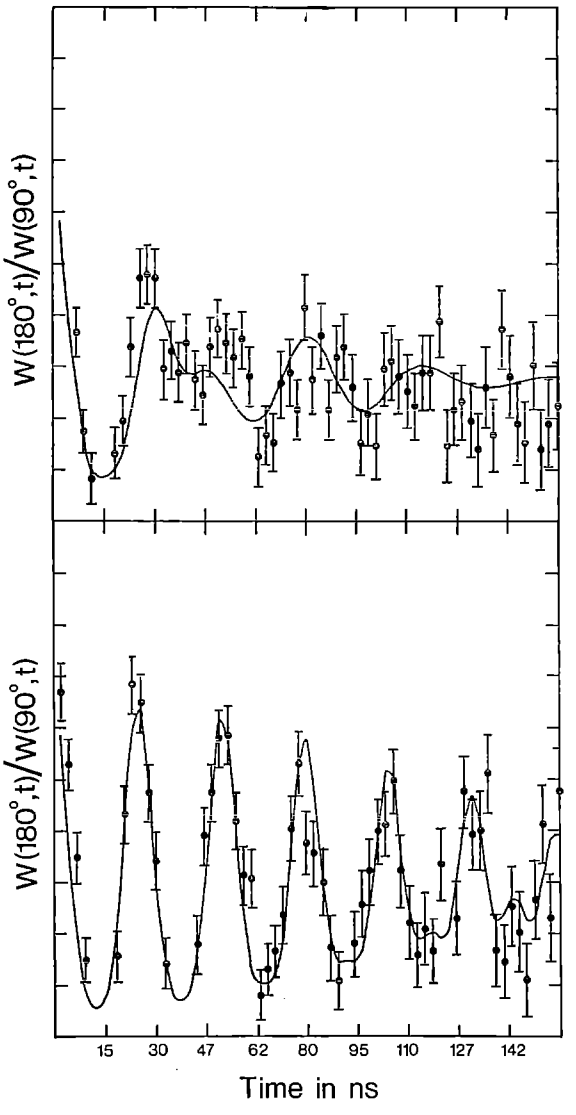

Fig. 2. PAC spectra from $^{111}CdZn_2LADH$ plus orthophenanthroline (upper panel) and $^{111}CdZn_2LADH$ plus NAD^+ and Cl^- (lower panel). The full drawn lines are least square fits to (1). The corresponding NQI parameters are listed in Table 1

A PAC experiment performed with excess DACA relative to NADH, and after all NADH was converted to NAD^+, gave a spectrum identical to that measured under turnover conditions. This means that the metal geometry for the ternary complex between DACA (either as aldehyde or corresponding alcohol) and NAD^+ is identical to the metal geometry for the ternary complex between DACA and NADH. However, a spectrum obtained under conditions of excess NADH relative to DACA with all DACA reduced to the corresponding alcohol gave a spectrum identical to the binary complex between LADH and NADH. The same result is obtained from spectra produced in the presence of NAD^+ and ethanol. No spectral difference between spectra from NADH plus DACA and $NADH_2$ plus DACA was observed, which proves that the Cd(II) geometry is identical (within the limits of error) for intact DACA and for DACA present under steady state turnover conditions (Table 1).

AOM analysis

The coordination geometry of Cd(II)-D-penicillamine is given in Table 2. The only unknown parameter in an AOM calculation of the NQI for Cd(II)-D-penicillamine is the partial NQI parameter for the mercapto group in penicillamine. The uniquely defined value for the partial NQI parameter for the mercapto group, which fits the experimentally determined NQI parameters (Table 1) is shown in Table 3. The fact that the sulphur atoms in Cd(II)-D-penicillamine bind to two cadmium atoms is not expected to influence the partial NQI parameter, because the partial NQI parameter for the Cl^- ion was identical for two different complexes in which the Cl^- ions were bound to either one or two cadmium ions respectively (Bauer et al. 1988). Table 3 lists the relevant partial NQI parameters used in the AOM calculation of NQI for $^{111}CdZn_2LADH$ and [^{111}Cd]Cd(II)-D-penicillamine.

Table 2. Coordination geometry in polar angles for Cd-D-penicillamine[a]

Ligand[c]		θ	φ
S	(mercapto)	0[b]	0[b]
S′	(mercapto)	171.9	−146.3
N	(amino)	80.7	0[b]
O^1	(carboxylate)	100.6	106.7
O^2	(carboxylate)	80.6	−153.6
$O^{2'}$	(carboxylate)	93.3	−81.0

[a] Personal communication G.G. Stevens, F. Huq and H.C. Freeman
[b] Choice of coordinate axes
[c] The ligands are a mercapto group, a primary amino group and a carboxylate group. The carboxylate group coordinates bidentate. For the structure see Freeman et al. (1976)

Table 3. Partial NQI parameters for ligands relevant to alcohol dehydrogenase

Ligand		Partial NQI parameter
Histidine	$(N)^a$	95± 4
Cysteine	$(S^-)^b$	300± 2
Water	$(O)^a$	207±11
Carbonyl	$(O)^a$	161± 8
Chloride	$(Cl^-)^a$	231± 5
Carboxylate	$(O^-)^{a,c}$	245± 5
Carboxylate	$(O^-)^{a,d}$	175± 5

[a] Bauer et al. (1988)
[b] This work
[c] monodentate
[d] bidentate

Table 4. Average metal coordination geometries for the catalytic site in apo and holo enzyme in polar coordinates. The data are derived from Eklund et al. (1986)

Angle	Enzyme	*his*67	*cys*46	*cys*174	H_2O
Polar	apo	0	106	109	100
Azimuthal	apo	0	0	143	246
Polar	holo	0	105	108	93
Azimuthal	holo	0	0	144	259

Table 5. Calculations of NQI parameters based on the geometry for the protein ligand in Table 4

Enzyme	ω_0 (calc)	η (calc)	ω_0 (exp)[a]	η (exp)[a]
Apo	291	0.54	268	0.87
Holo	305	0.66	330	0.82

[a] from Andersson et al. (1982)

Table 6. Angular movement required for the polar angle of the *cys*174- and solvent site ligand

Enzyme/substrate/inhibitor	$\Delta\theta$ (*cys*174)	$\Delta\theta$ (solvent)	ω_0 (calc)	η (calc)
Apo, –, –	4	4	260	0.81
Holo, –, –	6	6	323	0.81
Holo, daca, –	7	8	345	0.66
Holo, benzaldehyde, –	7	7	364	0.52
Holo, Cl^-, –	22	21	151	0.96
Holo, trifluoroethanol, –	23	21	134	0.82

The three different coordination geometries for the apo enzyme (without coenzyme) with either Zn(II), Co(II) or Cd(II) in the catalytic metal site are identical within the error limits of the X-ray diffraction data (Schneider et al. 1985). Table 4 shows the corresponding average set of ligand angles, transformed to polar angles with *his*67 as the *z*-axis and *cys*46 as the *x*-axis. The two ternary geometries (holo enzyme) for pyrazole and bromobenzylalcohol found by Eklund et al. (1986) are likewise averaged in angles. The result is given in Table 4. Table 5 lists the NQI parameters for the average geometry stated in Table 4 for the apoenzyme and holo enzyme, choosing water as the solvent ligand. The calculated ω_0 values are in good agreement with the experimental values. The η values are, however, significantly different from the experimental values. Table 6 gives the NQI parameters calculated by varying the angular positions for the *cys*174 and the solvent site ligand position and choosing the NQI parameters which come closest to the experimentally determined NQI parameters. As can be seen in all cases, except for the ternary complexes with either trifluoroethanol or Cl^-, less than 10° deviation from the X-ray angles (Table 4) is necessary to achieve agreement with the experimental values. This is consistent with the accuracy stated in Schneider et al. (1985).

For the ternary complex between LADH, coenzyme and an aldehyde, the solvent ligand is taken as a carbonyl group. The corresponding partial NQI parameter is given in Table 3. The data with trifluoroethanol and orthophenanthroline cannot be directly calculated within the AOM because no partial NQI parameters have been determined for these ligands. However, it appears reasonable to choose the value for a singly coordinated carboxylate group (Table 3) for a charged alkoxide group as they are both negatively charged oxygen, non-aromatic and bonded to a carbon atom. For orthophenanthroline, however, no obvious choice exists. For the ternary complex with Cl^- we know the partial NQI parameter (Table 3).

In the case of ternary complexes with trifluoroethanol and Cl^-, calculated values for the NQI parameters were in agreement with the corresponding experimental values if an additional fifth water ligand was positioned opposite *his*67.

Discussion

From X-ray diffraction data on LADH it has been concluded that the active site zinc ion has a nearly tetrahedral coordination geometry with cysteine 46, cysteine 174 and histidine 67 as protein ligands and a solvent molecule as a fourth ligand (Eklund et al. 1986). It was also deduced that the metal geometry has the same number and types of ligands in the presence of coenzyme, i.e. the coenzyme does not bind to the metal (Eklund et al. 1986). X-ray diffraction measurements on ternary complexes for NADH and bromobenzylethanol (Eklund et al. 1982) and for $NADH_2$ and DACA (Cedergren-Zeppezauer et al. 1982) showed that the substrates coordinate directly to zinc with the oxygen atom of either an alkoxide or carbonyl group respectively.

The geometry is very close to a regular tetrahedral geometry with two cysteines, one histidine and a solvent molecule as ligands. The only significant deviation from tetrahedral geometry occurs for the cysteine-metal-cysteine angle which is about 130°. The cadmium derivative exhibits a catalytic activity for ethanol oxidation (at pH 10) of the order of 10% of that of the native enzyme (Sytkowsky and Vallee 1979). This tenfold decrease of activity has been shown to be related to a correspondingly reduced rate of dissociation of the coenzyme (Zeppezauer 1986). For the reduction of aldehydes to alcohols however, the rate of hydride transfer is different for the cadmium enzyme relative to that for the zinc enzyme (Dunn et al. 1982).

The present AOM analysis of the NQI parameters for various cadmium derivatives of LADH shows that the experimental NQI parameters for the apoenzyme can be calculated with the geometry, within the limits of error of the angles, determined by X-ray diffraction. The NQI parameters for the ternary complexes with NADH and aldehydes can also be fitted, with the limits of error, by the X-ray geometry. However, the NQI parameters for the ternary complex with NAD^+ and Cl^-, with Cl^- coordinated at the solvent site, cannot be calculated, within the limits of error, for the angles determined from X-ray diffraction data (Tables 4 and 5). Adding a second chloride ion as a fifth ligand opposite to *his*67 does, however, result in a calculated NQI in agreement with the experimental set of NQI parameters. Additional support for such a five coordinated geometry derives from the fact that the binary complex between $^{111}CDZn_2LADH$ and orthophenanthroline has an NQI very close to that of the ternary Cl^- complex. With respect to the X-ray structure of the binary complex of the zinc enzyme with orthophenanthroline (Boiwe and Branden 1977) it is also reasonable to assume that Cd_2Zn_2LADH forms a five coordinated structure with this ligand.

The characteristic feature of the inhibitor complexes deduced to be five coordinated is the common reduction of the ω_0 value by more than a factor of 2 relative to the ω_0 value for the binary complex of $^{111}CdZn_2LADH$ and coenzyme.

Figure 3 shows the variation of the NQI parameters ω_0 and η as a function of the partial NQI parameter of the fourth ligand, based on the average ternary geometry

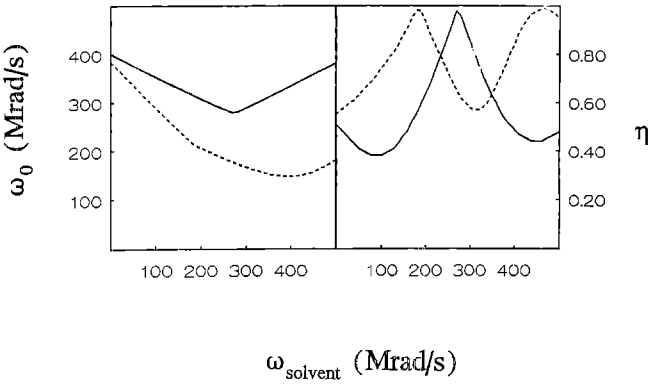

Fig. 3. Calculated NQI parameters ω_0 and η as a function of the partial NQI parameter for the solvent site ligand using the geometry given for the holo enzyme in Table 4 with (broken lines) or without (full drawn lines) a water molecule coordinated opposite *his*67 as a fifth ligand

given in Table 4 with or without a water ligand opposite *his*67. From this figure it is evident that achieving a low ω_0 value is not possible with any ligand at the fourth coordinating site. It is also evident, that low ω_0 values can be achieved by adding a water molecule as a fifth ligand.

From the X-ray diffraction analysis of native alcohol dehydrogenase, no zinc bound water molecule has been observed under conditions where either a substrate, trifluoroethanol, pyrazole or imidazole is bound to Zn(II) (Eklund et al. 1986).

Whether the metal ion is four or five coordinated during catalysis is of importance because mechanisms have been proposed for the native enzyme where the metal ion is five coordinated (Dworschak and Plapp 1977; and Dutler et al. 1986).

The NQI parameters for $^{111}CdZn_2LADH$, the binary complex between $^{111}CdZn_2LADH$ and coenzyme, and the ternary complex between $^{111}CdZn_2LADH$, NADH and aldehydes are in agreement with the four coordinated metal site structure as deduced from X-ray data of the cadmium enzyme (Eklund et al. 1986). It should further be stressed that the present result also agrees with the ligand position of substrates/inhibitors at the fourth tetrahedral position as found in the X-ray diffraction analysis (Eklund et al. 1986).

Our PAC data provide strong evidence for a five coordinated Cd(II) ion in the binary complex with orthophenanthroline and in the ternary complexes of this enzyme with NAD^+ and either trifluoroethanol or chloride ions. By inference, the Cd(II) ion in the productive complex Cd_2Zn_2LADH-NAD^+-alcohol may have a similar structure. This does not necessarily imply similar coordination environments in the native and in the Co(II) substituted enzymes. In fact the primary kinetic isotope effects for the hydride transfer step with DACA as substrate are 2.9 and 2.8 for Zn(II) and Co(II) in contrast to 1.7 for the Cd(II) enzyme (Dunn et al. 1982). This has been taken as evidence for a different structure of the metal ion environment in the transition state of the ternary complex interconversion in Cd_2Zn_2LADH. To derive information about whether this is so, we plan to perform experiment with alcohols under conditions where the hydride transfer

step is rate limiting (for ethanol the rate limiting step is the dissociation of NADH) in order to decide whether the steady state ternary (Cd_2Zn_2LADH-NAD^+-alcohol complex is five coordinated or not.

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Note added in proof. In the three dimensional structure of alcohol dehydrogenase, in its closed conformation with bound coenzyme and inhibitor, determined by X-ray diffraction to 1.8 Å resolution, a simple rotation of *glu*68 can bring one of the carboxyl group oxygen atoms at 2.4 Å distance to the active site metal atom. This buried, acidic residue could be considered a potential candidate for delivering a fifth ligand to Cd(II), when substituted for Zn(II) in the catalytic site, as an alternative to a water molecule (Eila Cedergren-Zeppezauer, personal communication).