Structural Information Concerning the Catalytic Metal Site in Horse Liver Alcohol Dehydrogenase, Obtained by Perturbed Angular Correlation Spectroscopy on ^{111}Cd

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Perturbed angular correlation spectra on horse liver alcohol dehydrogenase, measured on ^{111}Cd inserted specifically in the catalytic site, have been obtained under various conditions. Spectra have been obtained in the pH range 6–9 and in the presence of coenzyme and/or pyrazole or trifluoroethanol. No ionization at the metal site could be detected between pH 6 and 9, irrespectively of whether NAD^+ is present or not. From this we conclude that a water molecule ligated to Cd(II) must have a pK of ionization higher than 9.5. Both coenzyme, pyrazole and trifluoroethanol affect the spectra of Cd incorporated in the catalytic site of alcohol dehydrogenase. The interpretation of the present data is consistent with a four coordinated, nearly tetrahedral metal geometry, both with and without the coenzyme bound to the enzyme. The effect of the coenzyme on the metal coordination can best be explained by a reduction of the two cysteine metal bond lengths. From an analysis of the data we conclude that both pyrazole and trifluoroethanol enter as a fifth ligand not displacing the solvent ligand to the metal.

Introduction

Alcohol dehydrogenase is a dimer of MW 80,000, the main function of which is the conversion of various alcohols to the corresponding aldehydes, which requires the presence of the coenzyme NAD^+ [1]. Each identical monomer has one binding site for the coenzyme and furthermore binds 2 Zn atoms tightly [1]. One of the Zn atoms is directly involved in the catalytic event, whereas the second Zn atom has been shown to stabilize the correct conformation of the enzyme [2]. In the following, we refer to the first as the catalytic Zn atom and the second as the non-catalytic Zn atom. A question attracting the interest of many workers in this field is the role of the metal ion in catalysis, and especially the mode of substrate binding.

Two new methods of selective incorporation of divalent metals into the catalytic and non-catalytic Zn site of alcohol dehydrogenase have recently been described, which have opened the way for new spectroscopic studies on the enzyme [3–5]. ^{113}Cd NMR, Co absorption and Co/Cu and Mn proton relaxation spectroscopy have already been performed [5–7].

We shall present here PAC studies on horse liver alcohol dehydrogenase derivatives, where ^{111}Cd is specifically inserted in the catalytic Zn site. PAC detects the ,nuclear quadrupole interaction between a nucleus of an isotope (^{111}Cd in the case reported here) and its environment. The method has previously been applied to the study of the metal sites in carbonic anhydrase [8, 9], carboxypeptidase A [10], and superoxide dismutase [11], from which important information about the function of the metal in catalysis could be derived. The present results on LADH demonstrate that no metal ligand ionization takes place at the catalytic site below pH 9.0 in the CdZnLADH derivative, and calculations show that the competitive inhibitors pyrazole and trifluoroethanol enter the ligand sphere as a fifth ligand thereby not displacing the metal bound water molecule.

Experimental

Materials and Methods

Horse liver alcohol dehydrogenase (EC 1.1.1.1) and coenzymes were purchased from Boehringer/

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Mannheim/FRG and the enzyme was recrystallized prior to use. All other reagents were the purest available commercially. H_4Zn_2LADH was prepared in the crystalline state as described earlier [5]. This enzyme, depleted on the catalytic Zn ion, was dissolved shortly before (within 1 hour) addition of ^{111}Cd plus carrier Cd. All enzyme species were manipulated and kept under nitrogen or argon atmosphere throughout the experiments. All buffers used were freed of metal contaminants by extraction with 0.01% dithizone in carbon tetrachloride [12]. All glassware were 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 [13]. The specific activity is given in units of ΔA_{340} mg^{-1} min^{-1} at 23 °C.

The ^{111}Cd isotope was produced by bombardments of metallic Pd (98% enriched ^{108}Pd) with 21 MeV alpha particles. 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 [14]. If not specifically stated otherwise, CdZn-LADH was prepared by adding 1.7 eq. Cd (eq. Cd is used synonymously with g. at. Cd per mol enzyme; including the ^{111}Cd which was less than 10^{-12} mol) to a solution of 73 μM H_4Zn_2 LADH at pH 7 in 0.125 M Hepes. After 5 min this solution was passed over a Sephadex G25 column, equilibrated with 50 mM 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, thus enabling us to use only the radioactive protein peak for the PAC experiment. For a PAC experiment, 850 μl of CdZn-LADH from the column was transferred to 3.1 g sucrose dissolved in 2 ml of the desired buffer. All PAC experiments were run at 4 °C, and the final enzyme concentration was 10^{-5} M. The pH of each sample was measured at 4 °C, after the experiment.

PAC spectra were recorded with a four-detector, slow-fast coincidence spectrometer [8]. The theory and technique of PAC and its application to the studies of metalloenzymes have been described elsewhere [8]. The coincident counting rate $W(\theta, t)$ was measured at fixed angles 0–180°, and 0–90° as a function of the delay time t between the emission of the two gamma rays. θ is the angle between the detectors of the two gamma rays. From the coincident rates, which were determined experimentally, the NQI parameters ω and η were calculated according to equation (1) [8].

$$\frac{W(180°, t)}{W(90°, t)} = \frac{1 + A\,G(t, \omega, \eta)}{1 - \tfrac{1}{2}A\,G(t, \omega, \eta)} \qquad (1)$$

where A is the amplitude at t = 0 and $G(t, \omega, \eta)$ is a known function of t, ω and η [8]. The function of sucrose is to immobilize the enzyme in the time scale of the PAC experiments.

The NQI parameters ω and η can be related to an actual coordination geometry by the use of the AOM model. The application of the AOM model to NQI calculations has been described previously [14]. Briefly, the model as it is applied here assumes that bonding occurs via sigma orbitals and that ligand-ligand interaction can be neglected. We can then assign a partial nuclear quadrupole frequency ω_i to each ligand i. The NQI is proportional to the gradient of the electric field at the nucleus set up by the surroundings [8]. Thus, if we denote $\hat{a}_i$ for one of the three components of a unit vector in the x, y or z direction in a cartesian coordinate system, we can set up the electric field gradient tensor scaled to be equal to the NQI as [14]

$$V_{ab} = \Sigma_i \omega_i \left\{ \frac{3}{2}\hat{a}_i\hat{b}_i - \frac{1}{2}\delta_{\hat{a}_i,\hat{b}_i} \right\} \qquad (2)$$

where $\delta_{\hat{a}_i,\hat{b}_i} = 1$ for $\hat{a}_i = \hat{b}_i$ and 0 for $\hat{a}_i \neq \hat{b}_i$ and where a, b = x, y or z and the i summation runs over all the ligands. In a randomly oriented system such as a protein solution, one cannot determine the coordinate axis; therefore for simplicity the coordinate system in which the tensor in equation (2) is diagonal is chosen. In this coordinate system ω and η are defined as

$$\omega = V^p_{zz},\ \eta = \left| \frac{V^p_{xx} - V^p_{yy}}{V^p_{xx} + V^p_{yy}} \right|$$

where the index p refers to the principal coordinate system where the electric field gradient tensor is diagonal.

Results

Addition of 6 eq. of Cd to H_4Zn_2LADH (2.8×10^{-5} M) in 10^{-1} M Tes buffer pH 7 at 22 °C allowing 5 min for the metal to bind resulted in 1.8 eq. enzyme bound Cd (Table I). This is in agreement with the number of empty catalytic sites deduced from specific activity measurements on H_4Zn_2-LADH indicating about 0.1 eq. of residual Zn in the catalytic site (see Table II). Addition of 1.7 eq. of Cd to H_4Zn_2 LADH (7.3×10^{-5} M) under the same conditions gave 1.4 eq. enzyme bound Cd; by prolonging the binding time of Cd to H_4Zn_2LADH to 1 hour 1.6 out of 1.7 eq. Cd was bound. In contrast 2 eq. of Cd added to native LADH (7.3×10^{-5} M), the other conditions kept the same (5 min binding time), resulted in 0.5 eq. of enzyme bound Cd. Prolonging the binding time to more

TABLE I. Binding of Cd to Various Derivatives of LADH measured by Sephadex G 25 filtration by adding less than 10^{-12} mol radioactive ^{111}Cd to the total amount of Cd added, and then counting the percentage radioactive ^{111}Cd in the protein peak. The radioactivity is measured as the integral number of counts in the 247 keV line and corrected for the 49 min decay. The background counts for all measurements were below 1% of the ^{111}Cd counts The column is equilibrated with 50 m*M* Mes pH 6.0.

Enzyme	Added equivalent of Cd	Enzyme concentration, *M*	Binding time in minutes	Eq. Cd bound
H_4Zn_2LADH	1.7	7.3×10^{-5}	5	1.4
H_4Zn_2LADH	6.0	2.8×10^{-5}	5	1.8
H_4Zn_2LADH	1.7	7.3×10^{-5}	60	1.6
Native LADH	2.0	7.3×10^{-5}	5	0.5
Native LADH	2.0	7.3×10^{-5}	10	1.0

TABLE II. Catalytic Activity and UV Absorption Ratio between 255 nm and 280 nm for Various Derivatives of LADH.

	H_4Zn_2LADH	Native LADH*	CdZnLADH^{+}	CdZnLADH‡	CdZnLADH§
Specific activity	0.7	12–14	1.9	2.0	2.9
A_{255}/A_{280}	0.56	0.57	0.79	0.86	0.82

*A_{255}/A_{280} = 0.60 [4]. $^{+}$1.7 eq. Cd + H_4Zn_2LADH (5 min binding time). ‡as $^{+}$, but stored at 4 °C for 16 days in 50 m*M* Mes pH 6.0. §as ‡, except for a buffer change to 1:1 Tes/Mes 50 m*M* pH 7.0 for 1 hour at 22 °C.

TABLE III. Nuclear Quadrupole Interaction Parameters for Cd Added to Various Derivatives of LADH, pH = 5.7, 29 m*M* Mes. The errors quoted in this and all other tables are standard deviations.

Enzyme derivative	Binding time for Cd in minutes	ω_1 (MHz)	η_1	ω_2 (MHz)	η_2	P_1 (%)*
H_4Zn_2LADH	5	272 ± 8	0.89 ± 0.05	–	–	100
H_4Zn_2LADH	60	273 ± 23	0.90 ± 0.15	60 ± 4	0.85 ± 0.22	54 ± 8
Native LADH	10	65 ± 4	0.81 ± 0.16	–	–	100

*Percentage amplitude of the NQI characterized by ω_1, η_1.

than 40 min resulted in the denaturation of the enzyme at this high Cd concentration. The specific activity of the various derivatives of LADH are given in Table II.

The CdZnLADH preparation (5 min binding time) was stored for 16 days at 4 °C and pH 6.0, and the activity was then re-measured as 2.0, compared with 1.9 sixteen days before. The pH was then raised to 7.0 and the enzyme was left at 22 °C for an hour, before the activity was measured again. A significant rise in the specific activity was then observed (Table II).

The specific activity of preparations with Cd added to native LADH were identical to that of the native enzyme itself. The PAC spectra from CdZnLADH prepared by allowing Cd to bind during 5 min and 60 min to H_4Zn_2LADH, and during 10 min to native LADH, are shown in Fig. 1. The corresponding set of NQI parameters are listed in Table III. From Fig. 1 and Table III it can be seen that only one metal site is observed in the preparation, allowing Cd to bind to H_4Zn_2LADH within 5 min, in contrast to the case where the binding time of Cd is 60 min, where two approximately equal populated metal sites can be observed. Furthermore, for the two NQI interactions detected in the latter case one is identical to that detected for the derivative prepared by allowing Cd to bind during 5 min to H_4Zn_2-LADH, the other NQI being identical to that detected for Cd bound to native LADH.

TABLE IV. Nuclear Quadrupole Interaction Parameters for ^{111}Cd in the Catalytic Zn Site of LADH.

pH	Buffer	(conc.)	Coenzyme	(conc.)	ω (MHz)	η
5.7	Mes	(29 m*M*)	–		273 ± 11	0.87 ± 0.07
7.1	Tes	(42 m*M*)	–		273 ± 24	0.83 ± 0.13
9.0	Tes	(42 m*M*)	–		264 ± 13	0.91 ± 0.12
9.0	Tes	(42 m*M*)	NAD^+	(0.8 m*M*)	341 ± 16	0.78 ± 0.06
7.1	Tes	(42 m*M*)	NADH	(40 μ*M*)	329 ± 14	0.83 ± 0.07

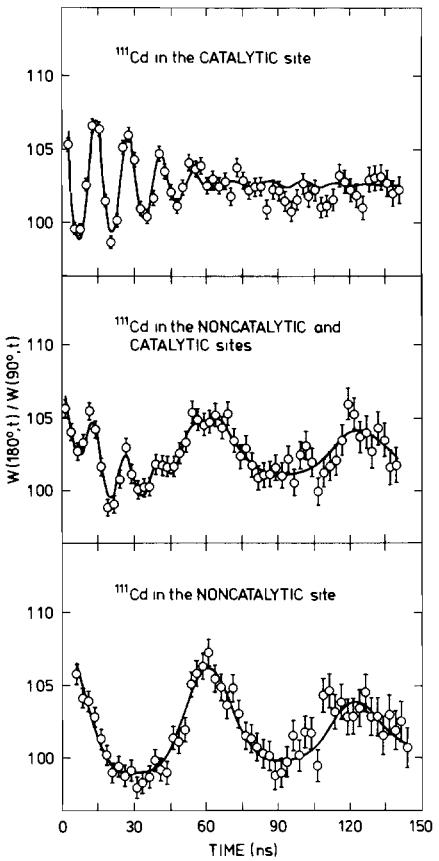

Fig. 1. PAC spectra of ^{111}Cd in LADH prepared by adding 1.7 eq. of Cd to H_4Zn_2LADH allowing 5 min binding time for Cd, upper panel; 60 min binding time, middle panel; and by adding 2 eq. of Cd to native LADH allowing 10 min binding time for Cd, lower panel. All spectra are measured at pH 5.7, in 29 m*M* Mes. For the identifications stated on the Figure, see the text.

We have measured the PAC spectra for ^{111}CdZnLADH at various pH values at 4 °C between pH 5.7 and 9.0. The NQI parameters deduced from some of these experiments by least square fitting to the spectra are given in Table IV. It can be seen from Table IV that, within the error limits, there is no change in the NQI parameters from pH 5.7 to 9.0. The presence of either NAD^+ or NADH at various pHs produces a different set of NQI parameters. There is no difference within the error limits between the NQI parameters from ^{111}CdZnLADH in the presence of NAD^+ at pH 9.0, and ^{111}CdZnLADH in the presence of NADH at pH 7.1.

TABLE V. Average Nuclear Quadrupole Interaction Parameters for ^{111}Cd in the Catalytic Site of LADH.

CdZnLADH	CdZnLADH + coenzyme
ω = 268 ± 5 MHz	ω = 330 ± 8 MHz
η = 0.87 ± 0.03	η = 0.82 ± 0.04

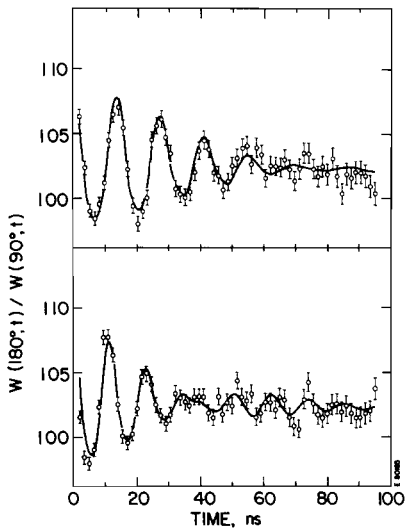

Fig. 2. Average PAC spectra of ^{111}Cd in the catalytic site of LADH. Upper panel: without coenzyme added. Lower panel: with coenzyme added.

In Fig. 2, PAC spectra of the Cd-substituted enzyme as well as its binary complex with coenzyme (NAD^+ or NADH) are presented as averages of all

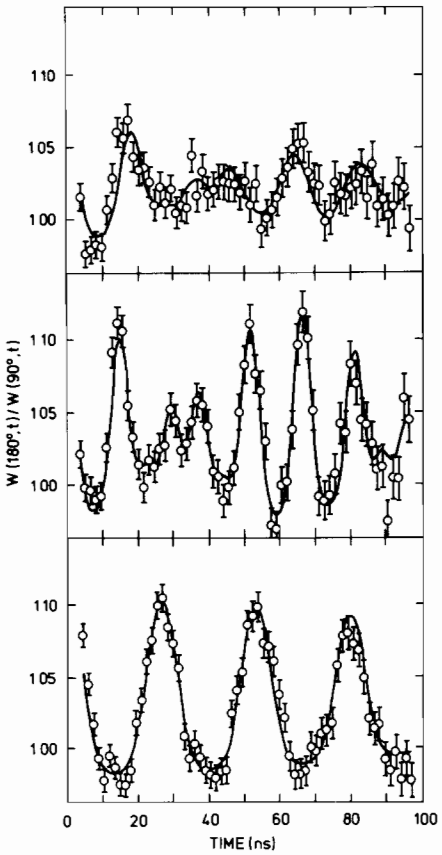

Fig. 3. PAC spectra of inhibitor complexes with ^{111}Cd in the catalytic site of LADH. Upper panel: binary complex with pyrazole. Middle panel: ternary complex with NAD^+ and pyrazole. Lower panel: ternary complex with NAD^+ and trifluoroethanol. Conditions as in Table VI.

between the catalytic and non-catalytic site of Cd during the 2–3 hours of measurements.

In nearly all PAC spectra obtained on LADH the NQI was not well-defined, and it has been necessary to introduce a 5% gaussian distribution of the NQI in order to obtain satisfactory fits. The only remarkable exception to this is the ternary complexes with either NAD^+ and pyrazole or NAD^+ and trifluoroethanol, where the frequencies are sharp (no damping can be observed in the spectra, see Fig. 3, middle and lower panel).

We have applied the AOM Model to the NQI parameters for CdZnLADH in order to arrive at a relevant interpretation of these data in terms of metal coordination geometry. Partial NQI frequencies have been determined for water and histidine (or imidazole), (R. Bauer, to be published). For the cysteine ligands, an estimate of the partial NQI frequency of 440 MHz is derived from the partial NQI of the sulfur ligand in Cd thiosemicarbazide [14] by adding the effect of the NQI frequency of a negative charge on the cysteine sulfur, assuming a bond length of 2.5 Å. For the PAC data on CdZnLADH in the presence or absence of coenzyme, we have assumed a pure tetrahedral geometry with two cysteine ligands, a histidine ligand and a ligand water molecule. Under these assumptions an acceptable fit to the NQI parameters was achieved by using partial NQI frequencies of 460 MHz and 420 MHz for the two cysteine ligands in the presence or absence of coenzyme, respectively (Table VII). A PAC experiment on CdZnLADH at pH 8.5 in the presence of 40 m*M* imidazole and 0.8 m*M* NAD^+ gave (within error limits) the same NQI parameters as that for the

TABLE VI. Nuclear Quadrupole Interaction Parameters for ^{111}Cd in the Catalytic Zn Site of LADH in the Presence of Inhibitors.

pH	Buffer	(conc.)	Inhibitor	(conc.)	Coenzyme	(conc.)	ω (MHz)	η
8.6	Tes	(42 m*M*)	pyrazole	(40 m*M*)	–		206 ± 12	0.72 ± 0.10
9.0	Tes	(42 m*M*)	pyrazole	(20 m*M*)	NAD^+	(0.8 m*M*)	256 ± 3	0.73 ± 0.02
9.4	Tes	(42 m*M*)	trifluoroethanol	(11 m*M*)	NAD^+	(0.8 m*M*)	136 ± 3	0.92 ± 0.06

recorded spectra for each case, and the corresponding average NQI parameters are given in Table V. We have further recorded the PAC spectrum for ^{111}CdZnLADH in the presence of pyrazole, in the presence of NAD^+ and pyrazole and in the presence of NAD^+ and trifluoroethanol. The spectra are shown in Fig. 3 and the corresponding NQI parameters are listed in Table VI. In all PAC spectra, except that shown in the middle panel of Fig. 1, there is no evidence of a second NQI to be present. Thus the experimental PAC condition (52% sucrose, 4 °C and pH values below 9.5) excludes any observable exchange

TABLE VII. Calculated Nuclear Quadrupole Interaction Parameters for ^{111}Cd in the Catalytic Site of LADH.

Condition	ω (MHz)	η
CdZnLADH	273	0.79
CdZnLADH + coenzyme	310	0.84
CdZnLADH + pyrazole	208	0.73
CdZnLADH + pyrazole + coenzyme	247	0.76
CdZnLADH + trifluoroethanol + coenzyme	145	0.98

corresponding ternary complex with pyrazole (R. Bauer, unpublished work). Thus we conclude that the partial NQI for pyrazole is identical to that for imidazole. The experiments with either pyrazole or trifluoroethanol were fitted under the assumption that they enter either as a fourth ligand excluding water, or as a fifth additional ligand. The parameters fitted were then the partial NQI frequencies for trifluoroethanol and the angular position of pyrazole and trifluoroethanol. No acceptable fit with only four ligands was possible. However, good agreement with the PAC data was obtained for a pentacoordinated geometry, where either pyrazole or trifluoroethanol enter as a fifth additional ligand in an angular position close to the opposite direction of the histidine ligand.

Discussion

There are still some fundamental questions to be answered concerning the function of the catalytic Zn atom in alcohol dehydrogenase. Three important issues which are dealt with in this paper are: 1) does the metal coordinated water molecule exhibit a pK in the region 7–8?; 2) is the metal four of five coordinated?, and 3) what is the effect of coenzyme binding on the metal ligand geometry?

In close connection to the present studies the question arises of the relevance of performing spectroscopy on Cd substituted for Zn in alcohol dehydrogenase. In this case it is noted that the fully Cd-substituted enzyme has about 12% activity towards alcohol at pH 10 as compared to the native zinc enzyme [4]. Thus, although a perturbation of catalysis is observed it is still possible to study the catalytic mechanism by using the Cd enzyme. Thereby, the distinction between ^{111}Cd in the catalytic and non-catalytic site during the PAC-experiment must be ascertained. In order to settle this question we examined the mode of Cd binding to H_4Zn_2LADH by means of column separations, catalytic activity measurements and PAC studies. A rise in the specific activity of 1.2 was observed, correlating with the uptake of 1.4 eq. Cd out of 1.7 eq. added during 5 min to H_4Zn_2LADH. This is close to the published value of 2.0 for the Cd_4LADH derivatives [4]; by inference 1.4 eq. Cd bound would mean a specific activity of 1.4. The fact that only one NQI is detected from preparations of CdZnLADH under these conditions leads us to conclude that this procedure gives a CdZnLADH derivative with Cd solely in the catalytic site. Prolonging the binding time of Cd to H_4Zn_2LADH at pH 7.0 to 60 min. results in a rise in the activity from 1.2 to 2.2 (Table II), indicating that exchange is possible within an hour between the catalytic and non-catalytic site at 22 °C pH 7.0. This is confirmed further by the PAC spectrum analysis from the corresponding CdZnLADH derivatives where two different NQI of about equal amplitude were derived. During 5 min. binding precisely 1.8 eq. Cd out of 6 eq. added to H_4Zn_2LADH is bound in the catalytic site. Therefore, under these conditions, only an insignificant fraction of Cd can directly exchange with Zn in the non-catalytic site. For Cd added to native LADH we conclude that Cd binds solely to the non-catalytic site under the conditions used in the present work, because the catalytic activity of these preparations are unchanged relative to the native enzyme. This conclusion is also verified by the derivation of a single NQI from a preparation with native LADH which differs significantly from that deduced for the catalytic site. Thus we can identify the NQI parameter ω = 65 MHz, η = 0.81 with Cd in the non-catalytic site. All other NQI determined in the present work are connected to Cd in the catalytic site because of the above conclusions.

The lack of pH-dependence of the PAC spectra from CdZnLADH between pH 6–9 proves that there is no metal-coordinated water molecule (or metal-coordinated amino acid) which ionizes in this region. As a consequence, if a hydroxyl ion is present as a metal ligand in CdZnLADH, the pK must be lower than 5.2, or higher than 9.5, of which the former choice must be considered unlikely for Cd.

The binary coenzyme/native enzyme complex shows a proton liberation with pK of 7.6 for NAD^+ and about 10 for NADH [15, 16]. In steady state kinetic experiments with CdZnLADH a pH dependence between pH 7 and 8 of K_m for NAD^+ is observed (M. Zeppezauer, personal communication). Furthermore, the equality of the NQI from CdZnLADH plus NADH at pH 7.1 and that from CdZnLADH plus NAD^+ at pH 9.0 shows that the charge state of the metal ligands is identical in these two latter cases. We can therefore deduce that the observed change in K_m between pH 7–8 for the binary complex between NAD^+ and CdZnLADH does not arise from any metal ligand ionization, because that would require a metal ligand to be in its ionized state for the complex NAD^+–CdZnLADH at pH 9, and in its un-ionized state for NADH-CdZnLADH at pH 7.1. This in turn would produce two different NQI for the two above-mentioned complexes, in contrast to what is observed (Table IV). The suggestion of an ionization of a metal-linked water molecule (or amino acid ligand) being responsible for the observed pK upon NAD^+ binding in the pH region 7–8 [15] can therefore be ruled out.

The observed sharpness of the NQI from the ternary complex of CdZnLADH, NAD^+ and pyrazole or trifluoroethanol means that the metal site is immobilized. This is probably due to the tightening of the active site cleft deduced from X-ray diffraction data [17]. In contrast, the diffuse character of

the NQI in all other cases must be related to an enhanced motion of the whole enzyme molecule.

The NQI frequency of ω = 65 MHz for the non-catalytic site (Table III) is almost an order of magnitude below the NQI frequencies for the catalytic site. This indicates that the four cysteines bound to the metal in the non-catalytic site are positioned very close to a pure tetrahedral geometry, in which case the NQI frequency would vanish. The NQI frequency of ω = 65 MHz suggests an average angular deviation from pure tetrahedral angles of maximum 4°/cysteine.

The AOM fit to the NQI parameters of the CdZn-LADH derivatives agrees with the X-ray diffraction results of a four coordinated nearly tetrahedral geometry [18]. The effect of the coenzyme on the conformation of LADH as judged from X-ray data is a tightening of the active site cleft. At the same time a minor rearrangement of the position of the metal and metal coordinated groups takes place (H. Eklund, personal communication). Correlated with this change of conformation the NQI of ^{111}Cd is changed, implicating a change in the metal coordination geometry. As explained in the results section this change in the NQI is consistent with a 10% increase in the partial NQI of the two metalbound cysteines upon coenzyme binding, keeping the binding mode of the histidine and water molecule unchanged. This increase signifies in the AOM model that the two cysteine bond lengths are reduced about 0.3 Å upon coenzyme binding.

The rather drastic reduction in NQI frequency upon addition of trifluoroethanol to the binary complex between CdZnLADH and NAD^+, leaves no doubt that trifluoroethanol coordinates directly to the metal. Furthermore, the AOM analysis of the PAC data from pyrazole and trifluoroethanol gave the result that these competitive inhibitors bind in an extra coordination position without displacing the metal-bound water molecule.

In summary, the PAC data suggest that no metal coordinated hydroxyl group is involved in the mechanism of LADH, and they suggest that the alcohol coordinates directly to the metal as an additional ligand in the enzyme/substrate complex.

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Abbreviations Used

PAC: perturbed angular correlation of gamma rays.
NQI: nuclear quadrupole interaction.
LADH: liver alcohol dehydrogenase.
H_4Zn_2LADH: LADH depleted of Zn in the catalytic site.
CdZnLADH: any LADH derivatives with bound Cd.
AOM: angular overlap model.
Tes: [2-{(tris(hydroxymethyl)methyl)amino}ethanesulfonic acid]
Mes. [2-(N-morpholino)ethanesulfonic acid]
Hepes: [4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid]

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