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Cadmium-113 and magnesium-25 NMR study of the divalent metal binding sites of isocitrate dehydrogenases from pig heart

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Abstract

The metal activator sites of NAD^+-dependent and $NADP^+$-dependent isocitrate dehydrogenases from pig heart have been probed using ^{113}Cd- and ^{25}Mg-NMR. In the presence of isocitrate and ADP, a broad resonance for cadmium bound to NAD-dependent isocitrate dehydrogenase was observed (~8 ppm) arising from exchange with isocitrate (−20 ppm) and/or ADP (27 ppm) complexes. The Cd shift with ADP suggests interaction of the metal with the nucleotide ring nitrogen. Increasing shifts with excess ADP are indicative of macrochelate formation. ^{25}Mg-NMR demonstrates that, unlike manganese, magnesium has a similar dissociation constant (1.8 mM) from NADP-dependent isocitrate dehydrogenase as from the enzyme-isocitrate complex (1.1 mM). The extrapolated line width of bound magnesium increases from 674 Hz in the binary complex to 10200 Hz in the ternary complex. The quadrupole coupling constant, calculated from relaxation rates, is larger in the ternary complex, indicative of greater distortion in the magnesium coordination sphere. The line widths of magnesium complexed to NAD-dependent isocitrate dehydrogenase are broader, as expected for the larger octamer. ^{113}Cd- and ^{25}Mg-NMR both show that the metal sites have anisotropic octahedral symmetry. ^{25}Mg relaxation rates yield correlation times corresponding to motions of a domain with motion independent of the enzyme multimers.

Keywords: NMR, ^{113}Cd-; NMR, ^{25}Mg-; Isocitrate dehydrogenase; Metal binding site; (Pig heart)

1. Introduction

Pig heart mitochondria contain two isocitrate dehydrogenases, NAD^+-dependent isocitrate dehydrogenase (NAD-IDH) [*threo*-D_S-isocitrate: NAD^+ oxidoreductase (decarboxylating), EC 1.1.1.41] and $NADP^+$-dependent isocitrate dehydrogenase (NADP-IDH) [*threo*-D_S-isocitrate: $NADP^+$ oxidoreductase (decarboxylating), EC 1.1.1.42]. The oxidative decarboxylation of isocitrate to form α-ketoglutarate catalyzed by NADP-IDH, a dimer of identical subunits [1], requires a divalent metal for catalytic activity [2,3]. NAD-IDH, composed of non-identical subunits in at least a tetramer [4–6], is activated by ADP which reduces the K_m for isocitrate. Catalysis and ADP activation require divalent metal and are dependent upon the metal used for enzyme assay.

The metal requirements of NAD-IDH may be satisfied by several divalent metals including manganese, magnesium, or cadmium [7,8]. The binding of manganese to NADP-IDH has been extensively studied by ultrafiltration [2,9] and by nuclear magnetic resonance [2,10,11]. The dissociation constant of manganese from the enzyme is reduced more than 20-fold by inclusion of isocitrate. Evidence that isocitrate changes the geometry of enzyme-bound metal is provided by changes in the electron spin resonance spectrum of enzyme-bound manganese [12], changes in the water proton relaxation rate [2] and by studies of the quenching of fluorescence of a covalently bound nucleotide analogue by the divalent metal activators, Ni^{2+} and Co^{2+} [13]. ^{113}Cd-NMR has indicated that cadmium in the ternary complex with isocitrate-NADP-IDH is coordinated to six oxygen ligands [14]. The ^{113}Cd resonance in the enzyme complex is shifted to 9 ppm compared with −23 ppm in Cd-isocitrate complexes. Studies using ^{25}Mg show increased line-width of the magnesium resonance upon addition of isocitrate to the Mg-NADP-IDH complex [14].

Binding experiments with manganese and NAD-IDH show that the metal binds to half of the subunits of this

Abbreviations: NAD-IDH, NAD-dependent isocitrate dehydrogenase; NADP-IDH, NADP-dependent isocitrate dehydrogenase; EDTA, ethylenediaminetetraacetic acid; Mops, 3-(*N*-morpholino)propanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid.

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multi-subunit enzyme and that the dissociation constant decreases tenfold in the presence of isocitrate [15]. ADP tightens the binding in the presence and absence of isocitrate but analysis of the binding data indicates that manganese does not bind as a Mn-ADP complex [15].

In the present study, the unique magnetic resonance properties of cadmium and magnesium are used to probe the enzyme binding site of NAD-dependent isocitrate dehydrogenase. Further measurements of ^{25}Mg-NMR in the presence of NADP-IDH permit comparison of the changes upon addition of isocitrate. ^{113}Cd chemical shifts are sensitive to changes in the chemical groups and symmetry of the first coordination sphere [16,17] with shifts occurring in the range from 800 to −110 ppm relative to cadmium in dilute solutions. Measurements of the cadmium shifts in the isocitrate dehydrogenase complexes provide information on the types of amino acids that may constitute the enzyme binding sites.

Magnesium-25 has a quadrupole moment and spin quantum number $j = 5/2$. While the differences in chemical shifts are expected to be small, the relaxation rates should be sensitive to changes in ligand binding [18–20]. In this study both transverse and longitudinal relaxation rates were measured for both isocitrate dehydrogenases. A preliminary version of a portion of this work has been presented [21].

2. Materials and methods

2.1. Materials

NADP-dependent isocitrate dehydrogenase was purified from pig hearts as described by Bacon et al. [22] and Ehrlich and Colman [23]. NAD-dependent isocitrate dehydrogenase was purified following Ramachandran and Colman [24], as modified by Ehrlich and Colman [8]. The enzymes were concentrated to 15–30 mg/ml by using Amicon ultrafiltration devices with PM-10 membranes and Amicon Centricon filters. They were dialyzed against the indicated buffers containing 10% D_2O. NADP-IDH concentration was determined using an extinction coefficient of 1.08 ml/mg at 280 nm [25] and a subunit molecular weight of 46,000. NAD-IDH concentration was determined using an extinction coefficient of 1.55 ml/mg at 280 nm [26].

^{113}Cd(92% enriched) was obtained from Icon as the free metal and dissolved in HCl. Cadmium nitrate (unenriched) was obtained from Matheson. The concentration of cadmium was measured by titration with EDTA and monitoring of the absorbance at 235 nm [27]. The absorbance decreases until an endpoint, corresponding to equimolar concentrations of cadmium and EDTA, is reached and the concentration of cadmium is determined from this endpoint. The concentration was also determined by monitoring the ^{113}Cd NMR spectrum as a function of EDTA concentration: at equimolar concentrations, the amplitude of the resonance due to free cadmium disappears. The concentrations obtained by these methods agree within 3%. ^{25}Mg (95% enriched) was obtained from Merck as the oxide and was dissolved in H_2SO_4.

2.2. NMR spectroscopy

^{113}Cd spectra were obtained at 55.5 MHz in a Bruker WM-250 spectrometer with a 10 mm variable frequency probe. A saturated solution of cadmium nitrate was used to tune the spectrometer. A sweep width of 10 000 Hz was used with 16 128 data points. Initial experiments with wider sweep widths were performed to ascertain that no resonances occur outside the range used. The signal-to-noise ratio was enhanced by using exponential line broadening. An external reference sample of $Cd(ClO_4)_2$ was used to determine chemical shifts. Positive shifts indicate lower shielding. Sample volumes were 2 ml and include 10% D_2O for spectrometer locking.

In the present study, preliminary ^{25}Mg spectra were obtained at 15.3 Mhz in a Bruker WM-250 spectrometer using a 15 mm variable frequency probe and additional measurements including relaxation measurements using a 10 mm probe. A saturated solution of unenriched $MgSO_4$ was used for initial tuning. Sample volumes of 1.5–2 ml were used in the 10 mm probe and 4 ml for the 15 mm probe. To avoid problems with ringing following the pulses, spectra were obtained from a spin echo. While this decreased the sensitivity, it avoided the problems of line distortion caused by ringing or acquisition delay. Typical acquisition and processing values are a sweep width of 10 000 Hz with 4096 data points and a pulse delay of 1–10 ms. Enzyme samples were initially dialyzed against buffers containing EDTA to remove extraneous metal, and then against the desired buffer. 20% D_2O was added as a field lock. Line widths were measured at half-height. A Lorentzian was fitted to the spectrum using software provided by Bruker on the Aspect 3000 computer of an AM-250 spectrometer. Transverse relaxation times ($1/T_1$) were measured using the Carr–Purcell–Meiboom–Gill 90°–180° sequence and variable pulse delays [28]. Longitudinal relaxation was measured with a 180°–90° pulse sequence followed by spin-echo detection. Magnesium speciation was calculated using association constants of 357 M^{-1} for Mg-isocitrate [29] and 2500 M^{-1} for Mg-ADP [30].

3. Results

3.1. ^{113}Cd-NMR

Attempts to obtain spectra of NAD-IDH bound cadmium in the absence of isocitrate were not successful because of precipitation of both enzyme and cadmium in

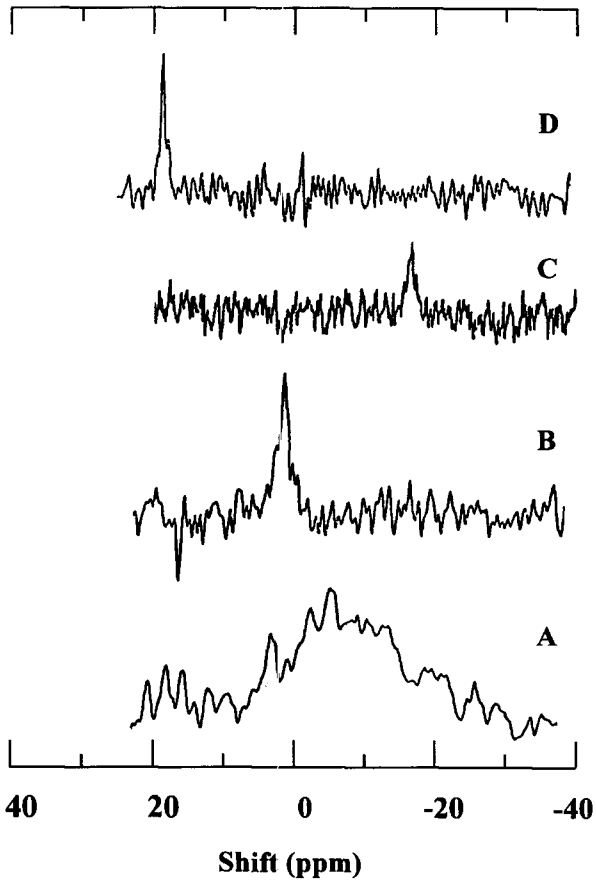

Fig. 1. ^{113}Cd-NMR Spectra. The samples are in sodium 50 mM Mops (pH 7.0). Composition of samples and NMR conditions are: (A) 1.34 mM Cd^{2+}, 0.37 mM NAD-IDH, 2 mM Mg^{2+}, 10 mM DL-isocitrate and 1 mM ADP. 72000 scans were acquired using a 25000 Hz sweep width and 0.5 s relaxation delay. An exponential line broadening of 50 Hz was used in processing the free induction decay. (B) 1.5 mM Cd^{2+}, 10 mM DL-isocitrate, 2 mM Mg^{2+} and 1 mM ADP. 1400 scans and 10 Hz line broadening. (C) 1.34 mM Cd^{2+}, 2 mM Mg^{2+}, and 10 mM DL-isocitrate. 3000 scans and 10 Hz line broadening. (D) 1.6 mM Cd^{2+} and 1 mM ADP. 1437 scans and 10 Hz line broadening.

these complexes. Similar difficulties were experienced previously for NADP-IDH [14] and attributed to the preference of cadmium for sulfur over oxygen ligation [31]. Precipitation did not occur in the ternary Cd-isocitrate-NAD-IDH complex. With 0.37 mM enzyme, 10 mM isocitrate and 0.45 mM ^{113}Cd, no signal was observed in range 600 to −200 ppm after 60000 scans in the presence or absence of 2 mM magnesium. Upon addition of 1 mM ADP a signal could be observed. The signal broadens when the cadmium concentration is increased. A typical spectrum is shown in Fig. 1A along with the spectra of ^{113}Cd in Cd-isocitrate-ADP (Fig. 1B), Cd-isocitrate (Fig. 1C) and Cd-ADP complexes. The resonance in the enzyme complex occurs at approx. −8 ppm (Fig. 1A). In a sample containing 1.5 mM Cd^{2+}, 1 mM ADP and 10 mM DL-isocitrate the resonance is observed to shift to 1.3 ppm (Fig. 1B). These are compared with resonances at −18 ppm (Fig. 1C) in the isocitrate complex and +18 ppm (Fig. 1D) in an ADP complex (1.7 mM Cd^{2+} and 1 mM ADP). The ^{113}Cd chemical shift is a function of nucleotide concentration (Fig. 2). The Cd^{2+}-ADP dissociation constant is 0.26 mM [32] and the dissociation constant for Cd-isocitrate is 2.8 mM [14]. Thus the chemical shift increases at nucleotide concentrations above the values where 1:1 complexes are formed. The signal in the Cd-enzyme-Mg-ADP-isocitrate complex (Fig. 1A) arises from exchange of cadmium between enzyme bound complexes and free Cd-ADP and Cd-isocitrate complexes. The line broadening is characteristic of intermediate exchange with a maximal line width obtained at a ratio of cadmium:enzyme of approx. 3 [33].

3.2. ^{25}Mg NMR with NADP-IDH

The magnesium resonance in buffered water is narrow (observed line width about 5 Hz). When enzyme is added, the line width increases. The line may be reasonably fitted by a Lorentzian [14]. The line width is a function of the line width in each of the complexes present and the proportion of magnesium in that complex:

$$\Delta\nu_{\text{observed}} = \sum_i f_i \Delta\nu_i \quad (1)$$

where f_i, the fraction of magnesium in a given form can be determined from the dissociation constant for the complex and $\Delta\nu_i$ is the line width of ^{25}Mg in that form. The dependence of line width on magnesium concentration in the presence of NADP-IDH is shown in Fig. 3A. Fig. 3B

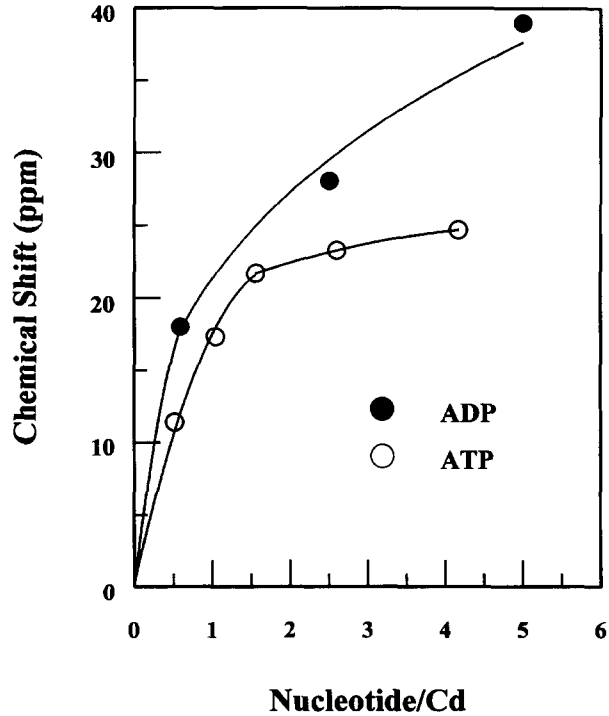

Fig. 2. Chemical shift of ^{113}Cd as a function of Cd/nucleotide ratio. The cadmium concentration was 1.7–2.0 mM.

which shows a similar magnesium titration obtained in the presence of isocitrate will be discussed below. From a non-linear least squares fit of the dependence of the observed line widths on magnesium concentration, a dissociation constant of 1.84 ± 0.19 mM and line width of bound magnesium of 674 ± 22 Hz is obtained. Previous studies on NADP-IDH have found that the binding of nucleotides is reduced by sodium sulfate. Titration of a sample containing magnesium-enzyme complex with sodium sulfate was accompanied by a decrease in the observed line width from 62 Hz at 5 mM Na_2SO_4 to 32 Hz at 50 mM Na_2SO_4. This result may be interpreted as a decrease in the magnesium-enzyme binding constant. While line widths can be used to obtain relaxation rates, $1/T_2$, these widths, particularly for narrow lines, are affected by magnetic field inhomogeneity and direct measurement of relaxation rates is preferable. From the ratio of T_1 to T_2, the correlation time, τ_c may be obtained provided $\omega_0\tau_c < 1.5$ [34]:

$$\frac{T_2}{T_1} = \frac{0.2J(\omega_0) + 0.8J(2\omega_0)}{0.3J(0) + 0.5J(\omega_0) + 0.2J(2\omega_0)} \quad (2)$$

with

$$J(\omega_0) = \frac{\tau_c}{1 + (\omega_0\tau_c)^2} \quad (3)$$

Using data shown in Table 1, a correlation time of 8 ns is obtained. The data in Table 1 also show that relaxation rates decrease (relaxation times increase) with increasing temperature implying that the rates are dominated by the intrinsic relaxation of the bound species and not by exchange as was concluded by Ehrlich and Colman [14]. The relaxation rates of the bound species $[(1/T_2)_{bound}$ and $1/T_1)_{bound})]$ are 2120 ± 70 and 1240 ± 200 s^{-1}. These values, together with the correlation time, can be used to obtain the quadrupole coupling constant, $X = 0.67$ MHz, calculated from [34]:

$$\frac{1}{T_1} = 0.95X^2(0.2J(\omega_0) + 0.8J(2\omega_0)) \quad (4)$$

The line width of ^{25}Mg in the presence of NADP-IDH is dependent upon pH. Fig. 4A shows a titration. The calculated pK_a arises from an amino-acid side chain, the ionization of which affects magnesium binding or site geometry. In the presence of isocitrate, the ^{25}Mg lines broaden, even in the absence of enzyme. A pH titration of the line width for magnesium in the presence of isocitrate is shown in Fig. 4B. The data cannot be fit to a simple titration curve. In the titration of the malonic acid-magnesium complex, dibasic and monobasic ions have been found to contribute equally to the line width of magnesium [35]. A satisfactory fit (solid line In Fig. 4) is obtained if both tribasic and dibasic isocitrate bind magnesium and yield the same line width in the bound complex. With this assumption, the data are fit assuming a dissociation constant for the tribasic form of 4.7 mM as found above. The

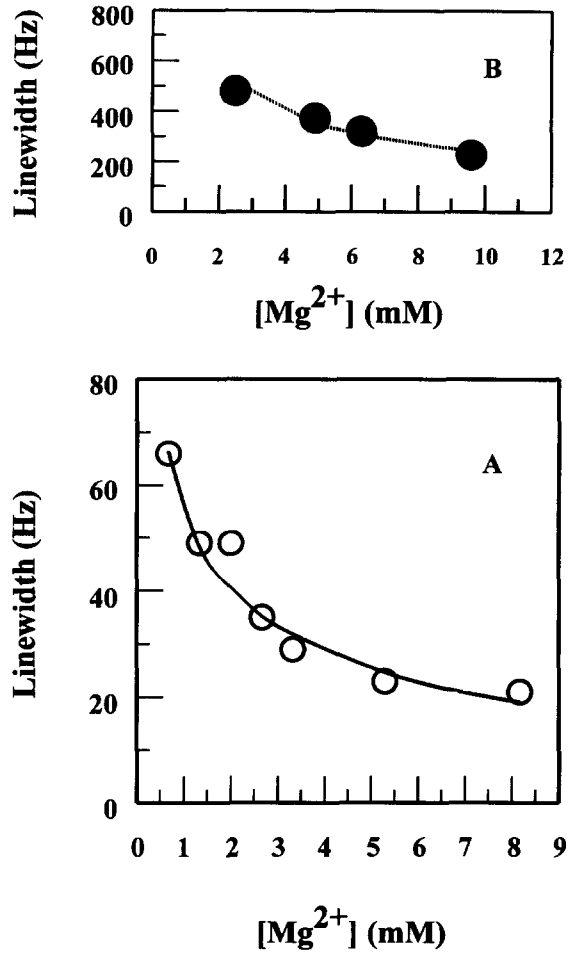

Fig. 3. Line widths of ^{25}Mg in the presence of isocitrate dehydrogenase. Increasing amounts of Mg were added to 0.27 mM NADP-dependent isocitrate dehydrogenase in 0.05 Mops (pH 7.5). The line width is the width at half-maximal amplitude. The widths were determined using a Lorentzian fit by means of Bruker software. measured line widths were corrected for the exponential line broadening (2–10 Hz) and line width of free magnesium (3–5 Hz). (A) No isocitrate. The solid line is a non-linear least squares fit to the data using Eq. (1) yielding values of 674 Hz and 1.84 mM for the line width of bound Mg and dissociation constant of the Mg-enzyme complex, respectively. (B) 10 mM DL-isocitrate. The line is a fit to Eq. (1) using: values given for Fig. 2A to determine $f_{Mg\text{-}NADP\text{-}IDH}$; a dissociation constant of 4.7 mM to determine $f_{Mg\text{-}isocitrate}$ and values obtained by fitting the data to get $f_{Mg\text{-}isocitrate\text{-}NADP\text{-}IDH}$ and $\Delta\nu_{Mg\text{-}isocitrate\text{-}NADP\text{-}IDH}$.

non-linear least squares fit gives a pK_a of 5.66 and a dissociation constant for the magnesium-dibasic isocitrate complex of 40 mM. This pK_a and dissociation constant are in excellent agreement with the values found by Grzybowski et al. [36] of 5.7 and 40 mM. For the complex containing magnesium, isocitrate and enzyme the data parallel the data obtained in the absence of enzyme, as shown by the dashed line (Fig. 4B). Thus, within the errors of the measurements, no additional information may be obtained about titratable groups on the enzyme near the magnesium binding site. In the presence of 0.025 M Na_2SO_4, the dissociation constant of Mg-isocitrate from

Table 1
Relaxation rates for ^{25}Mg in the absence and presence of enzyme NAD(P)-IDH

Enzyme	[Mg] (mM)	Temperature (K)	T_1 (ms)	T_2 (ms)
None	10	298	180	150
NADP-IDH	5.29	298	35	15.2
	8.17	293	29	16.8
		300	29	17.3 [a]
		303	–	18.2
		308	33	19.9 [a]
NAD-IDH	6	298	20	
			21(+ADP)	
	10	298	28	14.5 [a]
			30(+ADP)	

Isocitrate dehydrogenase (0.25–0.27 mM) was dialyzed into 0.05 M Mops buffer (pH 7.5), containing 10% glycerol. ^{25}Mg spectra were acquired at 15.3 MHz. T_1 was measured using a $\{180° - \tau - 90° - 0.5$ ms $- 180° -$ acquire$\}$ pulse sequence with echo detection and T_2 using a $\{90°_x(-\tau - 180°_y - \tau)_n -$ acquire$\}$ pulse sequence. T_2 was also calculated from the line widths at 1/2 maximal amplitude.
[a] Obtained from line width.

the ternary complex is determined to be 4.7 mM and line width 173 Hz at pH 7. This compares with reported dissociation constants for the magnesium-isocitrate complex of 1.9 mM [36], 0.7 mM [37], 2.0 mM [29] and 5.1 mM [38].

The line width of magnesium complexed with isocitrate is similar to that found for magnesium in a complex with malonate (170–270 Hz) [35]. Magnusson and Bothner-By [18] have measured the line widths for a series of carboxylic acids and obtained values ranging from 50 Hz for succinate to 490 Hz for tribasic citrate. The quadrupolar coupling constant, X, calculated from [34]:

$$\pi\Delta\nu_{1/2} = 0.95X^2\tau_c \quad (5)$$

is 1.5 MHz (assuming τ_c is $2.4 \cdot 10^{-10}$ s as for the malonate complex), indicating considerable asymmetry in the binary Mg-isocitrate complex compared with the aqueous complex (0.1 MHz) but similar in magnitude to the malonate complex [35].

The dissociation constants for the Mg^{2+}-isocitrate binary complex and the Mg^{2+}-enzyme complex obtained here may be used to calculate the concentration of magnesium-isocitrate for samples in which increasing amounts of magnesium were added to 10 mM isocitrate and 0.25 mM NADP-IDH. The experimental values of the line width are shown in Fig. 3B. Again, using Eq. (1), a least squares fit gives a dissociation constant of 1.12 ± 0.17 mM for magnesium-isocitrate and a line width of the magnesium resonance in the ternary complex of $10\,200 \pm 400$ Hz. The variation of line width with magnesium concentration using these values is also shown in Fig. 2B. Assuming the correlation time is the same for Mg^{2+}-$NADP^+$-IDH and the ternary complex (since the broad lines preclude direct measurement of T_1), the quadrupole coupling constant in the ternary complex is 2.6 MHz.

3.3. $^{25}Mg^{2+}$-NMR with NAD-IDH

Relaxation rates for ^{25}Mg in the presence of NAD-IDH are given in Table 1. Based upon these measurements a lower limit on $(1/T_2)_{bound}$ is 4000 s^{-1} and on $(1/T_1)_{bound}$ is 1800 s^{-1}. The estimated dissociation constant for the Mg-enzyme complex is 4 mM. The relaxation rates are about twice the values obtained for NADP-IDH. Addition of 1 mM ADP does not appreciably change the value of T_1. Since the line width of ^{25}Mg-ADP is 300 Hz [30] and dissociation constant 0.4 mM, this species has only a small contribution to the observed line width at the concentra-

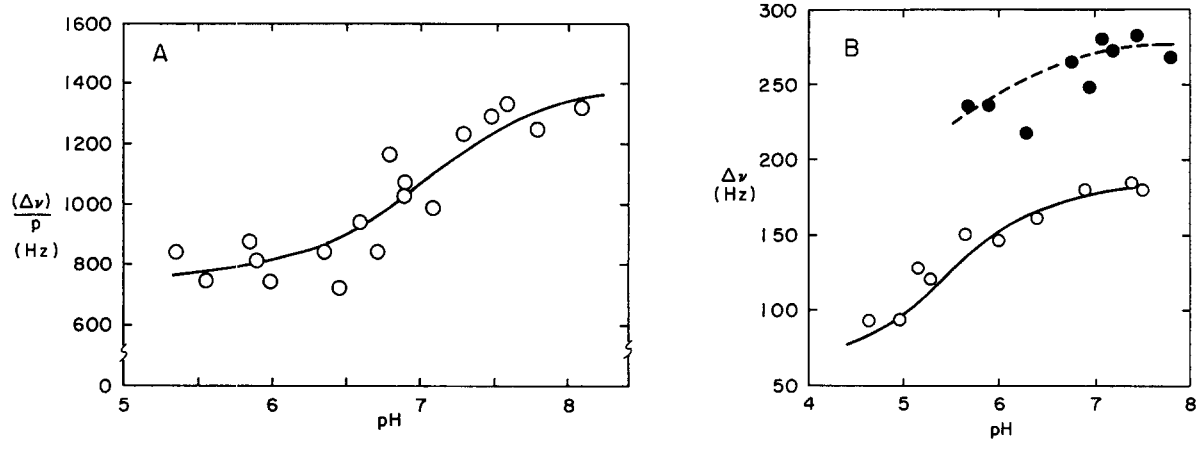

Fig. 4. Dependence of ^{25}Mg line widths on pH. (A) 0.23–0.25 mM isocitrate dehydrogenase present. The sample, initially in Mops buffer (as in Fig. 3A) was titrated down in pH with concentrated Mes or up with 1 mM NaOH. Values of the measured line widths $\Delta\nu$ were divided by the ratio of enzyme to magnesium. Experiments from three different samples are combined. The solid line is a non-linear least squares fit to the data with $\Delta\nu_{max} = 1405 \pm 150$ Hz, $\Delta\nu_{min} = 750 \pm 80$ Hz and $pK_a = 7.05 \pm 0.19$. (B) 10 mM DL-isocitrate with (0) or without (0) 0.19 mM isocitrate dehydrogenase. In the absence of enzyme the data are fit to a titration curve (solid line) with $\Delta\nu_{max} = 235$ Hz and dissociation constant 4.7 mM for tribasic isocitrate, dissociation constant 40 mM for dibasic isocitrate, and pK_a of 5.66. The dashed line is drawn parallel to the solid line indicating that the presence of enzyme does not change the pH variation of the observed line widths.

tions of magnesium and ADP used. The expected change in the relaxation rate due to changes in the enzyme bound complex was not observed.

4. Discussion

$^{113}Cd^{2+}$- and $^{25}Mg^{2+}$-NMR spectroscopy have yielded complementary information about the metal sites of NADP-dependent and NAD-dependent isocitrate dehydrogenases. For NADP-IDH the equilibrium dissociation constants for magnesium from the enzyme are similar in the presence and absence of isocitrate. In contrast, the dissociation constants for manganese differ by at least an order of magnitude, with tighter binding in the presence of isocitrate [2,15]. The relaxation rates in the enzyme bound complexes are two orders of magnitude faster than for magnesium-isocitrate, while the correlation time is at least two orders of magnitude greater in the macromolecular complex. If simple quadrupolar relaxation is assumed (Eq. 4), the quadrupolar coupling constants and hence the electric field gradients are similar in the Mg-isocitrate complexes but greater than those in the binary Mg-enzyme complex. This increase in electric field gradient is consistent with the greater asymmetry found for the Mn-isocitrate-enzyme complex than for the Mn-enzyme complex using electron spin resonance [12]. Data are not available on a wide range of magnesium-enzyme systems but the values obtained for isocitrate dehydrogenase are similar to those obtained for a prothrombin fragment, Factor XIII [35] and calmodulin [39]. The relaxation rate in the Mg-NADP-IDH is also similar to that determined by Lee and Nowak [40] for site I of yeast enolase, while the relaxation rate in the ternary complex with isocitrate is greater than that for site II of enolase. The correlation time found from measurements of T_1 and T_2 of 8 ns is less than the rotational correlation time calculated by Villafranca and Colman [10] for NADP-IDH monomer, suggesting that either the protein is asymmetrical or there is increased motion of the Mg binding domain. It should be noted that the analysis used in Eqs. (2)–(4) is only valid for shorter correlation times than the calculated rotational correlation time.

The three-dimensional structure of NADP-dependent isocitrate dehydrogenase from E.coli has been determined to 2.5 Å resolution in apo and ternary metal-isocitrate complexes. The coordination of magnesium in this enzyme involves the oxygens of isocitrate, two aspartate side chains and two water molecules [41]. The same coordination is observed for the inhibitory metal, calcium, but the distance between metal and isocitrate is greater [42]. The x-ray structure for the enzyme metal complex for E. coli isocitrate dehydrogenase in the absence of isocitrate has not been determined. While there is little overall similarity between the pig heart and *E. coli* enzymes, sequence alignments in the vicinity of the metal-isocitrate site indicate conservation of at least one of the aspartates participating in metal binding [43]. For the metal-isocitrate complex, the inner sphere coordination of the metal by oxygen containing ligands is in agreement with the conclusions obtained from the ^{113}Cd shifts in the metal-isocitrate-pig heart NADP-IDH complex [14]. The 28 ppm difference between the chemical shift of ^{113}Cd in the binary complex with isocitrate and the enzyme complex may be a result of alterations in the geometry of the octahedral complexes. A shift of 50 ppm was observed for Cd-phosphoglucomutase upon ligand binding and attributed to a change in coordination geometry of the inner sphere oxygens upon glucose phosphate binding [44]. Asymmetry in the Mg site of the *E. coli* enzyme is demonstrated by the Mg-oxygen distances which range from 1.86 to 2.34 Å [41]. The results for the *E. coli* enzyme suggest that differences in distances to isocitrate for different metals may be responsible for differences in the turnover rates for different activating metals.

The relaxation behavior of Mg bound to NAD-IDH is similar to that for NADP-IDH. The greater relaxation rates are consistent with the larger size of this protein and increased correlation time for motion of the Mg complex. The relaxation rate is not increased by ADP which produces an increase in the measured molecular weight [26]. This suggests that relaxation is dominated by local motions and not by the much slower rotation of the enzyme oligomer.

For NAD-dependent isocitrate dehydrogenase the absence of an observed resonance does not allow any conclusion on the nature of the cadmium binding site in the ternary Cd-isocitrate-enzyme complex. The observed resonance in the quaternary system with ADP is consistent with a bound oxygen-liganded cadmium in exchange with Cd-isocitrate and Cd-ADP. The broad resonance does not allow quantitation to demonstrate that all bound cadmium is accounted for and enzyme bound sites with ligands other than oxygen cannot be completely excluded. The ^{113}Cd resonance in the Cd-ADP and Cd-ATP complexes is shifted to 24 to 30 ppm. This shift probably arises from rapid equilibration between complexes in which Cd is complexed by the nucleotide phosphates and complexes in which Cd is coordinated with N-7 of the nucleotide ring. The ^{113}Cd resonance continues to shift upon addition of excess nucleotide indicative of macrochelate formation. This macrochelate complex has been proposed to account for potentiometric titration and proton NMR titration data [45–47]. Complexes in which this coordination with N-7 is inner sphere and outer sphere with an intervening water molecule are proposed. The small shift compared with the chemical shift in the complexes of Cd with nitrogen containing ligands [48] is consistent with the 41% macrochelate complex formed with ADP [45]. The increasing shift with ATP:Cd ratio suggests the existence of a concentration dependent equilibrium between macrochelates in the Cd-ATP and Cd-ATP_2 complexes. The slightly

greater shifts observed with ATP than with ADP are consistent with the greater extent of macrochelate formation for Cd-ATP [46].

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