Saturation Magnetization of Ureases from *Klebsiella aerogenes* and Jack Bean: No Evidence for Exchange Coupling between the Two Active Site Nickel Ions in the Native Enzymes

Edmund P. Day,*,† Jim Peterson,‡ Mariana S. Sendova,† Matthew J. Todd,§ and Robert P. Hausinger§

Physics Department, Emory University, 1510 Clifton Road, Atlanta, Georgia 30322, Department of Chemistry, University of Alabama, Tuscaloosa, Alabama 35487-0336, and Departments of Biochemistry and Microbiology, Michigan State University, East Lansing, Michigan 48824

Received October 2, 1991

Saturation magnetization data for native jack bean and *Klebsiella aerogenes* ureases in buffers ranging from pD 6.4 to pD 9.7 have been collected at four fixed fields over the temperature range from 2 to 200 K. There is no evidence in these data sets for exchange coupling between the two nickel(II) ions at the active site of either urease. This conclusion contrasts with that of a previous report that the active site nickel ions of native jack bean urease are weakly exchange coupled (Clark, P. A.; Wilcox, D. E. *Inorg. Chem.* **1989**, *28*, 1326–1333). Each of our eight saturation magnetization data sets can be fitted by assuming that all nickel ions are magnetically isolated and comprised of a population of high-spin ($S = 1$) and low-spin ($S = 0$) species, with the diamagnetic contribution increasing with increasing pD.

Introduction

Urease (urea amidohydrolase, EC 3.5.1.5), a nickel-containing enzyme, hydrolyzes urea to form ammonia and carbamate which spontaneously degrades to CO_2 and a second molecule of ammonia.[1,2] The very well characterized jack bean (*Canavalia ensiformis*) urease is homohexameric (subunit M_r = 90 770[3]) and contains 2 mol of nickel/mol of subunit.[1,4,5] One of the best characterized microbial ureases is that from *Klebsiella aerogenes* (currently *Klebsiella pneumoniae*) which possesses three subunit types (M_r = 60 304, 11 695, and 11 096[6]) in an $\alpha_2\beta_4\gamma_4$ stoichiometry and contains 4 mol of nickel/mol of enzyme.[7] For both the plant and bacterial ureases, the catalytic unit has been shown to contain two nickel(II) ions.[8,9]

Magnetic susceptibility data of jack bean urease at pH 7 have been interpreted in a previous study to indicate weak antiferromagnetic exchange coupling of the two nickel ions.[10] We have serious reservations, which we describe in the Discussion, about the methodology used in that study. Therefore, we have undertaken a saturation magnetization study of both jack bean and *K. aerogenes* ureases, including an investigation of the pD dependence of their magnetic properties. We find no evidence for Ni–Ni exchange coupling in either native enzyme.

Experimental Section

Growth of *K. aerogenes* CG253[pKAU19], purification of urease, and the enzyme assay followed published procedures.[9] The purified bacterial enzyme was shown to be homogeneous by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and possessed a specific activity greater than 2200 μmol of urea degraded min^{-1} mg^{-1}. This specific activity did not change on freezing of the samples for magnetization and EPR measurements except for the low pD samples which lost approximately half of their activity. Jack bean urease donated by Dr. Robert L. Blakeley and Dr. Burt Zerner was purified and assayed as described previously.[5]

Samples were deuterated to reduce hysteresis in the magnetization data introduced by $I = {}^1/_2$ protons. Purified urease was exchanged at least three times into D_2O (99.8%, Cambridge Isotope Laboratories) containing 20 mM buffer and 1 mM EDTA by repeated 4-fold dilution followed by reconcentration to the original volume using a Centricon-30 (Amicon) with a YM100 membrane. The buffers included 2-(*N*-morpholino)ethanesulfonic acid, pD (pH meter reading + 0.4)[11] 6.4 and 6.6; *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid, pD 7.7; and 2-(*N*-cyclohexylamino)ethanesulfonic acid, pD 9.0 and 9.7. The filtrate from the final concentration step was used as the magnetization control for each sample. Hysteresis due to remaining protons was monitored by collecting half of each saturation magnetization data set below 35 K on cooling and alternate points on warming. Very slight hysteresis is evident in Figures 1A and 2A as alternate points are slightly offset at the lowest temperatures.

The suprasil quartz sample holders for the magnetization experiment were etched overnight in 10% hydrofluoric acid to remove ferromagnetic impurities. There was no remaining magnetic field dependence in the Curie law intercepts of any of the samples or controls. In addition, samples and controls were subjected to three cycles of the freeze–pump–thaw procedure to remove paramagnetic spin $S = 1$ molecular oxygen. There was no remaining spin $S = 1$ oxygen signal in any of the magnetization controls.

Electron paramagnetic resonance (EPR) spectra were collected to screen for unwanted paramagnetic impurities and to search for integer spin signals of nickel(II). EPR spectra were recorded on one of two instruments: (i) a Bruker ESR 300 electron resonance spectrometer or (ii) a hybrid machine consisting of a Varian E109E spectrometer console used to provide the field modulation to a Bruker B-E 25 magnet with an ER 082 power supply and B-H 15 field controller, plus a Varian E102 microwave bridge. Both spectrometers were fitted with Oxford Instruments ESR 900 flow cryostats.

EPR samples were taken from the same degassed solutions as the magnetization samples and transferred to EDTA-rinsed suprasil tubes. Samples were rejected which showed more than 0.5% of the $g = 4.3$ ferric impurity signal relative to the total nickel when compared with a ferric EDTA integration standard. The EPR spectra of all samples reported in Table I were essentially featureless. No integer spin signals were found.

Nickel concentrations were measured in hydrolyzed samples (1 N HNO_3 overnight at 110 °C, evaporated, and resuspended in 50 mM HNO_3) by using a Varian atomic absorption spectrometer equipped with a graphite furnace, autosampler, and Zeeman background correction.

† Emory University.
‡ University of Alabama.
§ Michigan State University..

(1) Andrews, R. K.; Blakeley, R. L.; Zerner, B. In *The Bioinorganic Chemistry of Nickel*; Lancaster, J. R., Jr., Ed.; VCH Publishers, Inc: New York, 1988; pp 141–165.
(2) Mobley, H. L. T.; Hausinger, R. P. *Microbiol. Rev.* **1989**, *53*, 85–108.
(3) Takishima, K.; Suga, T.; Mamiya, G. *Eur. J. Biochem.* **1988**, *175*, 151–165.
(4) Blakeley, R. L.; Zerner, B. *J. Mol. Catal.* **1984**, *23*, 263–292.
(5) Dixon, N. E.; Gazzola, C.; Blakeley, R. L.; Zerner, B. *J. Am. Chem. Soc.* **1975**, *97*, 4131–4133.
(6) Mulrooney, S. B.; Hausinger, R. P. *J. Bacteriol.* **1990**, *172*, 5837–5843.
(7) Todd, M. J.; Hausinger, R. P. *J. Biol. Chem.* **1987**, *262*, 5963–5967.
(8) Dixon, N. E.; Gazzola, C.; Watters, J. J.; Blakeley, R. L.; Zerner, B. *J. Am. Chem. Soc.* **1975**, *97*, 4130–4131.
(9) Todd, M. J.; Hausinger, R. P. *J. Biol. Chem.* **1989**, *264*, 15835–15842.
(10) Clark, P. A.; Wilcox, D. E. *Inorg. Chem.* **1989**, *28*, 1326–1333.
(11) Zerner, B.; Bender, M. L. *J. Am. Chem. Soc.* **1961**, *83*, 2267–2274.

0020-1669/93/1332-0634$04.00/0

Magnetization data were collected using a Quantum Design superconducting susceptometer modified by the manufacturer to give a small flow of helium exchange gas through the sample region. This gas flow substantially reduces scatter in the magnetization data of metalloprotein samples. Each sample and its control were studied at four fixed fields (0.2, 1.375, 2.75, and 5.5 T) over the temperature range from 2 to 200 K. Higher temperatures were not investigated because unwanted sublimation of the frozen samples from the open quartz holders occurs above 210 K.

The magnetization data presented in Figures 1 and 2 were fitted as described below to find the amount of spin $S = 1$ paramagnetism in the data. This amount of spin was then used to scale the vertical axes of the plots. The plots therefore depend solely upon the magnetization measurement. The nickel concentration of each sample was separately determined by atomic absorption spectroscopy upon completion of the magnetization measurement. This methodology makes it possible to measure both the spin $S = 1$ and the spin $S = 0$ nickel(II) sites which may both be present.

The best least-squares fit to the saturation magnetization data was found by computer using sequential simplex optimization.[12,13] Powder average theoretical magnetization curves were calculated from the spin $S = 1$ Hamiltonian using a 15-point grid per octant.[14] The final fit was the best of 10, each started at a randomly selected point in parameter space.

Results

The saturation magnetization data of *K. aerogenes* urease at pD 6.6 measured over the temperature range from 2 to 200 K at four fields from 0.2 to 5.5 T are presented in Figure 1. The solid lines shown in Figure 1 were calculated from the spin $S = 1$ Hamiltonian

$$\mathcal{H} = D[S_z^2 - {}^2/_3 + (E/D)(S_x^2 - S_y^2)] + \beta \mathbf{S}\cdot\mathbf{g}\cdot\mathbf{H}$$

with $D = -10$ cm^{-1}, $E/D = 0.1$, and $\langle g \rangle = 2.1$. The Brillouin curve, shown as a dashed line in Figure 1A, was calculated with $D = E = 0$. Figure 1A, which is a plot of the magnetization in Bohr magnetons (β) versus $\beta H/kT$, highlights the low-temperature, saturation behavior of the data. Figure 1B, which is a plot of $\mu_{eff}^2 = g^2S(S + 1)$ versus temperature, emphasizes the high-temperature, Curie law region of the data. Both plots are required for a proper appraisal of the quality of the fit at both high and low temperatures. The relatively large scatter in the data in Figure 1B is due to the decrease in the paramagnetic signal with increasing temperature. The data in Figure 1B are scattered about the horizontal theoretical line at temperatures above 50 K, providing evidence that there is no spin in these data other than the spin $S = 1$ used in the fit. A substantial amount of a different spin in the sample would have resulted in a systematic slope in the data as the fitting program varied the intercept parameters to minimize χ^2 while using the incorrect theoretical model. The fact the data are scattered about the horizontal indicates the spin $S = 1$ theoretical model used to fit the data was adequate for this data. The amount of spin $S = 1$ paramagnetism determined by the fit was 140 nmol, and the amount of nickel in the sample determined by atomic absorption spectroscopy was 170 nmol.

A similar presentation of data for a sample of *K. aerogenes* urease at pD 9.7 is given in Figure 2. In this case the spin Hamiltonian parameters were $D = -35$ cm^{-1}, $E/D = 0.1$, and $\langle g \rangle = 2.2$. The contrast in the relationship of the data to the Brillouin curve between Figures 1A and 2A is due to the far larger value of D in Figure 2A at high pD. The amount of spin $S = 1$ paramagnetism determined from the fit was 170 nmol while the amount of nickel for this sample determined by atomic absorption spectroscopy was 230 nmol. Both here and in Figure 1 the percentage of spin $S = 1$ nickel found using the particular amount for the "best fit" shown in the figure is consistent with the uncertainties of the amount listed in column 5 of Table I. The

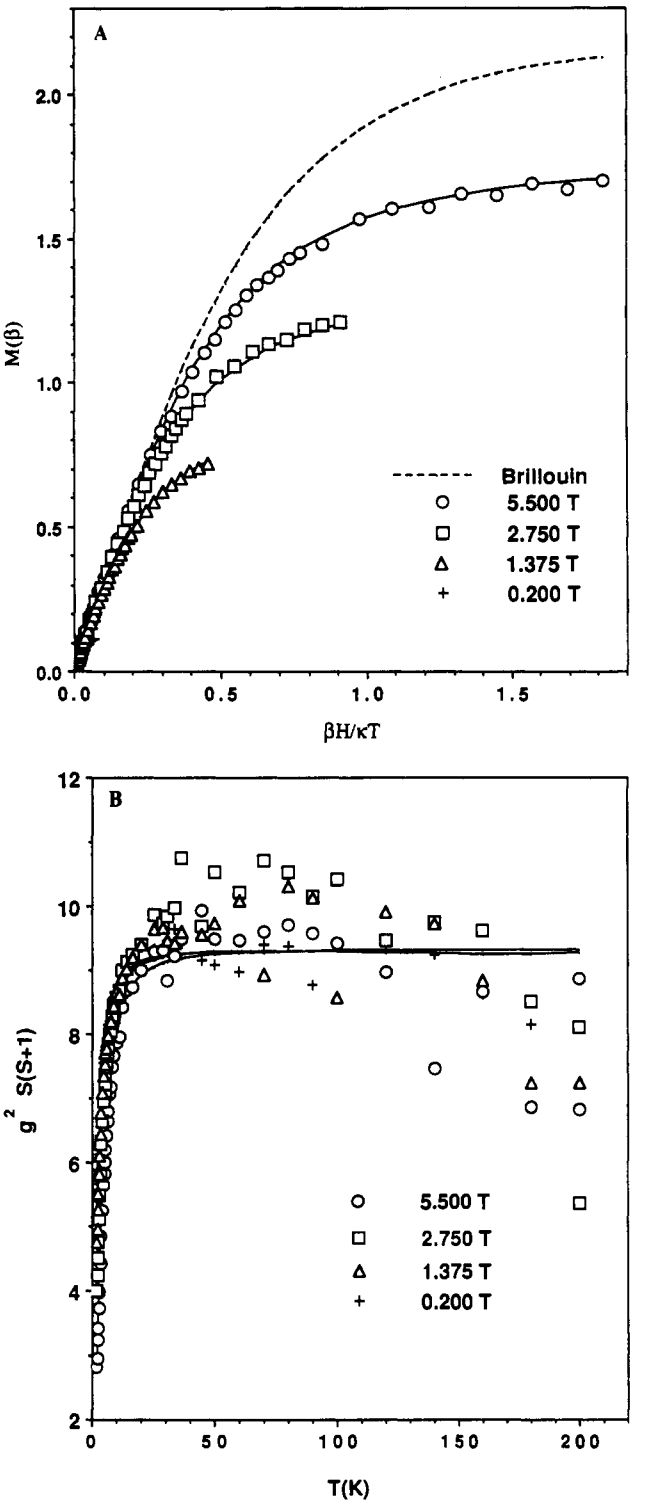

Figure 1. (A) Sample saturation magnetization of *K. aerogenes* urease at pD 6.6 plotted in Bohr magnetons (β) versus $\beta H/kT$ at four fixed fields (+, 0.2; Δ, 1.375; □, 2.75; and ○, 5.5 T) over the temperature range from 2 to 200 K. The solid curves were calculated from the spin $S = 1$ Hamiltonian with $D = -10$ cm^{-1}, $E/D = 0.1$, and $\langle g \rangle = 2.1$. The dashed line Brillouin curve was calculated with $D = E = 0$. The amount of nickel in the sample determined by atomic absorption spectroscopy was 170 nmol. The amount of spin $S = 1$ paramagnetism determined from this fit to the data was 140 nmol. This was used to scale the vertical axis. Both here and in Figure 2 the percentage spin $S = 1$ nickel found for the particular best fit shown in the figure is consistent with the amount and uncertainty given in column 5 of Table I. (B) The same data and theory presented as $\mu_{eff}^2 = g^2S(S + 1)$ versus temperature.

(12) Nelder, J. A.; Mead, R. *Comput. J.* **1965**, *7*, 308–313.
(13) Walters, F. H.; Parker, L. R., Jr.; Morgan, S. L.; Deming, S. N. *Sequential Simplex Optimization: A Technique for Improving Quality and Productivity in Research, Development, and Manufacturing*; CRC Press, Inc: Boca Raton, FL, 1991.
(14) Day, E. P.; Kent, T. A.; Lindahl, P. A.; Münck, E.; Orme-Johnson, W. H.; Roder, H.; Roy, A. *Biophys. J.* **1987**, *52*, 837–853.

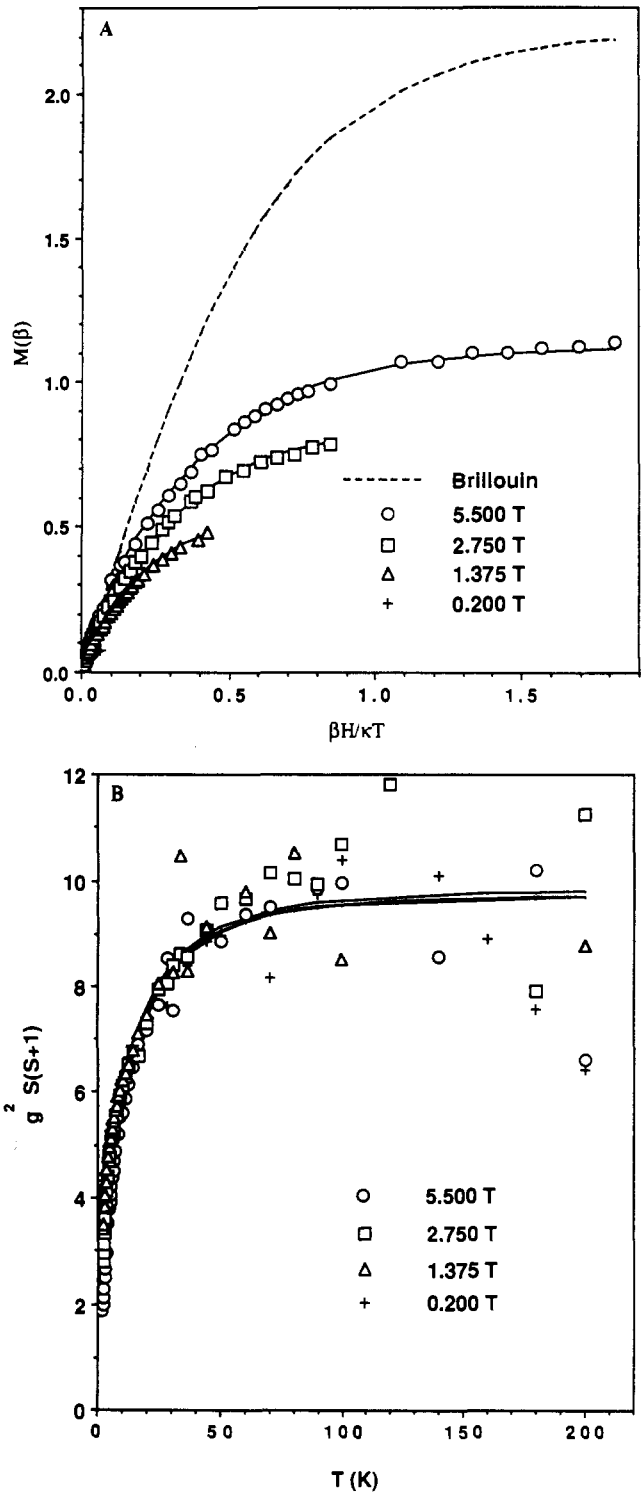

Figure 2. Similar data to Figure 1 with pD = 9.7. The spin $S = 1$ Hamiltonian parameters for this fit are $D = -35$ cm^{-1}, $E/D = 0.1$, and $\langle g \rangle = 2.2$. The amount of nickel in the sample determined by atomic absorption spectroscopy was 230 nmol. The amount of spin $S = 1$ paramagnetism determined from the fit to the data was 170 nmol.

amount of Table I represents an average found by exploring the neighborhood of the best fit to determine the uncertainties in the parameters. (See footnotes *c* and *e* of Table I.)

The results of these and similar measurements on several preparations of *K. aerogenes* and one of jack bean urease over the pD range from 6.4 to 9.7 are summarized in Table I. The ratios of the amount of spin $S = 1$ determined from the magnetization data to the amount of nickel determined by atomic absorption spectroscopy are listed as percentages in column 5 of Table I. Although there is unexpected variability in the fitting

Table I. Magnetic Properties of Urease Samples

pD (±0.1)	$\langle g \rangle$[a] (±0.05)[b]	D[c] (cm^{-1})	E/D (±0.05)[d]	Ni(S=1)[e]/ Ni(tot) (%)
		K. aerogenes		
first preparation				
6.4	2.0	−12 (3)	0.1	116 (15)
7.7	2.0	−20 (8)	0.1	66 (11)
9.0	2.7[f]	−35 (15)[f]	0.1	50 (6)
second preparation				
6.6	2.1	−10 (2)	0.1	91 (7)[e]
third preparation				
7.7	2.1	−30 (10)	0.1	84 (10)
9.7	2.2	−35 (8)	0.1	71 (5)[e]
		Jack Bean		
6.4	2.5[f]	−35 (15)[f]	0.1	ND[g]
9.7	2.7[f]	−43 (17)[f]	0.1	ND[g]

[a] Saturation magnetization data are sensitive to the average value of g. These data are not able to resolve the separate components of the g tensor. Although the fits were carried out with $g_x = g_y = g_z = \langle g \rangle$ (in order to limit the number of free parameters), this does not mean that g is necessarily isotropic. [b] The uncertainty in $\langle g \rangle$ was estimated by considering the change in $\langle g \rangle$ upon removing 0.5% of high-spin $S = {}^5/_2$ impurity iron. [c] The estimated uncertainty in D was arrived at by determining the range of D within which the quality of fit parameter (χ^2) was no worse than twice that of the best fit. [d] The estimated uncertainty in E/D is generous and emphasizes that saturation magnetization data are not strongly dependent upon E/D. [e] The estimated uncertainty in the percentage of nickel in the high-spin $S = 1$ state combines the uncertainty in the amount of spin $S = 1$ found from fitting the saturation magnetization data (found as described for D[c]) and that due to the atomic absorption determination of the nickel contained in the sample (±5%). The numbers in this column summarize the results of a full exploration of the neighborhood of each best fit to arrive at a representative average value and its uncertainty. [f] The spin Hamiltonian is most appropriate when g is close to 2 and $|D|$ is close to 0. When the fitting process yields g values approaching 3 and $|D|$ values approaching 50 cm^{-1}, the spin Hamiltonian formalism is being stretched to its limits. In this region of parameter space we are using the spin Hamiltonian to parameterize the data. This allows for systematic comparison of saturation magnetization, EPR, (MCD), and, in the case of Fe-containing proteins, Mössbauer data even though the assumptions underlying the spin Hamiltonian are beginning to break down. [g] There are no data from atomic absorption spectroscopy on the nickel content of the jack bean samples.

parameters generated for samples within each pD value, a trend of decreasing percent of spin $S = 1$ with increasing pD is observed for samples from each preparation. Futhermore, increasing pD leads to a general increase in $\langle g \rangle$ and in the magnitude of D. These results are consistent with a change from six-coordination to five-coordination for a portion of the nickel as the pD increases.

There is no evidence for exchange coupling between the two nickel ions at the active site of either *K. aerogenes* or jack bean urease in the magnetization data of this study. It was possible to fit the family of curves of saturation magnetization data for each sample assuming a spin $S = 1$ Hamiltonian. Systematic attempts were made to fit each data set assuming the sample consisted of a spin-coupled dimer. These fits yielded values for the exchange coupling consistent with zero (<0.5 cm^{-1} $S_1 \cdot S_2$).

The results of a magnetic circular dichroism (MCD) study[15] were interpreted to indicate the presence of a ferromagnetically coupled spin $S = 2$ component in native jack bean urease. Neither MCD nor saturation magnetization data can distinguish high-spin $S = 1$ components having a large g value from spin $S = 2$ having a g value of 2. In light of this ambiguity, we have limited our model to spin $S = 1$ to be fully consistent with the known magnetochemistry of nickel(II).[16]

Discussion

These measurements indicate that all of the nickel of native urease can be accounted for by a mixture of magnetically isolated

(15) Finnegan, M. G.; Kowal, A. T.; Werth, M. T.; Clark, P. A.; Wilcox, D. E.; Johnson, M. K. *J. Am. Chem. Soc.* **1991**, *113*, 4030–4032.

high-spin ($S = 1$) and low-spin ($S = 0$) nickel(II) and that the proportion of each spin changes with pD. There are many examples in the inorganic literature of nickel(II) systems which have a fraction of the sites with spin $S = 1$ and a fraction of the sites with spin $S = 0$.[17,18] Futhermore, the spin population of these inorganic compounds can be altered by solvent properties, including pH. We have looked for and can find no evidence of exchange coupling in the native enzyme as was reported earlier.[10]

The discrepancy between our results, using both bacterial and jack bean native ureases, and those of Clark and Wilcox for jack bean enzyme requires comment. All of the evidence for weak exchange coupling in the native state of jack bean urease was presented in their report in the fits shown in Figure 2A at 5 T over the temperature range from 2.4 to 240 K and Figure 2B at 1 T over the temperature range from 6.3 to 240 K.[10] The researchers tried but were unable to present a single parameter set to describe these two fields of data for the native enzyme. Instead they described the 5-T data as a composite consisting of 28% spin $S = 1$ Ni(II) monomer with $g = 2.2$, $D = 0$, and $E = 0$ and 72% Ni(II) dimer formed by antiferromagnetic exchange coupling (11.0 cm^{-1} $S_1 \cdot S_2$) between the $g = 2.2$, $D = 0$, $E = 0$ monomers. The 1-T data were described as a different composite consisting of 22% spin $S = 1$ Ni(II) monomer with $g = 2.2$, $D = -6.9$ cm^{-1}, and $E = 0$ and 78% Ni(II) dimer formed by antiferromagnetic exchange coupling (12.6 cm^{-1} $S_1 \cdot S_2$) between the $g = 2.2$, $D = -6.9$ cm^{-1}, $E = 0$ monomers. Curves calculated from these parameters are shown in Figure 3 in a single plot of susceptibility against inverse temperature. The form of these curves indicates that the two data sets fit by these parameters were collected from separately prepared samples which had different magnetic properties. It would be impossible to find a single parameter set to describe both data sets under these circumstances.

The evidence of Clark and Wilcox for weak antiferromagnetic exchange coupling between the nickel(II) sites of native jack beam urease is thus derived from two separate single-field saturation magnetization measurements. Rarely does a fit to saturation magnetization data collected at a single field stand the test of comparison with data collected at other fields on the same sample. For example, as a demonstration exercise we show in Figure 4 a fit patterned after that of Clark and Wilcox to a single field (5.5 T) of our data taken from Figure 1A. In Figure 4 we have subtracted 25 nmol of spin $S = 1$ monomer (15% of the Ni) with $g = 2.1$, $D = -5.8$ cm^{-1}, and $E/D = 0.3$ from the raw data of Figure 1A before fitting the difference to 75 nmol of a dimer (90% of the Ni) with identical spin $S = 1$ sites ($g = 2.1$, $D = -5.8$ cm^{-1}, and $E/D = 0.3$) antiferromagnetically coupled with $J = -1.0$ cm^{-1} (2 cm^{-1} $S_1 \cdot S_2$). (The 5% difference between the amount of nickel assumed by our fit and the amount of nickel determined from atomic absorption spectroscopy is within the uncertainty of the atomic absorption measurement.) The single-field demonstration fit, though convincing when examined in isolation (main Figure 4), is found to be inadequate when compared with all four fields of data collected from the same sample (inset Figure 4).

For a fit to saturation magnetization data to be valid, it is necessary that data be collected at several (four, for example) fixed fields on the same sample and that this entire family of curves be fit with a single parameter set as has been done here in Figures 1 and 2. This procedure was not followed by Clark and Wilcox,[10] leaving their interpretation open to question.

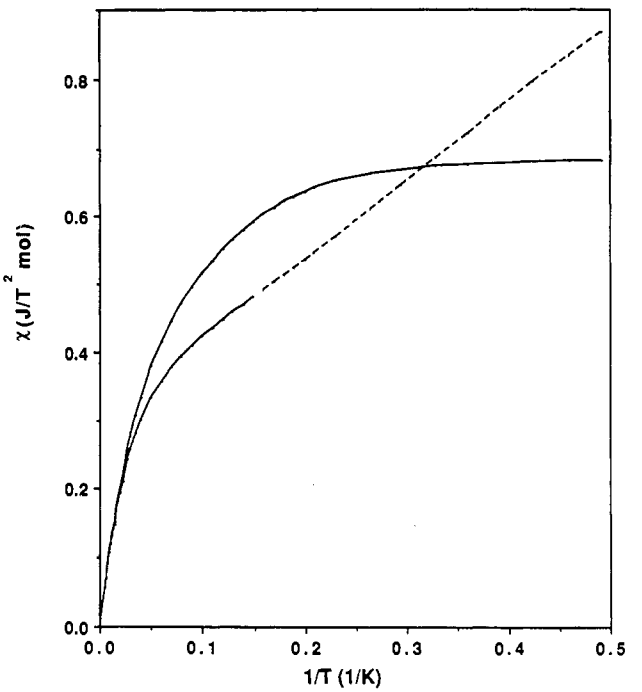

Figure 3. Susceptibility versus inverse temperature curves calculated using the parameters given by Clark and Wilcox[10] to describe their data for native jack bean urease. The solid portion of the partially dashed curve described data collected at 1 T over the temperature range from 6.5 to 240 K. Their fit assumed a composite sample with 22% of the nickel(II) as isolated spin $S = 1$ monomer with $g = 2.2$, $D = -6.9$ cm^{-1}, and $E/D = 0$ and 78% as antiferromagnetically coupled dimer (12.6 cm^{-1} $S_1 \cdot S_2$) of identical spin $S = 1$ nickel(II) sites ($g = 2.2$, $D = -6.9$ cm^{-1}, $E/D = 0$). The solid curve describes data collected at 5 T over the temperature range from 2.4 to 240 K. This fit assumed a different composite sample with 28% of the nickel(II) as isolated spin $S = 1$ monomer with $g = 2.2$, $D = 0$, and $E/D = 0$ and 72% as antiferromagnetically coupled dimer (11.0 cm^{-1} $S_1 \cdot S_2$) of identical spin $S = 1$ nickel(II) sites ($g = 2.2$, $D = 0$, $E/D = 0$).

Finally, it is important not to assume that all of the nickel in a sample is paramagnetic since there are many examples in the inorganic literature where nickel(II) sites are partially paramagnetic (high spin, $S = 1$) and partially diamagnetic (low spin, $S = 0$).[17,18] Therefore, the procedure of first converting the susceptibility data to molar susceptibility per nickel which was followed by Clark and Wilcox is not recommended. Instead four fields of saturation magnetization (susceptibility) data should be fitted to determine the amount of spin $S = 1$ paramagnetism in the sample. The nickel content should then be determined separately to measure the amount of spin $S = 0$ diamagnetism that is present.

Taken together, the optical and magnetic data of urease indicate the two nickel(II) ions at the active site may have different coordinations. On the one hand, the optical absorption spectrum of jack bean urease has been interpreted to indicate six-coordinate octahedral nickel(II) although there are additional features in the observed spectrum which are not accounted for by this interpretation.[19] On the other hand, six-coordinate nickel(II) compounds are high spin $S = 1$ with magnetic properties which generally fall in the range of $2.05 < g < 2.2$ with $|D| < 10$ cm^{-1}.[17,18] The magnetic properties of jack bean urease given in column 5 of Table I lie well outside these values, ruling out the possibility of only six-coordination. Extended x-ray absorption fine structure

(16) We have examined the urease data for spin $S = 2$ and can rule out the possibility of fitting any of the eight data sets using only spin $S = 2$. We have also tried a model combining spin $S = 1$ and spin $S = 2$. For jack bean the fits combining spin $S = 1$ and spin $S = 2$ were within one standard deviation ($\Delta\chi^2 < 1$) of the fits using only spin $S = 1$ which had two fewer free parameters. For the *Klebsiella* data the fits combining the two spins were worse by more than three standard deviations ($\Delta\chi^2 > 3.3$) on the average than the single-spin fit. In summary, the fits are comparable or better for the simpler model involving only spin $S = 1$ despite the fact this model has two fewer parameters.

(17) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 5th ed.; John Wiley & Sons: New York, 1988; pp 741–755.

(18) Sacconi, L. In *Transition Metal Chemistry. A Series of Advances*; Carlin, R. L., Ed.; Marcel Dekker, Inc.: New York, 1968; Vol. 4, pp 199–298.

(19) Blakeley, R. L.; Dixon, N. E.; Zerner, B. *Biochim. Biophys. Acta* **1983**, *744*, 219–229.

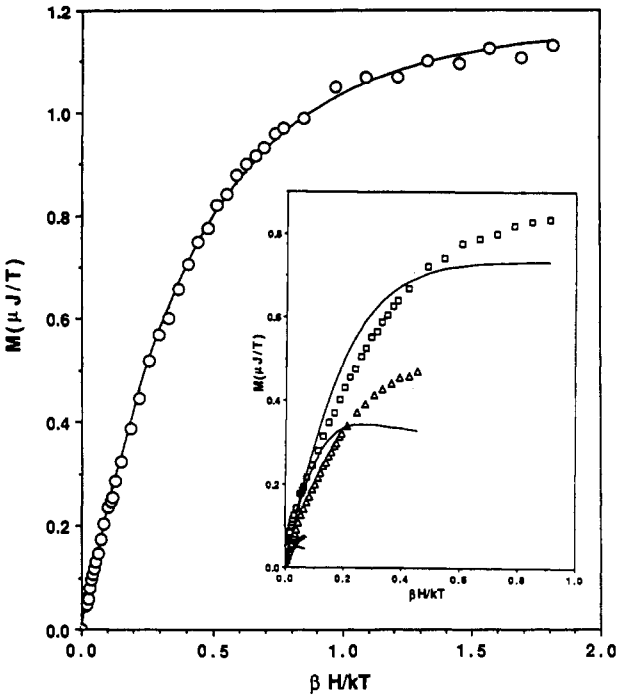

Figure 4. Demonstration fit to a single field of magnetization versus $\beta H/kT$ data. First, 25 nmol (15% of the Ni(II)) with spin $S = 1$, $g = 2.1$, $D = -5.8$ cm^{-1}, and $E/D = 0.3$ was subtracted from the data shown in Figure 1A. Second, the difference data were fit with 75 nmol of dimer (90% of the Ni(II)) formed by antiferromagnetically coupling (2 cm^{-1} $S_1 \cdot S_2$) identical spin $S = 1$ sites ($g = 2.1$, $D = -5.8$ cm^{-1}, $E/D = 0.3$). (The 5% difference between the assumed spins in the sample and the measured nickel content of the sample is within the uncertainty of the atomic absorption measurement of the sample's nickel content.) The main figure shows the resulting convincing fit to the single field of data at 5.5 T. The inset shows the very poor fit to the other three fields of data at fields of 2.75, 1.375, and 0.2 T.

(EXAFS) data are consistent with either six- or five-coordinate nickel(II).[20-22] It may be that the two nickel(II) ions at the active site of urease have different coordination, with one site being six-coordinate and one five-coordinate. The magnetic data then represent an average of the properties of these two different sites. This model cannot be ruled out by the present data. Whether this "two coordination sites" model is the only model consistent with all the data needs further investigation.[16]

Our results are consistent with the model for the mechanism of urease catalysis proposed by Zerner and colleagues[23] in which one nickel coordinates water or the oxygen atom of urea, polarizing the carbonyl group, and a second nickel coordinates hydroxide ion which functions as a catalytic nucleophile. The proposed mechanism requires that the two nickel ions be less than 0.6 nm apart, but does not require them to be coupled in the native enzyme. Antiferromagnetic coupling in the presence of the inhibitor β-mercaptoethanol[15] is evidence for the proximity of the nickel ions required by the proposed mechanism. Finally, the proposed mechanism invokes several groups (a carboxylic acid, a general base, a thiol, and a nickel-coordinated hydroxide group) that are sensitive to pH. One or more of these groups may explain the observed pD dependence in the magnetic properties of the active site nickel ions of urease.

Acknowledgment. The superconducting susceptometer was purchased with a National Institutes of Health Shared Instrumentation Grant RR05273 (E.P.D.). This research was supported by National Institutes of Health Grant GM32394 (E.P.D.), Biomedical Research Support Grant S07RR07151-11 (J.P.), the Australian Research Committee and the University of Queensland (B.Z.), and USDA Competitive Research Grant 8902262 (R.P.H.). We thank Dr. Robert Blakely and Dr. Burt Zerner, who donated the jack bean urease sample, Dr. Michael Hendrich, who helped us in our search for integer spin EPR signals, and Dr. Thomas A. Kent, who developed the software used to fit the magnetization data.

Registry No. Ni, 7440-02-0; urease, 9002-13-5.

(20) Hasnain, S. S.; Piggott, B. *Biochem. Biophys. Res. Commun.* **1983**, *112*, 279–283.
(21) Alagna, L.; Hasnain, S. S.; Piggott, B.; Williams, D. J. *Biochem. J.* **1984**, *220*, 591–595.
(22) Clark, P. A.; Wilcox, D. E.; Scott, R. A. *Inorg. Chem.* **1990**, *29*, 579–581.
(23) Dixon, N. E.; Riddles, P. W.; Gazzola, C.; Blakeley, R. L.; Zerner, B. *Can. J. Biochem.* **1980**, *58*, 1335–1344.