Proton and Deuteron Nuclear Magnetic Relaxation Dispersion Studies of Ca^{2+}–Mn^{2+}–Lentil Lectin and Ca^{2+}–Mn^{2+}–Pea Lectin: Evidence for a Site of Solvent Exchange in Common with Concanavalin A†

Lokesh Bhattacharyya and C. Fred Brewer*

Departments of Molecular Pharmacology and of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461

Rodney D. Brown, III, and Seymour H. Koenig*

IBM Thomas J. Watson Research Center, Yorktown Heights, New York 10598

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ABSTRACT: Measurements of the magnetic field dependence of the longitudinal magnetic relaxation rates (NMRD profiles) of solvent protons and deuterons led to the discovery of two classes of solvent binding sites in Ca^{2+}–Mn^{2+}–concanavalin A (CMPL) [Koenig, S. H., Brown, R. D., III, & Brewer, C. F. (1985) *Biochemistry* (second of three papers in this issue)]. In this paper, we compare proton and deuteron NMRD profiles of Ca^{2+}–Mn^{2+}–lentil lectin (CMLcH) and Ca^{2+}–Mn^{2+}–pea lectin (CMPSA) with those of CMPL. All three metalloproteins are D-mannose/D-glucose-specific lectins that have a high degree of structural similarity and require the metal ions for their biological activities. We have developed a method for the preparation of fully active metal ion derivatives of lentil lectin (LcH) and pea lectin (PSA), including the diamagnetic derivatives Ca^{2+}–Zn^{2+}–LcH and Ca^{2+}–Zn^{2+}–PSA [Bhattacharyya, L., Brewer, C. F., Brown, R. D., III, & Koenig, S. H.(1984) *Biochem. Biophys. Res. Commun. 124*, 857–862]. The behavior of these two lectins with regard to their NMRD profiles is essentially identical, for both the paramagnetic and diamagnetic forms. Together with CMPL, all three lectins have a common paramagnetic contribution with a negative temperature dependence of the rates, while CMPL contributes an additional component with a positive temperature dependence. The common contribution derives from the class of fast exchanging water molecules observed in the proton NMRD profile of CMPL (Koenig et al., 1985); their protons are calculated to be relatively remote from the Mn^{2+} ions (4.4 Å for CMPL and 5.5 Å for LcH and PSA). These waters are tentatively assigned to the coordination sphere of the Ca^{2+} ions in all three proteins, and their relaxation contributions are insensitive to the presence of bound saccharide.

In the preceding paper in this issue (Koenig et al., 1985), measurements of the magnetic field dependence of solvent proton and deuteron magnetic relaxation profiles (NMRD)[1] were used to demonstrate the existence of two classes of solvent binding sites in Ca^{2+}–Mn^{2+}–concanavalin A (CMPL). The deuteron NMRD profile, which is sensitive to sites of slowly exchanging solvent molecules, indicated such a site at S1 (the transition metal ion site), while the proton NMRD data, which, in addition, are sensitive to sites of faster exhanging water molecules, indicated such a site approximately 4.4 Å from Mn^{2+}, well beyond its inner coordination sphere, but otherwise not identified.

In this paper, we report a study of solvent proton and deuteron NMRD profiles of Ca^{2+}–Mn^{2+}–lentil lectin (CMLcH) and Ca^{2+}–Mn^{2+}–pea lectin (CMPSA). Both are D-mannose/D-glucose-specific lectins like CMPL (Goldstein & Hayes, 1978), and all three have a high degree of structural similarity (Foriers et al., 1981; Meehan et al., 1982). Though these lectins have similar binding specificities toward simple carbohydrates (Allen et al., 1976), they show significant differences in their affinities toward glycopeptides (Kornfeld et al., 1981), differences that are believed to be responsible for their differing biological activities (Brown & Hunt, 1978; Lis & Sharon, 1981). Thus, it is of considerable interest to elucidate the molecular properties of these lectins in order to find the basis for their distinct biological activities.

The recent discovery of a method of preparing Ca^{2+}–Zn^{2+} derivatives of LcH and PSA (Bhattacharyya et al., 1984, 1985) now allows comprehensive studies of both paramagnetic and diamagnetic metal ion derivatives of these two lectins. We show in the present study, from a comparison of the proton and deuteron NMRD profiles of CMLcH and CMPSA with those of CMPL, that all three proteins contain a common class of sites characterized by rapidly exchanging solvent molecules with their protons about 4.4–5.5 Å from the Mn^{2+} ions. In addition, CMPL has a water ligand (or ligands) directly bonded to Mn^{2+}, in relatively slow exchange, that contributes observably to both the proton and deuteron solvent relaxation

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[1] Abbreviations: NMRD, nuclear magnetic relaxation dispersion, the magnetic field dependence of the longitudinal solvent nuclear magnetic relaxations rates ($1/T_1$); Con A, concanavalin A with unspecified metal ion content; MP, binary Mn^{2+}–Con A complex in the unlocked conformation; CMPL, Con A with Mn^{2+} and Ca^{2+} at S1 and S2, respectively, in the locked conformation; CZPL, Con A with Zn^{2+} and Ca^{2+} at S1 and S2, respectively, in the locked conformation; LcH, lentil lectin; CMLcH, native LcH containing 1 equiv of Mn^{2+} and 1.7–2 equiv of Ca^{2+} per monomer; CZLcH, LcH containing 1 equiv of Zn^{2+} and 1.5–2 equiv of Ca^{2+} per monomer; PSA, pea lectin; CMPSA, native PSA containing 1 equiv of Mn^{2+} and 1.5–2 equiv of Ca^{2+} per monomer; CZPSA, PSA containing 1 equiv of Zn^{2+} and 1.5–2 equiv of Ca^{2+} per monomer; 3-MDG, 3-*O*-methyl-D-glucopyranose; EDTA, ethylenediaminetetraacetic acid; EPR, electron paramagnetic resonance.

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rates (Koenig et al., 1985). The location of the common, faster exchanging solvent binding site in the lectins is tentatively assigned to the coordination sphere of the Ca^{2+} ions in all three proteins.

Materials and Methods

Preparation of Proteins. Lentil lectin, LcH (*Lens culinaris* Med. sub. *Macrosperma*), was purified by affinity chromatography on Sephadex G-100 (Ticha et al., 1970). Separation of the two isolectins, LcHA and LcHB, was accomplished by CM-cellulose chromatography (Howard et al., 1971). Pea lectin, PSA (*Pisum sativum* L. var. Columbian), and its two isolectins, A and B, were obtained as previously described (Trowbridge, 1974).

Ca^{2+}–Zn^{2+}-substituted lentil lectin (CZLcH) and Ca^{2+}–Zn^{2+}-substituted pea lectin (CZPSA), which were prepared by a method discovered only recently, possess the same saccharide binding properties as the native Ca^{2+}–Mn^{2+} lectins (Bhattacharyya et al., 1984, 1985). The concentrations of native lentil and pea lectins and their separated isolectins were determined in pH 6.4 buffer by using the absorbance $A^{1\%,1cm}$ = 12.6 at 280 nm (Howard et al., 1971) for the lentil proteins and $A^{1\%,1cm}$ = 15.0 at 280 nm for the pea proteins, the latter value being determined in the present study. All concentrations are given in monomeric units, 23.5 kilodaltons (kDa). About 20 μM EDTA was added to all CMLcH and CMPSA samples to complex any small amount of free Mn^{2+} present, an amount so small as to not contribute to the observed NMRD rates.

Metal Ion Analysis. Analysis for Mn^{2+} was carried out in two ways [cf. Brewer et al. (1983)]. One method involved acidifying the protein samples to pH 1.2 and determining the total liberated Mn^{2+} by using proton NMRD. Atomic absorption spectroscopy was also used to determine the concentrations of Mn^{2+} and other metal ions present in the samples. The results showed that CMLcH contained 1 equiv of Mn^{2+} and 1.7–2.0 equiv of Ca^{2+} per monomer; the same results were found for CMPSA. CZLcH and CZPSA were found to have 0.8 and 0.9 equiv of Zn^{2+} per monomer, respectively, and 1.5–2.0 equiv of Ca^{2+} per monomer. The Mn^{2+} content in these derivatives was less than 0.1%.

NMRD Measurements. Longitudinal (spin–lattice) relaxation rates of solvent protons and deuterons were measured by using an improved field-cycling apparatus used previously (Brown et al., 1977; Koenig et al., 1985). Sample volumes were routinely 0.6 mL. Reproducibility of the data for a given sample for proton rates was generally better than ±1.5% and for deuterium rates was generally better than ±2.5%.

Results

Proton NMRD Profiles of CMLcH and CZLcH. CMLcH, the native form of lentil lectin, consists of a mixture of two isolectins, A and B, which differ slightly in amino acid composition and charge (Howard et al., 1971). The two isolectins were separated by ion-exchange chromatography, and their metal ion content (Mn^{2+} and Ca^{2+}) and proton NMRD profiles were measured. The results (not shown) indicated that the two isolectins have identical NMRD profiles; therefore, all NMRD data presented here were taken on preparations of the native protein (CMLcH), which is approximately a 1:1 mixture of LcHA and LcHB.

Representative solvent proton NMRD profiles for solutions of 0.81 mM CMLcH and 0.80 mM CZLcH at 5 and 25 °C are shown in Figure 1. The rates for both metal ion complexes of the protein were insensitive both to changes in pH from 5 to 8 and to changes in salt concentration from 0.01 to 1 M NaCl or KCl. (Higher salt concentrations have a small effect on CMLcH, as will be shown below).

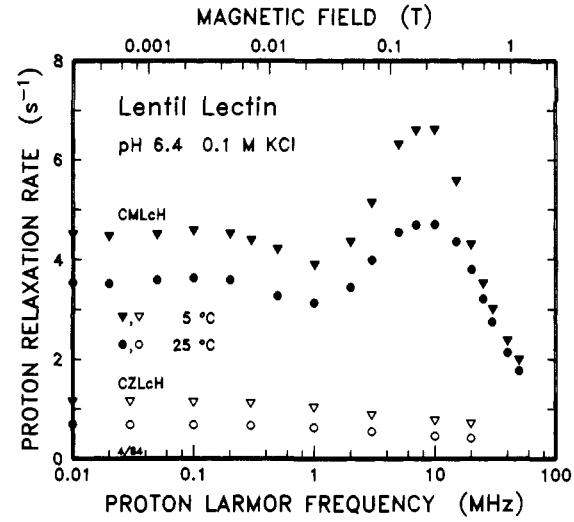

FIGURE 1: Longitudinal magnetic relaxation rates of solvent protons as a function of magnetic field strength (NMRD profiles) of 0.81 mM CMLcH (▼, ●) and 0.80 mM CZLcH (▽, ○), in pH 6.4 buffer containing 0.1 M potassium acetate and 0.1 M potassium chloride, at 5 and 25 °C.

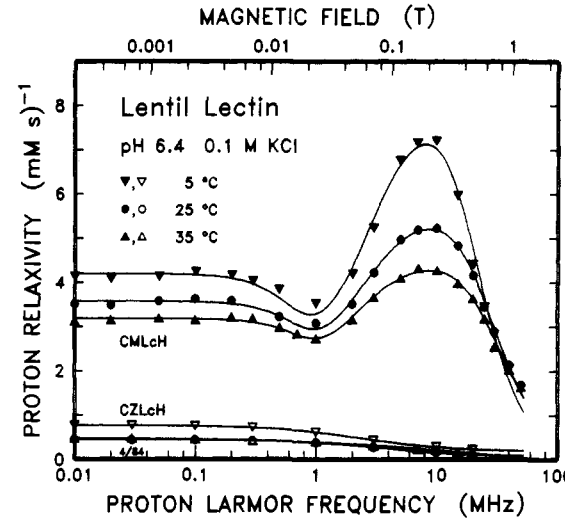

FIGURE 2: Paramagnetic contribution to the proton NMRD profile of CMLcH, expressed as rates per millimolar bound Mn^{2+} (mM^{-1} s^{-1}), at 5 (▼), 25 (●), and 35 (▲) °C. The paramagnetic contribution was obtained by subtracting the CZLcH relaxation rate data from that for CMLcH in Figure 1, after adjusting for the small difference in protein concentrations. The solid lines through the data points for CMLcH are a visual guide and have no other significance. The solid lines through the data points of CZLcH represent a least-squares comparison of the data with a heuristic theoretical expression useful for diamagnetic proteins and correspond reasonably well with those expected for a globular protein of the molecular weight of the LcH dimer (47 kDa) (Hallenga & Koenig, 1976).

Figure 2 shows the paramagnetic contributions of the Mn^{2+} ions to the NMRD profiles of CMLcH at 5, 25, and 35 °C, expressed as relaxivity, the contribution to the NMRD profile per millimolar bound Mn^{2+} ions, and obtained as previously described (Brown et al., 1977), by subtracting the CZLcH relaxation rate data from that for CMLcH after adjustment for the slight differences in protein concentration. (We have observed a small concentration dependence of the relaxivity of CZLcH and have therefore used relaxivity values of CZLcH derived frm essentially the same concentration of protein used as for the CMLcH data.) No concentration dependence of the paramagnetic contribution to the relaxivity values of CMLcH was observed. The paramagnetic relaxivities of

CMLcH decrease with increasing temperature, in contrast to those of solutions of CMPL, which increase with temperature from 5 to 35 °C.

Mn^{2+} aquo ions in solution contribute a characteristic variation of the NMRD profile in the low-field region [cf. Brown et al. (1977)]. The paramagnetic relaxivities in the low-field region between 0.01 and 0.2 MHz are flat for CMLcH, indicating less than 20 μM free Mn^{2+} in solution. The paramagnetic contribution to the NMRD profiles displays a peak toward the high-field region that is characteristic of Mn^{2+}–protein complexes [cf. Koenig et al. (1971)] and here is centered at approximately 7 MHz. Addition of either 0.15 M D-mannose or 0.1 M 3-MDG had no effect on the observed rates, in contrast to the behavior of solutions of CMPL, which show a drop in rates of approximately 15% at 25 °C at all fields upon addition of methyl α-D-mannopyranoside or D-glucose (Koenig et al., 1973; Brewer & Brown, 1979).

The LcH dimer is approximately 47 kDa (Lebrun et al., 1983), and the relaxivity data in Figure 2 at 25 °C for CZLcH are characteristic of globular diamagnetic proteins of this molecular weight (Koenig & Schillinger, 1969; Hallenga & Koenig, 1976). CZLcH has the same molecular weight as native CMLcH (Bhattacharyya et al., 1984, 1985), supporting its use as a diamagnetic correction for CMLcH.

Proton NMRD Data of CMPSA and CZPSA. CMPSA consists of a mixture of two isolectins, A and B, that differ somewhat in their charge and amino acid composition (Trowbridge, 1974). The two isolectins are present in a ratio of approximately 9:1 B:A in the pea lectin, var. Columbian, used for this study. The proton NMRD profiles and metal ion contents (Mn^{2+} and Ca^{2+}) of the two isolectins were shown to be the same. All subsequent measurements were accordingly done with the native pea mixture, CMPSA.

Proton NMRD data were recorded at 5, 25, and 35 °C for a solution of 0.90 mM CMPSA in pH 6.4 buffer (not shown). The profiles were insensitive both to changes in pH from 5 to 8 and to changes in salt concentrations from 0.01 to 1 M KCl or NaCl (higher salt concentrations have a small effect) and were essentially indistinguishable from those for CMLcH. Like CMLcH, addition of saccharide had no effect on the observed rates, and the temperature dependence was the same. Data were also recorded for 0.72 mM Ca^{2+}–Zn^{2+}–PSA (CZPSA) under the same conditions.

The temperature dependence of the paramagnetic contribution of the bound Mn^{2+} of CMPSA to the NMRD profiles, expressed as relaxivity, is shown in Figure 3, as are the profiles for the diamagnetic protein, CZPSA. The latter data at 25 °C were characteristic of a diamagnetic protein with the molecular weight of the PSA dimer (Hallenga & Koenig, 1976), approximately 47 kDa (Meehan et al., 1982). CZPSA has the same molecular weight and saccharide binding properties as CMPSA (Bhattacharyya et al., 1984, 1985). CZPSA also showed a concentration-dependent relaxivity, and therefore similar concentrations of CZPSA and CMPSA were used to determine the paramagnetic contribution to the relaxivity (Figure 3). No concentration dependence of the paramagnetic contribution to the NMRD profile of CMPSA was observed. The results for CMLcH and CZLcH (Figure 2) are essentially identical in all details with those for CMPSA and CZPSA (Figure 3), respectively.

Proton NMRD Data of CMPSA in 5 M NaCl Buffer. The temperature dependence of the paramagnetic contribution to the proton NMRD profile of CMPSA in 5 M NaCl at −8 and 5 °C is shown in Figure 4. Data (not shown) were recorded for CZPSA under the same conditions in order to obtain the

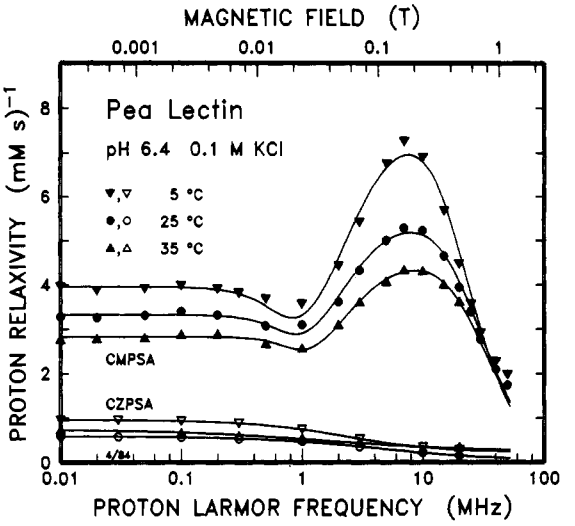

FIGURE 3: Paramagnetic contribution to the proton NMRD profile of CMPSA, expressed as rates per millimolar bound Mn^{2+} (mM^{-1} s^{-1}), at 5 (▼), 25 (●), and 35 (▲) °C, in pH 6.4 buffer containing 0.1 M potassium acetate and 0.1 M potassium chloride. The paramagnetic contribution was obtained by subtracting CZPSA relaxation rate data, normalized to the CMPSA concentration (not shown), from CMPSA relaxation rate data (not shown) at the appropriate temperature. The concentration of CZPSA was 0.72 mM and that of CMPSA 0.90 mM. The solid lines through the data points for CMPSA are a visual guide and have no other significance. The solid lines through the data points of CZLcH represent a least-squares comparison of the data with a heuristic theoretical expression useful for diamagnetic proteins and correspond reasonably well with those expected for a globular protein of the molecular weight of the PSA dimer (47 kDa) (Hallenga & Koenig, 1976).

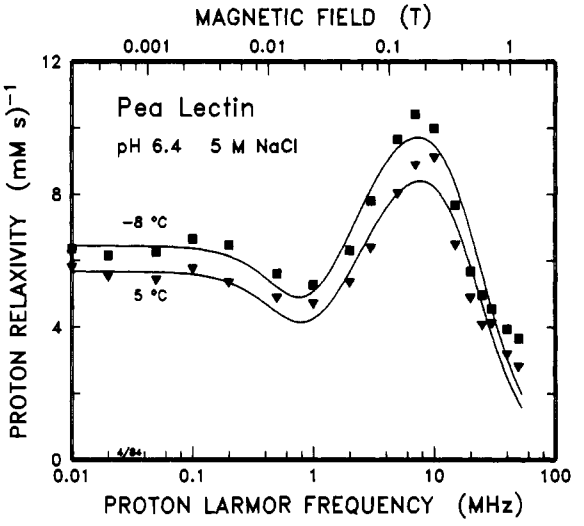

FIGURE 4: Proton NMRD profiles of CMPSA at −8 and 5 °C in pH 6.4 buffer containing 0.1 M potassium acetate and 5 M sodium chloride. The concentration of CMPSA from which the data were derived was 0.46 mM. The solid lines through the data points represent a visual guide and have no other significance.

paramagnetic contribution for CMPSA. The results at 5 °C are similar to those in Figure 3, although the peak at 7 MHz is somewhat greater. The proton NMRD profile of CMPSA has somewhat larger relaxivities at −8 °C than at 5 °C. Nonetheless, over the range −8 to 35 °C (Figures 3 and 4), the paramagnetic relaxivity for CMPSA increases with decreasing temperature. Comparable results were found for CMLcH (not shown).

Deuteron NMRD Data of CZLcH and CMLcH Solutions. The deuterium NMRD profiles of CZLcH and CMLcH at 5 °C are shown in Figure 5. The solvent contained 90% deuterons and 10% protons, and the samples had nearly the

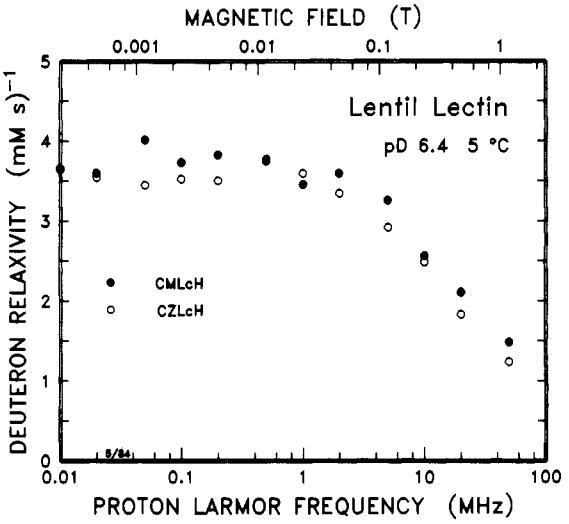

FIGURE 5: Deuteron NMRD profiles of CMLcH (●) and CZLcH (○) at 5 °C in pH 6.4 buffer, 0.1 M potassium acetate and 0.1 M potassium chloride, containing 90% D_2O and 10% H_2O. Concentrations of CMLcH and CZLcH from which the data were derived were 1.16 and 0.95 mM, respectively.

same protein concentrations. The proton NMRD profile of CMLcH in the mixed solvent (not shown) showed the same relaxivity as a 100% H_2O solution of the same protein, demonstrating the absence of any detectable solvent isotope effects in the mixed solvent system. In contrast to analogous deuterium NMRD data for CZPL and CMPL, which showed significant differences in rates between the paramagnetic and diamagnetic complexes (Koenig et al., 1985), there is little, if any, observable paramagnetic component in the deuteron NMRD profiles of solutions of CMLcH and CZLcH.

Discussion

Structures of LcH and PSA. Native forms of LcH and PSA are metalloproteins that bind Mn^{2+} and Ca^{2+} with relative stoichiometries of metal to protein monomer of approximately 1:1 for Mn^{2+} and approximately 1.7–2.0:1 for Ca^{2+} (Bhattacharyya et al., 1985). PSA and LcH possess a great deal of structural similarity with Con A (Meehan et al., 1982). One distinction is that Con A can form tetramers at pH above 6.4, whereas PSA and LcH are found only as dimers, with molecular weights of approximately 47 kDa [cf. Trowbridge (1974) and Foriers et al. (1981), respectively]. Low-resolution X-ray crystallographic data for PSA (Riskulov et al., 1984; Meehan et al., 1982) indicate the dimensions of the dimer to be 90 × 50 × 40 Å^3, nearly the same as those of the Con A dimer, 84 × 40 × 39 Å^3 (Hardman & Ainsworth, 1972). The presence of high electron density regions at the ends of the dimer are believed to indicate the sites of the Mn^{2+} and Ca^{2+} ions, which may correspond to the S1 and S2 sites in Con A, respectively (Hardman et al., 1982). Preliminary sequence data for PSA suggest strong homology with LcH (van Driessche et al., 1976), as discussed below.

Low-resolution X-ray crystallographic data of LcH have been reported. Less is known about the three-dimensional structural features of LcH than those of PSA, although both are believed to be similar. The complete amino acid sequence of LCH has been reported (Foriers et al., 1981). All of the amino acids that provide ligands for the S1 and S2 metal ion sites in Con A are conserved in LcH, with the exception of Tyr-12 at S2, which is replaced by Phe-125. All amino acids believed to be part of the saccharide binding site in Con A are also conserved in LcH, as are those associated with the so-called hydrophobic cavity (Foriers et al., 1981).

Proton NMRD Comparison of CMLcH, CMPSA, and CMPL. The proton relaxivity profiles for the paramagnetic contributions in CMLcH and CMPSA (Figures 2 and 3, respectively) are essentially identical, indicating strong conservation of the environment of the Mn^{2+} and Ca^{2+} binding sites in the two proteins. EPR spectra for PSA (Brewer and Friedman, unpublished results) and LcH (Tichy et al., 1971) show similar, six-line, hyperfine structure at room temperature, both similar to that observed for Con A (Reed & Cohn, 1970), indicating high octahedral symmetry at the Mn^{2+} site and concomitant low ligand field splittings. Spin-echo EPR spectroscopy shows a conserved histidine ligand at the Mn^{2+} site in PSA and LcH similar to that found in Con A (Brewer et al., unpublished results).

The low relaxivities of CMLcH and CMPSA and the structures of their NMRD profiles (Figures 2 and 3) indicate that the relaxation cannot be due to a water molecule exchanging from the inner coordination sphere of the Mn^{2+} ion but is due to a complexed, rapidly exchanging water molecule (or molecules) further away. The nature and location of the exchanging solvent molecules are posited below.

The results for CMLcH and CMPSA differ from those for CMPL in important ways. First, the magnitudes of the relaxivities of CMLcH and CMPSA at 25 °C at low fields are nearly 3-fold lower than those for CMPL [cf. Figure 3 of Koenig et al. (1985)], with a smaller difference at higher fields. Second, the temperature dependence of the ^1H NMRD profiles for CMLcH and CMPSA between 5 and 35 °C, Figures 2 and 3, respectively, is different from that for CMPL [cf. Figure 3 of Koenig et al. (1985)]. CMLcH and CMPSA have a negative temperature coefficient between 5 and 35 °C, whereas CMPL has a positive temperature coefficient over this range. However, a change in the sign of the temperature dependence of the ^1H NMRD profile of CMPL is seen below this range [cf. Figure 3 of Koenig et al. (1985)]. Furthermore, like the profiles for CMLcH, the profile for CMPL is similar to that for CMPSA at −8 °C (Figure 4), though somewhat different in magnitude. In summary, the shape of the profile of CMPL at −8 °C has changed significantly from that at higher temperatures and resembles those of CMLcH and CMPSA, suggesting that at −8 °C the determinants of the solvent proton relaxation profiles of the three lectins are similar. At higher temperatures, activated exchange from another class of sites begins to dominate the relaxivity of CMPL, but not that of the other two lectins, with a concomitant change in both shape and temperature dependence of the demonstrably composite ^1H NMRD profile. These results argue for two classes of sites for CMPL, with each class of site having opposite temperature dependences. Only one of these sites, that with waters in relatively rapid exchange, appears to play a role in the ^1H NMRD profiles of CMLcH and CMPSA over the entire temperature range examined. These conclusions are futher supported by the following observations.

Deuteron NMRD Results for CMLcH. In the preceding paper (Koenig et al., 1985), a comparison of ^1H and ^2H NMRD profiles of CMPL demonstrated the existence of two classes of exchanging solvent molecules, both of which contributed to the ^1H NMRD profiles of the lectin, while only one class contributed substantially to the paramagnetic ^2H NMRD profiles. By contrast, the ^2H NMRD profiles of CMLcH and CZLcH at 5 °C (Figure 5) in the present study show little if any paramagnetic contribution. However, deuteron rates should be only 1/40 of the proton paramagnetic contribution when fast exchanging waters dominate the relaxation process, because of the lower gyromagnetic ratio of

the deuteron compared to the proton. The inference from Figure 5, therefore, is that the ^1H paramagnetic contributions to the NMRD profiles in Figures 2 and 3 for CMLcH and CMPSA, respectively, are due to fast exchanging water molecules. Their low relaxivities, on the other hand, indicate that these waters are rather far from the Mn^{2+} ion. The deuteron results for CMPL, containing a solvent exchange mechanism not seen in CMLcH and CMPSA, involve an exchange-limited solvent contribution, known to be due to water molecules directly liganded to the Mn^{2+} in CMPL (discussed in the preceding paper). These water molecules, if present in CMLcH and CMPSA, must be exchanging too slowly over the entire temperature range to contribute to the ^1H data of Figures 2 and 3.

Effects of Saccharide Binding. Additional confirmation of a single exchange contribution in CMLcH and CMPSA, in contrast to the composite nature of the proton NMRD profiles in CMPL, comes from examining the effects of saccharide binding on the ^1H NMRD profiles of the three lectins. CMLcH and CMPSA show no effect upon addition of saccharide from −8 to 35 °C, while CMPL shows a relative large effect (approximately 18% reduction) at 35 °C, with progressively smaller decreases at lower temperatures, until at −8 °C a negligible reduction occurs (Koenig et al., 1985). These results indicate that the exchange contribution observed in CMLcH and CMPSA is insensitive to bound saccharide at all temperatures examined, which is similar to the exchange contribution for CMPL at low temperatures (−8 °C). At higher temperatures CMPL has another exchange contribution that is sensitive to bound saccharide.

The Site of the Rapidly Exchanging Solvent Molecules in CMPL, CMLcH, and CMPSA. The X-ray crystallographic structure of CMPL has been determined to 1.75-Å resolution (Hardman et al., 1982). Two of the ligands of the Mn^{2+} at the S1 site are water molecules hydrogen bonded to nearby residues. It is this class of exchanging water molecules that dominates the ^1H NMRD profile of CMPL between about 5 and 35 °C (Koenig et al., 1985). This contribution is exchange limited, which accounts for the positive temperature coefficient of the proton data over this range. Solvent exchange from these sites is also responsible for the deuteron paramagnetic relaxivity since the exchange limitation is less severe for ^2H than for ^1H. The relaxivity contributions of this class of sites are also sensitive to the presence of bound saccharide on CMPL, judging from the temperature dependence of this effect. Bound saccharide results in a rate decrease as well as a flattening of the ^1H NMRD profile, indicating a decrease in the rate of solvent exchange (Koenig et al., 1973; Brewer & Brown, 1979).

The second class of exchanging water molecules in CMPL, which are in fast exchange, was calculated to be approximately 4.4 Å from the Mn^{2+} ion, according to a comparison of theory with the paramagnetic relaxivity profile of CMPL at −8 °C, a temperature at which only this site contributes to the proton relaxation data (Koenig et al., 1985). The relaxivity profiles of CMLcH and CMPSA are similar in form but lower in magnitude than the profile of CMPL at −8 °C, suggesting that there is one water molecule rather than two water molecules involved or that the distance is somewhat greater. An estimate of the distance of the protons of the water molecule(s) at this site in CMLcH (and CMPSA) can be obtained by comparing theory with the paramagnetic relaxivity data of CMLcH, as was done for CMPL (Koenig et al. 1985). The results of the fit at 5 and 35 °C are shown in Figure 6; values for the parameters obtained here are compared with those for CMPL

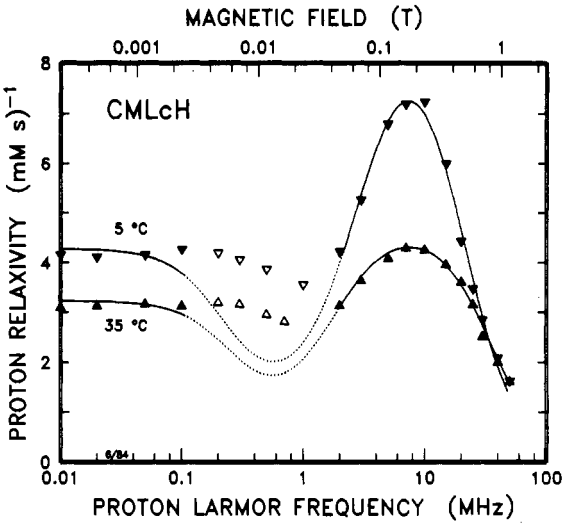

FIGURE 6: Results of a least-squares comparison of relaxation with the paramagnetic NMRD profiles of CMLcH at 5 (▼) and 35 °C (▲) are shown. In the theory (Koenig & Brown, 1985) it is argued that for Mn^{2+}–protein complexes for which the ligand field splitting of the electronic levels of the Mn^{2+} is very small, which is true for CMLcH (Tichy et al., 1971), the comparison should give good results for the parameters of the theory so long as the data in the decade of field centered about 1 MHz are excluded. The solid lines show the results of the comparison of the theory with the data; the dotted lines show the region where the comparison is expected to be inappropriate. Values for the parameters are shown in Table I.

Table I: Values for Parameters[a] Derived from a Comparison of a Theory of Relaxation with Paramagnetic NMRD Profiles of CMLcH at 5 and 35 °C (Figure 6) and CMPL[b] at −8 °C

	5 °C, CMLcH	35 °C, CMLcH	−8 °C, CMPL
τ_m (ns)	12	8.6	5.2
$T_{1so} = T_{2so}$ (ns)	1.3	1.2	6.5
τ_v (ps)	140	190	100
τ_R (ns)	22	9.4	42
r (Å)	5.5	5.6	4.4
q	2	2	2

[a] τ_m is the residence time of the exchanging water ligands; T_{1so} and T_{2so} are the longitudinal and transverse relaxation times, respectively, of the unpaired electrons of Mn^{2+}; τ_v is the correlation time describing the interaction that couples the Mn^{2+} magnetic moment with the protein; τ_R is the appropriate rotational correlation time of the protein computed from Stokes' law; r is the distance separating the protons of the exchanging water ligands from Mn^{2+}; and q, the number of exchanging water ligands, is assumed to be 2. [b] From Koenig et al. (1985).

in Table I. The results give an average value of 5.5 Å, assuming two exchanging waters at this site in CMLcH, compared to the value of 4.4 Å calculated for CMPL. The exchange time τ_m for the waters at this site is approximately 10^{-8} s, significantly shorter than that (approximately 10^{-5} s) for the exchanging waters at the Mn^{2+} ions in CMPL (Koenig et al., 1985), and contributes to the negative temperature coefficient of the data for CMLcH and CMPSA, and the similar behavior of CMPL at the lower temperatures, through its influence on the electron–nuclear correlation times for solvent proton relaxation at these sites [Tables I and II in Koenig et al. (1973)].

Since the more remote solvent exchange site appears to be quite similar in all three lectins, with lifetimes of exchange significantly longer than those found for waters solvating amino acid side chains (approximately 10^{-10} s), we propose that the sites represent waters coordinated with the Ca^{2+} ions at S2 in the proteins. This is consistent with the distance calculations separating the water protons at these sites from the Mn^{2+} ions,

assuming similar geometries of the S1 and S2 sites in CMLcH and CMPSA, relative to CMPL [cf. Hardman et al. (1982)]. Moreover, the exchange time is in the range observed for water ligands of Ca^{2+} ions (Eigen & De Maeyer, 1963). Indeed, X-ray crystallographic data of CMPL show two water molecules as ligands of the Ca^{2+} ion (Hardman et al., 1982), which can be expected to exchange with bulk solvent. The greater distance of this site in CMLcH and CMPSA, 5.5 Å as contrasted with 4.4 Å in CMPL, suggests that the position(s) of the water ligands at the Ca^{2+} sites in the former two proteins is (are) further from the Mn^{2+} ions than in CMPL, perhaps due to a slight difference in the binding configuration of the Ca^{2+} to one of the two bridging aspartic acid residues found in CMPL (Hardman et al., 1982).

In summary, we have found a class of rapidly exchanging water molecules, relatively remote from the coordination sphere of the Mn^{2+} ions, that dominates the proton relaxivity profiles of CMLcH and CMPSA and is the second contributing class of exchanging water molecules in CMPL. We suggest that these are ligands of the Ca^{2+} ions at S2 in the proteins, with distances that differ somewhat between CMPL, on the one hand, and CMLcH and CMPSA, on the other. These findings are a unique example of the assignment of two solvent exchange sites in a single metalloprotein (CMPL) and of a common solvent exchange site in a group of related metallolectins. These results may have general applicability to the study of binary metal clusters in other proteins. Finally, the metal ion binding sites of CMLcH and CMPSA are shown to be very similar, as indicated by their essentially identical paramagnetic relaxivity NMRD profiles.

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