Nuclear Magnetic Resonance Investigation of Cadmium 113 Substituted Pea and Lentil Lectins*

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The lentil (LcH) and pea (PSA) lectins, which are members of the class of D-glucose/D-mannose binding lectins, are Ca²⁺-Mn²⁺ metalloproteins that require the metal ions for their saccharide binding and biological activities. We have prepared a variety of Cd²⁺ derivatives of PSA and LcH, with Cd²⁺ in either the transition metal (S1) or calcium (S2) sites, or in both. Thus, Cd²⁺, Zn²⁺, Cd²⁺-Mn²⁺, and Ca²⁺-Cd²⁺ derivatives were prepared, in addition to the Cd²⁺-Cd²⁺ derivatives which we have recently reported. This is the first report of stable mixed metal Cd²⁺ complexes of lectins. The physical and saccharide binding properties of the Cd²⁺ derivatives of both lectins were characterized by a variety of physicochemical techniques and found to be the same as those of the corresponding native proteins.

113Cd NMR spectra of mono- and disubstituted 113Cd²⁺ complexes of LcH and PSA were recorded and compared with 113Cd NMR data for concanavalin A (ConA) (Palmer, A.R., Bailey, D.B., Behnke, W.D., Cardin, A.D., Yang, P.P., and Ellis, P.D. (1980) Biochemistry 19, 5063–5070). The data for the PSA and LcH derivatives were found to be very similar, indicating close homology of their metal ion binding sites. 113Cd resonances at 44.6 ppm and -129.4 ppm for 113Cd²⁺, 113Cd³⁺ LcH, and at 45.6 and -130.4 for the corresponding PSA derivative, are chemical shifts very similar to those observed for 113Cd²⁺, 113Cd³⁺ ConA. Assignment of the resonances to the transition metal (S1) and calcium (S2) sites were unambiguous since the Ca²⁺, 113Cd²⁺ and 113Cd³⁺-Zn²⁺ derivatives of both lectins showed single resonances characteristic of the S1 and S2 sites, respectively. The results indicate that, unlike ConA, 113Cd²⁺ binds tightly to PSA and LcH. Binding of monosaccharide to both lectins induce small (2 ppm) upfield shifts in their 113Cd resonances, in contrast to the larger shift (8 ppm) observed in ConA. The 113Cd²⁺-Mn²⁺ complexes of PSA and LcH fail to show a 113Cd resonance characteristic of these derivatives, which provides evidence for the close proximity of the metal ions in the two proteins. The present findings indicate that the coordinating ligand atoms to the metal ions at the S1 and S2 sites in LcH, PSA, and ConA are the same.

Lentil (LcH) and pea (PSA) lectins have been extensively employed as tools in many areas of biological research due to their unique carbohydrate specificities (1, 2). Along with ConA, they have been classified as D-glucose/D-mannose specific lectins (3). However, unlike ConA, LcH and PSA display enhanced binding affinities toward certain fucosylated glycopeptides (4, 5).

Metal ions are required for activities of a large number of lectins; including LcH, PSA, and ConA (3, 6, 7). However, only in the case of ConA has the role of metal ions been studied in detail (6). ConA possesses two metal-binding sites per monomer: S1, the so-called “transition metal” site, and S2, the “calcium” site (3). There are two conformational states of ConA which possess different binding properties: a “locked” configuration which strongly binds metal ions and saccharides; and an “unlocked” configuration which binds them very weakly (6, 8). The apoprotein which exists predominantly in the unlocked conformation sequentially binds Mn²⁺ and Ca²⁺ and undergoes a first-order conformational transition to the locked or fully active conformation (6). Binary, ternary, and quaternary complexes of both conformations of ConA involving metal ions and saccharides have been characterized (6, 8). Thus, the relationship between metal ion and saccharide binding properties of ConA are well established.

Although LcH and PSA also bind Mn²⁺ and Ca²⁺, little is known about the role of the metals in regulating the activities of the lectins. It is known that both proteins are dimers of molecular mass 47 kDa (9, 10), and that each monomer of both lectins consists of one  and one  chain, with molecular masses of 6.5 and 17 kDa, respectively (9, 11). Each monomer of LcH and PSA also contains one Mn²⁺ and approximately two Ca²⁺ ions (7, 12). Amino acid sequence data show conservation of the amino acids in LcH and PSA which constitute the S1 and S2 sites in ConA, except that Tyr-12 at S2 of ConA is replaced by a Phe residue in LcH (Phe-123) and PSA (Phe-123) (9, 10). EPR (13-15) and magnetic circular dichroism data (16) show the metals in the S1 sites to be in octahedral or slightly distorted octahedral environments in all three lectins, in agreement with the x-ray crystallographic data for ConA (18, 19) and recent data for PSA (20) which show that the metal ions are located at positions similar to that in ConA. However, LcH and PSA differ from ConA in their metal ion exchange properties (7, 12) and kinetics of

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1 The abbreviations used are: LcH, *Lens culinaris* hemagglutinin, lentil lectin; PSA, *Pisum sativum* agglutinin, pea lectin; ConA, concanavalin A, the jack-bean lectin; CD, circular dichroism; NMRD, nuclear magnetic relaxation dispersion, the magnetic field dependence of nuclear magnetic relaxation rates, in the present case, the longitudinal relaxation rate, 1/\(T_1\), of solvent protons; 3-MDG, 3-O-methyl-d-glucopyranose.

2 E. Stafford, A. D. Cardin, W. D. Behnke, L. Bhattacharyya, and C. F. Brewer, manuscript in preparation.
exchange of solvent water molecules at the Mn$^{2+}$ and Ca$^{2+}$ sites (17, 21). Furthermore, we have recently substituted Cd$^{2+}$ for Mn$^{2+}$ in LcH and PSA (7), and observed differences in their visible CD spectra compared to Cd$^{2+}$-substituted ConA (22). Thus, it is important to characterize the metal ion binding properties of LcH and PSA in order to understand the role of the metals in the structure and activity of the lectins.

$^{115}$Cd NMR is an important tool for characterizing the metal ion-binding sites of metalloproteins because of its sensitivity to the nature of the coordinating ligands (23). This technique has been used to characterize the S1 and S2 sites of ConA (24, 25). However, in these studies, Cd$^{2+}$ formed weak complexes with ConA and various mixed metal complexes of Cd$^{2+}$ and the lectin were formed in solution under equilibrium conditions which did not yield well-defined complexes in certain instances (e.g. Cd$^{2+}$-Mn$^{2+}$). We have recently reported the preparation of stable Cd$^{2+}$-Cd$^{2+}$ derivatives of LcH and PSA (7). In the present study, we report the preparation and properties of stable Cd$^{2+}$-Mn$^{2+}$, Cd$^{2+}$-Zn$^{2+}$, and Ca$^{2+}$-Cd$^{2+}$ derivatives of the two lectins, and $^{115}$Cd NMR spectra of these derivatives, as well as those of Cd$^{2+}$-Cd$^{2+}$-derivatives. The availability of stable mixed metal derivatives of $^{115}$Cd at either the S1 or S2 sites of LcH and PSA has allowed unequivocal assignments of their $^{115}$Cd resonances. Perturbations of these resonances in the presence of bound saccharide are also reported. The results are compared to $^{115}$Cd NMR data for ConA (25) and recent x-ray crystallographic data for PSA (20). The CD studies of Ca$^{2+}$-Cd$^{2+}$ derivatives of LcH and PSA indicate some differences in the environments of the metal ions compared to ConA (22). The present findings, however, show that the coordinating ligand atoms of the metal ions at both the S1 and S2 sites in all three lectins are the same.

Materials and Methods

Seeds of lentil (Lens culinaris Med. sub. Macrospora) and pea (Pisum sativum L. var. Columbian) were purchased from a local food store. The respective lectins were purified by affinity chromatography on Sephacryl G-100 (11, 26). Salts of different metals were the highest purity products available from either Mallinckrodt or Fisher. $^{115}$Cd (95.8% enriched) was obtained from U. S. Services, Inc. Known weights of the metal were dissolved in 1:1 diluted HCl or H$_2$SO$_4$ to get the corresponding $^{115}$Cd salt. Monosaccharides were obtained from Sigma and Pfanstiehl Laboratories. Polysaccharides PGM and GM of P. quinatus were gifts from Dr. M. Slodki, Northern Regional Research Center, United States Department of Agriculture.

Preparation of Cd$^{2+}$ Derivatives—A modification of the procedure described earlier for the preparation of other metal derivatives of LcH and PSA was used (7, 12). LcH or PSA were dissolved at about 10 mg/ml in 50 mM sodium acetate buffer, pH 4.0, containing appropriate amounts of salts of different metals. The following combinations of salts were used: 0.2 M CdSO$_4$ for the Cd$^{2+}$-Cd$^{2+}$ derivatives; 0.1 M each of CdCl$_2$ and CaCl$_2$ for the Ca$^{2+}$-Cd$^{2+}$ derivatives; 0.2 M CdSO$_4$ with 0.5 M MnSO$_4$ or ZnSO$_4$ for the Cd$^{2+}$-Mn$^{2+}$ or Cd$^{2+}$-Zn$^{2+}$ derivatives, respectively. The solutions were incubated at 37°C for 16 days for LcH and 30 days for PSA. Any precipitate formed was removed by centrifugation, and the metal derivatives were then dialyzed against water at 4°C and stored as salt-free lyophilizates. $^{115}$Cd derivatives of the two lectins were obtained by using the appropriate $^{115}$Cd salts.

Protein Concentrations—The concentrations of LcH and its derivatives were determined using $A_{280}$ = 12.6 at 280 nm (7, 27). The extinction coefficients ($A_{280}$) at 280 nm of PSA and its derivatives were taken as 15.0 (7). Unless otherwise stated all protein concentrations are reported in terms of monomer concentration.

Metal Ion Analyses—The metal concentration of known concentrations were acidified to pH 1.2 with concentrated HCl and allowed to stand overnight at room temperature; the precipitates were removed by centrifugation. The supernatants were used for metal ion analysis by atomic absorption measurements using a Perkin-Elmer model 603 spectrophotometer (28). Mn$^{2+}$ in these solutions was also determined by proton NMRD techniques (29).

Hemagglutination Assays—These were done at room temperature in phosphate-buffered saline at 1 mg/ml protein concentrations using 3% suspensions of rabbit erythrocytes (29).

Turbidity Reaction and Inhibition by Monosaccharides—These were done at room temperature in phosphate-buffered saline following Bhattacharyya et al. (7, 12).

$^{115}$Cd NMR Measurements—$^{115}$Cd NMR spectra were acquired on a Bruker WH-400 NMR spectrometer using a 10-mm broadband probe tuned to 88.756 MHz. Lyophilized protein samples (100-200 mg) were dissolved in 2.0 ml of D$_2$O buffer, 0.1 M potassium acetate, and 0.1 M potassium chloride. To this solution was added 0.5 ml (20% v/v) of D$_2$O. Spectra were acquired under deuterium field/frequency lock without proton decoupling at 22°C. A 22° pulse and a 0.4-s delay between scans was employed for a total of 130,000 scans/spectrum.

RESULTS

Preparation of Cd$^{2+}$ Derivatives—We have previously used 10 mM sodium acetate buffer, pH 4.0, to prepare Ca$^{2+}$Zn$^{2+}$, Ca$^{2+}$-Co$^{2+}$, Ca$^{2+}$-Ni$^{2+}$, and Cd$^{2+}$-Cd$^{2+}$ derivatives of LcH and PSA (7, 12). However, preliminary experiments with LcH showed that the yields of Cd$^{2+}$ derivatives listed in Table I were below 50% under these conditions. The yields of these derivatives were found to depend on the concentration of acetate in solution, with optimum yields of 70-80% obtained at 50 mM acetate. Therefore 50 mM sodium acetate buffer, pH 4.0, was used to prepare the derivatives reported here. This also allowed an increase in the protein concentrations used in the exchange reactions from the previously reported 5 mg/ml (7, 12) to 10 mg/ml during the preparation of these derivatives.

Table I shows the results of metal ion analysis of Cd$^{2+}$, Mn$^{2+}$, Cd$^{2+}$-Zn$^{2+}$, and Ca$^{2+}$-Cd$^{2+}$ derivatives of LcH and PSA. The results show approximately 1:1 stoichiometry between the metal ions and the 23.5-kDa monomer of the proteins. Cd$^{2+}$-Mn$^{2+}$ derivatives were found to have a slight excess of Cd$^{2+}$ and less than the stoichiometric amounts of Mn$^{2+}$, presumably due to the formation of small amounts of the corresponding Cd$^{2+}$-Cd$^{2+}$ derivatives during the exchange reactions (see below). The preparation and metal ion analysis of the Cd$^{2+}$-Cd$^{2+}$ derivatives of LcH and PSA have been reported earlier (7). Thus, conditions have been found in which Cd$^{2+}$ occupies both the S1 and S2 sites of both lectins.

Table I

Metal ion content of lentil and pea lectin derivatives

| Derivatives | Cd$^{2+}$ | Mn$^{2+}$ | Zn$^{2+}$ | Ca$^{2+}$ |
|-------------|----------|----------|----------|----------|
| LcH        | 1.33     | 0.98     | 0.97     | 0.93     |
| PSA        | 1.18     | 1.07     | 1.00     | 1.00     |

Derivatives

| Cd$^{2+}$-Mn$^{2+}$-LcH | 1.34 | 0.86 | 0.91 | 1.13 |
|-------------------------|------|------|------|------|
| Cd$^{2+}$-Zn$^{2+}$-LcH | 0.97 | <0.01| 0.97 | ND   |
| Ca$^{2+}$-Cd$^{2+}$-LcH | 0.93 | 0.02 | 0.93 | 1.37 |
| Ca$^{2+}$-Mn$^{2+}$-PSA | 1.18 | 0.91 | 0.91 | 0.50 |
| Cd$^{2+}$-Zn$^{2+}$-PSA | 1.07 | <0.01| 1.07 | ND   |
| Ca$^{2+}$-Cd$^{2+}$-PSA | 1.00 | 0.01 | 1.37 | 1.44 |
or can selectively bind to one or the other site.

**Physical Properties of the Cd**\(^{2+}\)** Derivatives**—The Cd\(^{2+}\)** derivatives of LcH and PSA (Table I) have essentially the same physical properties as the respective native lectins. A mixture of all of the Cd\(^{2+}\) derivatives of LcH and the native protein coelutes in a single symmetrical peak (not shown) from a Bio-Gel P-100 column, indicating the same shape and size for the native lectin and Cd\(^{2+}\) derivatives. The same results are also obtained with native PSA and its Cd\(^{2+}\) derivatives. The near ultraviolet (240–320 nm) absorption and CD spectra of the Cd\(^{2+}\) derivatives are superimposable with those of the respective native proteins (7, 22) at the same protein concentrations (not shown). The results show that the conformations and overall structures of the Cd\(^{2+}\) derivatives of LcH or PSA are identical to those of the corresponding native lectins.

The NMRD profiles of the Cd\(^{2+}\)-Mn\(^{2+}\) derivatives of LcH and PSA were found to be identical to those of the corresponding native lectins containing Mn\(^{2+}\) and Ca\(^{2+}\) at the same temperature (Fig. 2 and 3 of Ref. 21). The results indicate that the Cd\(^{2+}\)-Mn\(^{2+}\) derivative of each lectin has the same coordination sphere of the Mn\(^{2+}\) ion as that of the corresponding native protein. Thus, Mn\(^{2+}\) is at the S1 sites and, as will be shown (below), Cd\(^{2+}\) at the S2 sites in these derivatives.

**Hemagglutination, Precipitation, and Precipitation-Inhibition Assays**—Native LcH and its Cd\(^{2+}\) derivatives are equally active in hemagglutinating rabbit red blood cells, each having a titer of 1024 at 1 mg/ml. The same results were obtained with the native PSA and its derivatives (Table I), each having a titer of 2048 at 1 mg/ml.

The development of turbidity as a function of time was followed for Cd\(^{2+}\)-Mn\(^{2+}\), Cd\(^{2+}\)-Zn\(^{2+}\), and Ca\(^{2+}\)-Cd\(^{2+}\) derivatives of LcH and PSA with the two polysaccharides from *P. pinus* PGM and GM, and compared with the corresponding curves obtained with the respective native lectins (Fig. 4 of Ref. 7). The native and the metal derivatives of each lectin were found to give overlapping curves.

For the determination of concentrations of monosaccharides required for 50% inhibition of precipitation, readings were taken 45 min after mixing (7). The concentrations of methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, and 3-MDG required for 50% inhibition of precipitation of PGM by native lectins and their Cd\(^{2+}\) derivatives are shown in Table II. The results indicate that all of the Cd\(^{2+}\) derivatives have essentially the same sugar binding activities as the respective native lectins. The Cd\(^{2+}\) NMR spectra of 111\(^{Cd}\)-Cd\(^{2+}\)-PSA, with resonances at 46.6 and −130.4 ppm. Two resonances at 44.6 and −129.4 ppm were obtained with 111\(^{Cd}\)-Cd\(^{2+}\)-LcH (Table III). Binding of 3-MDG to 111\(^{Cd}\)-Cd\(^{2+}\)-PSA had no effect on the 46.6-ppm resonance, however, the −130.4-ppm resonance was shifted upward by 2.2 ppm (Fig. 1b, Table III). Similar results were obtained for 111\(^{Cd}\)-Cd\(^{2+}\)-LcH in the presence of 3-MDG (Table III).

The 111\(^{Cd}\) NMR spectrum of 111\(^{Cd}\)-Zn\(^{2+}\)-LcH (Fig. 2a) shows one resonance at −122.2 ppm. This resonance is approximately 7 ppm downfield of the corresponding resonance of the double 111\(^{Cd}\) derivative (Table III). Similarly, the single resonance at −123.7 ppm for 111\(^{Cd}\)-Zn\(^{2+}\)-PSA is about 7 ppm downfield of the corresponding resonance of 111\(^{Cd}\)-Zn\(^{2+}\).

### Table II

| Derivatives  | Concentration of monosaccharides required for 50% inhibition of precipitation | ppm |
|-------------|--------------------------------------------------------------------------------|-----|
|             | methyl α-D-glucopyranoside | methyl β-D-glucopyranoside | 3-MDG |
| Native LcH  | 2.2                          | 19.4                          | 0.37  |
| Cd\(^{2+}\)-Cd\(^{2+}\)-LcH | 2.1                          | 19.7                          | 0.38  |
| Cd\(^{2+}\)-Mn\(^{2+}\)-LcH | 2.2                          | 21.0                          | 0.38  |
| Cd\(^{2+}\)-Zn\(^{2+}\)-LcH | 2.2                          | 19.4                          | ND*   |
| Ca\(^{2+}\)-Cd\(^{2+}\)-LcH | 2.2                          | 18.6                          | 0.39  |
| Native PSA  | 1.0                          | 12.2                          | 0.18  |
| Cd\(^{2+}\)-Cd\(^{2+}\)-PSA | 1.1                          | 13.5                          | 0.19  |
| Cd\(^{2+}\)-Mn\(^{2+}\)-PSA | 1.1                          | 12.8                          | 0.18  |
| Cd\(^{2+}\)-Zn\(^{2+}\)-PSA | 1.2                          | 13.2                          | 0.19  |
| Ca\(^{2+}\)-Cd\(^{2+}\)-PSA | 1.1                          | 14.0                          | 0.19  |

* ND, not determined.

### Table III

| Derivatives  | S1 | S2 |
|-------------|----|----|
| Cd\(^{2+}\)-Cd\(^{2+}\)-LcH | 44.6 | −129.4 |
| Cd\(^{2+}\)-Cd\(^{2+}\)-LcH + 3-MDG | 44.1 | −130.6 |
| Cd\(^{2+}\)-Cd\(^{2+}\)-PSA | 46.6 | −130.4 |
| Cd\(^{2+}\)-Cd\(^{2+}\)-PSA + 3-MDG | 46.9 | −132.6 |
| Cd\(^{2+}\)-Zn\(^{2+}\)-LcH | −122.2 |
| Cd\(^{2+}\)-Zn\(^{2+}\)-LcH + 3-MDG | −124.0 |
| Cd\(^{2+}\)-Zn\(^{2+}\)-PSA | −123.7 |
| Cd\(^{2+}\)-Zn\(^{2+}\)-PSA + 3-MDG | −124.8 |
| Ca\(^{2+}\)-Cd\(^{2+}\)-LcH | 42.9 |
| Ca\(^{2+}\)-Cd\(^{2+}\)-LcH + 3-MDG | 42.5 |
| Ca\(^{2+}\)-Cd\(^{2+}\)-PSA | 45.0 |
| Ca\(^{2+}\)-Cd\(^{2+}\)-PSA + 3-MDG | 44.9 |
The same result was obtained with the PSA derivative. In each case, the intensity did not account for the Cd\(^{2+}\) incorporated in the proteins (Table I).

Preparation of Cd\(^{2+}\) Derivatives of LcH and PSA—Bhattacharyya et al. (7, 12) have previously shown that the Mn\(^{2+}\) and Ca\(^{2+}\) in PSA and LcH can be selectively substituted by other metal ions at pH 4.0 and 37 °C in the presence of high concentrations of the desired salts. Thus, Mn\(^{2+}\) in PSA and LcH was substituted with Co\(^{2+}\), Zn\(^{2+}\), Ni\(^{2+}\), and Cd\(^{2+}\) to give the corresponding Ca\(^{2+}\)-Mn\(^{2+}\) complexes. Cd\(^{2+}\) was the only metal to replace both the Mn\(^{2+}\) and Ca\(^{2+}\) (at S1 and S2 sites, respectively) to give the double Cd\(^{2+}\) derivatives. All of the metal derivatives were found to be as active as the respective native proteins.

The present report shows that Cd\(^{2+}\) can be selectively substituted into the S1 and S2 sites of PSA and LcH to give either diamagnetic complexes, such as the Ca\(^{2+}\)-Cd\(^{2+}\) and Cd\(^{2+}\)-Zn\(^{2+}\) complexes in which Cd\(^{2+}\) occupies S1 and S2, respectively, or paramagnetic complexes, such as the Cd\(^{2+}\)-Mn\(^{2+}\) complexes, with Cd\(^{2+}\) at S2. These complexes are stable, fully active, and can be isolated and characterized. This is in contrast to Cd\(^{2+}\) complexes of ConA in which the metal is weakly bound to the protein and various mixed metal complexes of Cd\(^{2+}\) are formed in solution under equilibrium conditions (25). In fact, ConA does not yield well defined Cd\(^{2+}\)-Zn\(^{2+}\) or Cd\(^{2+}\)-Mn\(^{2+}\) complexes, and the interpretation of the \(^{113}\text{Cd}\) NMR results for such complexes have been tentative (25). Thus, the ability to prepare \(^{113}\text{Cd}\) derivatives of PSA and LcH with selective substitutions at the S1 and S2 sites has permitted us to define fully the \(^{113}\text{Cd}\) NMR spectral properties of the S1 and S2 sites in both lectins and compare these results with the \(^{113}\text{Cd}\) NMR data for ConA (25).

Properties of Cd\(^{2+}\) Derivatives—By a variety of criteria, the Cd\(^{2+}\) derivatives of LcH and PSA (Table I) are identical in their intrinsic molecular and spectroscopic properties to the respective Mn\(^{2+}\) and Ca\(^{2+}\) containing native lectin (12, 21, 22). The near ultraviolet absorption and CD spectra indicate the same conformations of the derivatives as the respective native lectins. Gel-filtration chromatography show that the derivatives have the same hydrodynamic properties as the respective native lectins. NMRD studies indicate that solvent relaxation by Mn\(^{2+}\) in the Cd\(^{2+}\)-Mn\(^{2+}\) complexes of LcH and PSA occurs via the same mechanisms as in the respective native proteins (21), which suggest that the Mn\(^{2+}\) in the former complexes have the same coordination environments as in the respective native proteins.

The results of hemagglutination, precipitation, and precipitation-inhibition studies (Table II) indicate that saccharide binding activities of these derivatives are also equal to those of the respective native lectins. Similar results have been reported for other metal derivatives (7, 12). These results for LcH and PSA are also similar to those observed for metal-substituted ConA in which the derivatives are equally active as the native protein (32), indicating that the saccharide binding properties of these three lectins are not sensitive to the nature of the metals present at the S1 and S2 sites.

\(^{113}\text{Cd}\) NMR of Double \(^{113}\text{Cd}\) Complexes—\(^{113}\text{Cd}\) NMR studies of ConA (24, 25) showed that the ternary locked \(^{113}\text{Cd}\) complex of ConA gave rise to resonances at 46 and −125 ppm, which were assigned to the S1 and S2 sites, respectively. The 46-ppm resonance is characteristic of nitrogen- and oxygen-coordinated Cd\(^{2+}\) (33, 34), and the −125-ppm resonance of the \(^{113}\text{Cd}\) derivatives of both lectins up-field by approximately 2 ppm for LcH (Fig. 2b) and 1 ppm for PSA (Table III).

The \(^{113}\text{Cd}\) NMR spectrum of Ca\(^{2+}\)-Cd\(^{2+}\)-PSA (Fig. 3a) shows a resonance at 45.0 ppm and Ca\(^{2+}\)-\(^{113}\text{Cd}\)-LcH a resonance at 42.9 ppm. Neither spectrum was perturbed by the addition of 3-MDG.

The \(^{113}\text{Cd}\) NMR spectrum (not shown) of \(^{113}\text{Cd}^{2+}\)·Mn\(^{2+}\)-LcH gave two small resonances of equal intensities which were nearly within the noise level and with the same chemical shifts as that of the corresponding double \(^{113}\text{Cd}\) derivatives. The same result was obtained with the PSA derivative. In each case, the intensity did not account for the Cd\(^{2+}\) incorporated in the proteins (Table I).
resonance is characteristic of oxygen-coordinated Cd" (35-37), both in an octahedral environment. The data corroborate with the presence of Glu-8, Asp-10, Asp-19, and His-24 at the S1 site, and Asp-10, Tyr-12, Asp-14, and Asp-19 at the S2 site of ConA (18).

The $^{113}$Cd NMR spectrum of the double $^{113}$Cd" complex of PSA shows resonances at 46.6 and $-130.4$ ppm (Fig. 1, Table III). $^{113}$Cd resonances at 44.6 and $-129.4$ ppm were observed for the double $^{113}$Cd" complex of LcH (Table III). By analogy with ConA (25), the resonances at 46.6 and 44.6 ppm for PSA and LcH, respectively, can be tentatively assigned to $^{113}$Cd at the S1 sites of the proteins, whereas the resonances at 130.4 and $-129.4$ ppm tentatively assigned to $^{113}$Cd at the S2 sites. Spin-echo EPR studies of native LcH and PSA show the presence of a His residue as the ligand for Mn" at the S1 site in both lectins, in agreement with the findings of conserved His residues at this site in the primary sequences of LcH, PSA, and ConA (9, 10) and recent x-ray crystallographic data at 3 A resolution for PSA (20). Thus, the presence of $^{113}$Cd resonances at 44.6 and 46.6 ppm in LcH and PSA, respectively, is consistent with binding of $^{113}$Cd" to a His residue in the S1 sites of these proteins.

The resonances at $-129.4$ and $-130.4$ ppm for the double $^{113}$Cd complexes of LcH and PSA, respectively (Table III), are characteristic of purely oxygen-ligated $^{113}$Cd" (35-37) and indicate that these resonances are associated with binding of $^{113}$Cd to the S2 sites of LcH and PSA. Thus, substitution of a Phe residue in LcH and PSA for a Tyr residue in ConA at positions nearly identical to the S1 resonances of the double $^{113}$Cd" complex of ConA (25). The double $^{113}$Cd" resonance of LcH and PSA produced essentially the same small effects. $^{113}$Cd resonances associated with the S2 sites in both proteins were shifted upfield by approximately 1-2 ppm, while resonances associated with the S1 sites were essentially unchanged (Table III). This contrasts with the larger upfield shift of 8 ppm for the double $^{113}$Cd" complex of ConA (25). Like PSA and LcH, the $^{113}$Cd resonance for the S1 site of the ConA complex also was insensitive to saccharide binding. These results suggest that the S2 sites of LcH and PSA undergo a larger conformational change than the S1 sites upon monosaccharide binding but that the magnitude of this change is somewhat less than that in ConA. The lack of any perturbation in the EPR spectra of the Mn" (15, 38) and magnetic circular dichroic spectra of Cd" (16) at S1 sites in the three lectins supports the conclusion that there is little conformational change at S1 upon saccharide binding. The near ultraviolet CD spectra of LcH and PSA, however, does show that a conformational change occurs in the proteins upon saccharide binding (22).

Other Comments—Recent NMRD studies of Ca", Mn", and Cd" NMRD data show that complexes of LcH and PSA with Cd" at the S1 sites give resonances that are very close to the chemical shift position(s) of Cd" at the S1 sites of ConA (24, 25). Since $^{113}$Cd NMR chemical shifts are sensitive to the nature and number of ligand atoms, and geometry of the complex (25), these results provide evidence that the water ligands of the metal ion at S1 are present in LcH and PSA, as well as in ConA, and that their exchange kinetics

\[ \text{Ca}^{2+}, \text{Cd}^{2+} \text{and Mn}^{2+} \text{LeH has a resonance at 42.9 ppm compared to} \]

\[ \text{44.6 ppm for the double }^{113}\text{Cd derivative (Fig. 1b). Thus, there is a small chemical shift dependence for the }^{113}\text{Cd resonance at S1 on whether Cd}^{2+} \text{or Ca}^{2+} \text{is present at S2 for both proteins. These results also confirm the chemical shift assignment of the downfield resonances of the double }^{113}\text{Cd" complexes of LeH and PSA to the S1 sites.} \]

$^{113}$Cd NMR of $^{113}$Cd"-Mn" Complexes—The $^{113}$Cd NMR spectra of the $^{113}$Cd"-Mn" complexes of both LcH and PSA failed to show any resonance other than those associated with smaller amounts of double $^{113}$Cd complexes which were formed during the preparation of the former complexes. The presence of Mn" at the S1 sites in the $^{113}$Cd"-Mn" complexes of both lectins was verified by NMRD analysis and the amount of Mn" and Cd" by NMRD and atomic absorption spectroscopy (Table I). Since divalent manganese is paramagnetic, nearby nuclei experience enhanced $T_1$ and $T_2$ relaxation, and thus undergo line-broadening of their resonances. The extent of line-broadening depends upon the distance between the two nuclei (39). The results obtained with the other Cd" derivatives already presented indicate close proximity between S1 and S2 sites. The distance between these two sites in LcH and PSA has been calculated (21) to be approximately 5 Å, in agreement with recent x-ray crystallographic data for PSA (20). Thus, the broadening of $^{113}$Cd resonances in the $^{113}$Cd"-Mn" derivatives are so large that they are unobservable. These results provide direct evidence for binding of $^{113}$Cd at the S2 sites in LeH and PSA.

Effects of Saccharide Binding—Binding of the monosaccharide 3-MDG to the various $^{113}$Cd complexes of LeH and PSA produced essentially the same small effects. $^{113}$Cd resonances associated with the S2 sites in both proteins were shifted upfield by approximately 1-2 ppm, while resonances associated with the S1 sites were essentially unchanged (Table III). This contrasts with the larger upfield shift of 8 ppm for the S2 resonance of the double $^{113}$Cd" complex of ConA (25). Like PSA and LeH, the $^{113}$Cd resonance for the S1 site of the ConA complex also was insensitive to saccharide binding. These results suggest that the S2 sites of LcH and PSA undergo a larger conformational change than the S1 sites upon monosaccharide binding but that the magnitude of this change is somewhat less than that in ConA. The lack of any perturbation in the EPR spectra of the Mn" (15, 38) and magnetic circular dichroic spectra of Cd" (16) at S1 sites in the three lectins supports the conclusion that there is little conformational change at S1 upon saccharide binding. The near ultraviolet CD spectra of LeH and PSA, however, does show that a conformational change occurs in the proteins upon saccharide binding (22).

\[ \text{J. McCracken and C. F. Brewer, manuscript in preparation.} \]
must be slower in the former two lectins such that they do not contribute to the observed solvent proton relaxation rates in the respective Ca\textsuperscript{2+}-Mn\textsuperscript{2+} complexes.

Similar considerations indicate that both ligand waters of Ca\textsuperscript{2+} in ConA (19) are also present in LcH and PSA.

**SUMMARY**

We have prepared and characterized a series of stable mono- and disubstituted Cd\textsuperscript{2+} derivatives of LcH and PSA (the first time for any lectin) and found them to have the same physical and saccharide binding properties as the respective native time for any lectin) and found them to have the same physical and also similar to that of ConA, which indicates conservation of the coordinating ligand atoms to metal ions both proteins. The \textsuperscript{113}Cd NMR data for LcH and PSA are similar, and also similar to that of ConA, which indicates conservation of the coordinating ligand atoms to metal ions at the S1 and S2 sites in all three proteins. These results can be compared to recent CD studies of Ca\textsuperscript{2+}-Co\textsuperscript{2+} derivatives of the three lectins, which show some differences in the environments of the metal ions in LcH and PSA, as compared to ConA (22). The results also agree with recent limited x-ray crystallographic data for PSA (20).

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