Magnesium as a natural substitute for manganese in concanavalin A and other lectins

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Addition of magnesium to apo-concanavalin A in the presence of calcium was shown by ultraviolet difference spectroscopy to generate a holoprotein spectroscopically identical to the MnCa-holoprotein. The MgCa- and MnCa-forms bound equally strongly to Sephadex G-75. In kinetic experiments, the binding of Mg^{2+} was much slower than Mn^{2+} binding; K_d for Mg^{2+} was estimated as 7.4 mM. The combined Mg^{2+} and Mn^{2+} contents of 10 lectins specific for D-galactose or *N*-acetyl-D-galactosamine were each close to one atom per subunit, suggesting occupancy of the Mn^{2+} site by Mg^{2+} is common in plant lectins.

Concanavalin A *Lectin* *Manganese* *Metalloprotein* *Ultraviolet difference spectroscopy*

1. INTRODUCTION

Many plant lectins contain manganese and calcium, and in concanavalin A (con A) these atoms occupy a double site that has been well characterized by X-ray crystallography [1]. Extensive studies of metal-binding by apo–con A have led to an overall kinetic model [2] that has a key feature of a conformational change from the 'unlocked' MnCa-protein, which does not bind carbohydrate, to the 'locked' fully-active form. Though the transition metal or S1 subsite is usually regarded as specific for manganese, preparations of native con A contain far less than stoichiometric amounts of Mn^{2+}. Occupancies of 0.11–0.21 mol/mol subunit have been reported [3], while other transition elements are present in only trace amounts. Higher Mn^{2+} contents are found in other lectins, except for *Griffonia (Bandeiraea) simplicifolia* [4] and *Dolichos biflorus* [5] lectins which contain 0.11 and 0.16 Mn^{2+}/subunit, respectively and significantly more magnesium. Peanut agglutinin contained only trace amounts of Mn^{2+}, but 0.78 Mg^{2+}/subunit [6]. Metal analyses of the pea and sainfoin lectins [7] showed the sum of Mg^{2+} and Mn^{2+} contents was close to 1 mol/mol subunit, suggesting these metals might share the S1 type sites. However, Mg^{2+} had been reported not to be bound by con A at either S1 or S2 subsites [8]. In the present work, it was found that several more lectins have combined Mg^{2+} and Mn^{2+} contents close to 1 atom/subunit, and that Mg^{2+} can substitute for Mn^{2+} in con A, generating an active holoprotein.

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2. MATERIALS AND METHODS

Con A was prepared by affinity chromatography on Sephadex G-75 [9]. *Bauhinia purpurea, Vicia villosa* and *Wisteria floribunda* lectins were obtained from E.-Y. Labs Inc. and *D. biflorus* lectin from Sigma Chemical Co. Other lectins were prepared by affinity chromatography on a *p*-aminophenyl-2-acetamido-2-deoxy-D-galactose derivative of AffiGel 10 (BioRad Labs) prepared in a similar manner to the column previously described for peanut agglutinin [6]. The two *Cytisus scoparius* lectins were obtained by sequential elution of the column with D-galactose, lectin I, and *N*-acetyl-D-galactosamine, lectin II [10]. Lectin

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samples were dialysed extensively against water containing Chelex 100 (BioRad Labs) prior to analysis for metals by atomic absorption spectroscopy.

Con A was demetallised by dialysis against 0.5 M NaCl, 0.015 M HCl [11] then dialysed against 0.1 M NaCl, 0.04 M acetate buffer (pH 5.0). The preparations of apoprotein were allowed to equilibrate at room temperature for several days before use, to form predominantly 'unlocked' apoprotein [12]. Metal binding to apo–con A was measured by ultraviolet difference spectroscopy [11], using a Varian Superscan 3 spectrophotometer with automatic baseline correction. Protein concentrations were 9×10^{-5} M and 5 mm pathlength cuvettes thermostatted at 23°C were used. The wavelength for the kinetic measurements was 288.5 nm.

For gradient affinity chromatography of con A samples, Sephadex G-75 columns (1.6×15 cm) were eluted with linear gradients to 3 mM methyl α-D-glucoside [7].

3. RESULTS

The magnesium, manganese and calcium contents of 10 lectins specific for D-galactose or *N*-acetyl-D-galactosamine were determined (table 1). In all but one, the Mg^{2+} content exceeded the Mn^{2+} content. The sum of the 2 metals was close to 1 mol/mol subunit, the average of the 10 being 0.96.

Table 1

Metal contents of lectins

Lectin	Metal atoms/subunit[a]			
	Mn	Mg	Mn +Mg	Ca
B. purpurea	0.10	0.70	0.80	1.19
G. simplicifolia	0.14	0.68	0.82	1.50
D. biflorus	0.15	0.61	0.76	1.80
Sophora japonica	0.19	0.91	1.10	2.29
C. scoparius II	0.20	1.01	1.21	1.80
C. scoparius I	0.25	0.69	0.94	1.52
V. villosa	0.26	0.84	1.10	1.50
W. floribunda	0.27	0.78	1.05	1.05
Glycine max	0.31	0.55	0.86	1.54
Phaseolus lunatus	0.73	0.18	0.91	1.23

[a] Subunit M_r-values were all assumed to be 30000

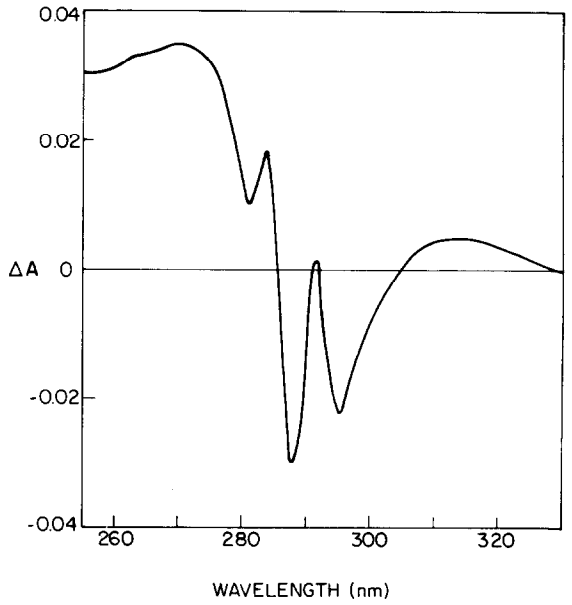

Fig.1. Ultraviolet difference spectrum of apo–con A reconstituted with Mg^{2+}, 50 mM, and Ca^{2+}, 1 mM, *vs* apo–con A. No difference spectrum arose when Mg^{2+} or Ca^{2+} alone was added at these concentrations.

Addition of Mg^{2+} and Ca^{2+} to apo-con A produced a difference spectrum (fig.1) identical to that given by Mn^{2+} and Ca^{2+}, and very similar to the MnCa-con A difference spectrum in [11]. No spectral change occured with Mg^{2+} in the absence of Ca^{2+}, unlike the binding of Mn^{2+} [11]. Reaction with Mg^{2+} was much slower than with Mn^{2+}. For a batch of apo-protein that had an apparent first-order rate constant of 0.32 min^{-1} with Mn^{2+} and Ca^{2+} at 1 mM each, the rate with Mg^{2+} and Ca^{2+} at 5 mM each was 0.067 min^{-1}. Further experiments were conducted with Mg^{2+} at 1.5–35 mM and Ca^{2+} at 10 mM. At 10 mM Ca^{2+}, the rate of formation of CaCa-con A [13] was much below the rates for MgCa-con A. From an Eadie-Hofstee plot of the data (fig.2), a K_d value for Mg^{2+} of 7.4 mM was obtained.

The carbohydrate-binding activity of apo-con A reconstituted with Mg^{2+} and Ca^{2+} was tested by gradient affinity chromatography. The major peak of MgCa-con A emerged at the same position in the methyl α-D-glucoside gradient as MnCa-con A (fig.3), and the major component of native con A

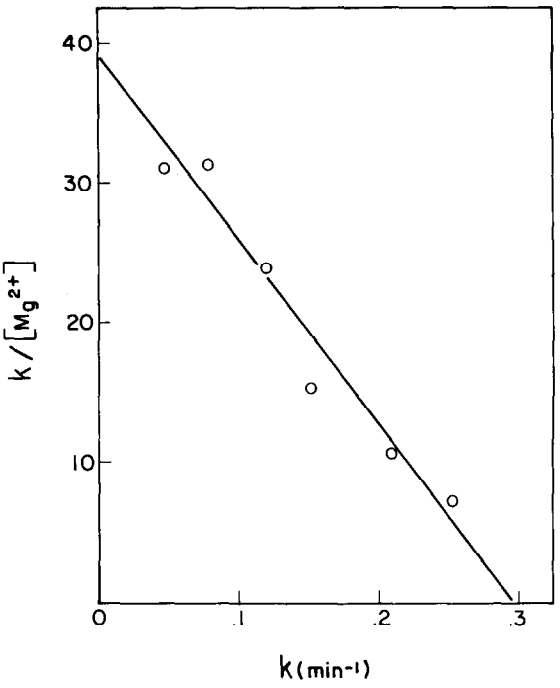

Fig.2. Eadie-Hofstee plot of Mg^{2+} binding to apo-con A in the presence of 10 mM Ca^{2+}.

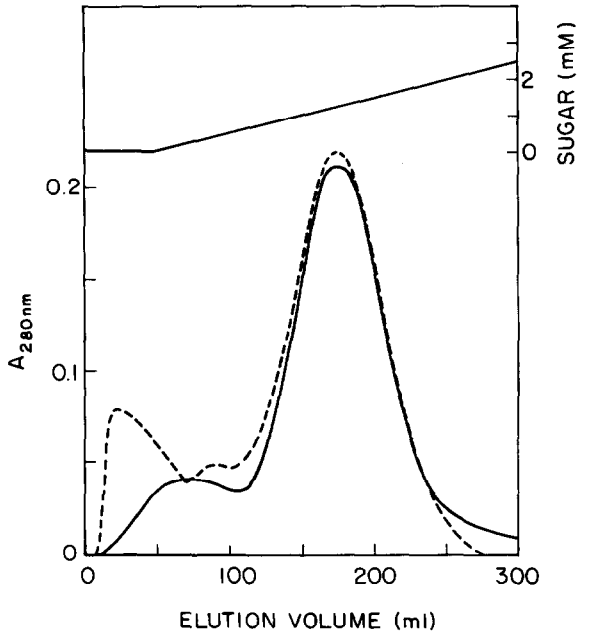

Fig.3. Gradient affinity chromatography of 18 mg MgCa-con A (——) and 21.5 mg MnCa-con A (– – –). The gradient of methyl α-D-glucoside is indicated at the top of the figure.

[7]. The metal contents of the major MgCa-con A species were 0.65 Mg^{2+}, 1.53 Ca^{2+} and <0.03 Mn^{2+}/subunit, assuming subunit $M_r = 26000$.

4. DISCUSSION

The Mn–O distances in the S1 subsite of con A average 2.28 Å [1]. Though this distance would be at the high end of the range for Mg–O distances, apo-con A was able to bind $Mg^{2>}$ at the S1 site in the presence of $Ca^{2>}$, and formed a holoprotein capable of carbohydrate binding. The failure to demonstrate $Mg^{2>}$ binding previously [8] is probably due to this requirement for $Ca^{2>}$. No MgMg-con A appeared to be formed, though MnMn-con A and CaCa-con A are known [2,13]. $Ca^{2>}$ is not essential for $Mn^{2>}$ binding to apo-con A at pH values similar to that used here, but $Mn^{2>}$ is more strongly bound when $Ca^{2>}$ is present [14,15]. Recent values for the $Mn^{2>}$ K_a are 9×10^5 M^{-1} in the presence of $Ca^{2>}$ and 5×10^3 M^{-1} in its absence [15]. At higher pH values $Mn^{2>}$ binding is independent of $Ca^{2>}$.

The analytical data on the 10 lectins presented here, and previous data on others [3,5,7] suggest occupancy of the S1 site by $Mg^{2>}$ may be a general phenomenon in plant lectins, with the ratio of $Mg^{2>}$ to $Mn^{2>}$ perhaps being governed by the natural availability of $Mn^{2>}$. $Mg^{2>}$ substitution for $Mn^{2>}$ occurs in other proteins, for example, phosphoenol pyruvate carboxykinase [16]. The preliminary kinetic experiments showed that $Mg^{2>}$ is bound much more slowly than $Mn^{2>}$, but these simple experiments may not be a guide to the situation during biosynthesis of con A. The 'locked' MnCa form can release $Mn^{2>}$ [17]. This could allow $Mg^{2>}$ binding at the pre-formed S1 site at a more rapid rate than for binding to the 'unlocked' form. Hence, the formation in vivo of the mixed MgMnCa forms of con A could proceed at higher rates than shown here for in vitro $Mg^{2>}$ binding.

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