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CONFORMATIONAL CHANGES FOLLOWING Mn(II) BINDING TO DEMETALIZED CONCANAVALIN A[1]

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SUMMARY

A temperature-dependent conformational change occurs following the binding of only one Mn(II) to a concanavalin A monomer. This change is independent of Ca(II) near pH 7 and is characterized by an activation energy of 22.3 kcal mol^{-1}, a value similar to that attributed to a cis-trans peptide isomerization. Two conformations have been detected in magnetic resonance experiments on solvent water protons where spin lattice relaxation times are influenced by bound Mn(II). Both conformations possess saccharide binding activity and Ca(II) stoichiometrically enhances the rate of conversion to the final, more stable conformation.

Determining the mechanism for activation of saccharide binding in concanavalin A (Con A)[4] is of fundamental importance to the understanding of how this plant lectin interacts with cell membranes. Near pH 5 saccharide binding by each 25,500-dalton subunit of Con A requires that two specific metal binding sites, S1 and S2, be occupied by a transition metal ion and Ca(II), respectively (1,2,3). This condition, however, is not essential near pH 7 where it was recently shown that a single Ca(II) will activate each Con A subunit in the absence of transition metal ions (4).

We recently demonstrated that when Mn(II) alone is added to demetalized Con A, a state is produced which can be detected by a large enhancement in

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[4]Abbreviations used: Con A, concanavalin A; MUM, 4-methylumbelliferyl α-D-mannopyranoside.

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the relaxation time T_1 of water protons. A subsequent change, independent of a second metal ion, occurs which alters the Mn(II) site and reduces the relaxation enhancement (5). Saccharide binding data is presented here showing that both states are active. In addition, we have shown that Ca(II) will enhance the conversion rate for a stoichiometric number of Con A monomers. The activation energy has been determined and is similar to that measured by others. We now present a simplified description of steps which occur following Mn(II) and Ca(II) binding to demetalized Con A.

MATERIALS AND METHODS

Con A was prepared from jack bean meal (Sigma) by affinity chromatography as previously described (6). Concentration of protein solutions were determined spectrophotometrically at pH 6.4 using an absorbance $A^{1\%}_{280nm} = 13.7$ (7). Protein solutions were buffered at pH 6.4 in 0.05 M Mops (Sigma) and 1.0 M NaCl which had first been treated with Chelex-100 (Bio-Rad Laboratories) to remove extraneous divalent metal ions.

Demetalized Con A was prepared by dialysis of freshly isolated Con A in a 0.1 N HCl bath as described elsewhere (4). Solutions prepared in this way contained less than 0.01 mol total Ca(II) per ml of 25,500-dalton monomers as determined by atomic absorption. In the course of all experiments described here, Ca(II) contamination did not exceed 0.05 mol total Ca(II) per mol of Con A subunits. No contaminaton by Mn(II) was detected. Demetalized Con A was stored at 1°C in buffer with no observable loss of protein due to precipitation. Samples of Con A and Mn(II) were prepared by incubating aliquots of demetalized Con A with the appropriate final concentration of Mn(II) at 1°C for three to seven days.

Water proton relaxation experiments were carried out with triplicate samples of Con A and Mn(II) initially stored at 1°C and then warmed to various temperatures from 5°C to 32°C. Samples were kept at these temperatures from 24 h to 7 days. For temperature-dependent experiments, samples were incubated at the appropriate temperature and aliquots (0.25 ml) were taken at various times and run immediately at ambient temperature (23°C). The conformational change is slow and spin lattice relaxation times T_1 could be measured before any changes in relaxation time occurred. A Bruker SXP NMR Spectrometer operating at 20.5 MHz was used and T_1 values were determined by the inversion-recovery method.

Saccharide binding studies were carried out by measuring the unquenched fluorescence of mixtures of Con A and MUM (Pierce) as previously described (4). A Perkin-Elmer MPF-3L fluorescence spectrophotometer with a thermostated cuvette holder was used to measure the fluorescence.

RESULTS

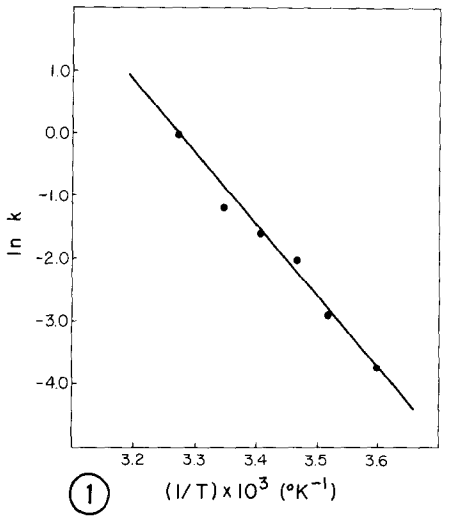
The experiments reported herein were designed to examine our recent findings showing that only one Mn(II) binds to a demetalized Con A monomer and

that two states for this monomer can be detected in water proton relaxation rate enhancement studies (5). The initial state characterized by a fast proton relaxation rate slowly converts at 5°C to a new state characterized by a slower relaxation rate. At 32°C the conversion proceeds relatively rapidly (approx. 4 hrs). The final conformation which arises from altering of the Mn(II) binding site will not convert back to the initial state.

Analysis of the time-dependent change by standard procedures (8) showed that the rate-limiting step observed was the conformational change for the conversion of the Con A-Mn(II) monomer from its initial to its final form. At 32°C the rate constant was 0.96 h^{-1}. An Arrhenius treatment of the rate constants is shown in Figure 1. At the concentration of Mn(II) employed, one Mn(II) would be bound per monomer (5), although all concentrations where Mn(II) occupied between 0.5 and 1.0 sites per monomer gave similar results.

When Ca(II) was added to the initial Con A-Mn(II) complex, conversion to the apparent final state occurred rapidly with the extent of conversion being equal to the amount of Ca(II) added. At 0.4 or 0.8 equivalents of Ca(II) per Con A monomer, a rapid conversion to the final conformation occurred for the respective fraction of monomers. Even after 6.5 days at 1°C, no further changes could be detected. This result was observed for solutions containing 1.0, 2.0, or 3.0 equivalents of Mn(II) per monomer. Addition of Ca(II) to Con A containing only Mn(II) and in the final state produced no change in the water proton relaxation rate. Once the final conformation developed, Ca(II) did little to the conformation of Con A, insofar as the states are monitored in these relaxation rate experiments.

The results of saccharide binding studies for the two states of Con A with only one Mn(II) are shown in Fig. 2. These Scatchard plots (9) for MUM binding show that Con A in both conformations has essentially 100% activity in terms of one molecule of MUM being bound by each 25,500-dalton subunit.



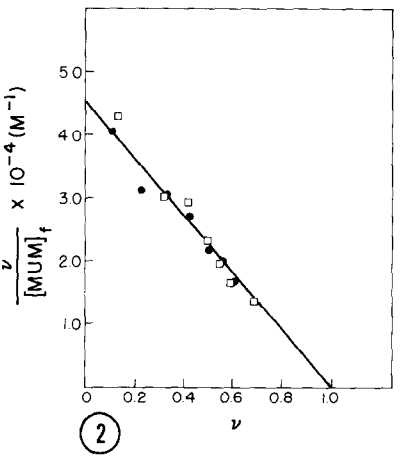


Figure 1: Arrhenius treatment of the rate constants for conversion to the more stable form of Con A containing only Mn(II). The solutions were 0.4 mM in Con A monomers and 0.8 mM in Mn(II), a concentration which assures that nearly 100% of all monomers contain a single Mn(II).

Figure 2: Scatchard analysis of MUM binding to both conformations of Con A containing only Mn(II). MUM binding was analyzed at 5°C for both forms. The less stable form was prepared by incubating 0.16 mM Con A monomers with 1.6 mM Mn for 72 h at 5°C. The more stable form is prepared by a similar incubation at 25°C. In solutions of the less stable form, calculations using the rate constants reported here show that approximately 15% of the Con A would be converted to the more stable form. The line is the best fit for the less stable form (●); data for more stable conformation (□) are superimposed on plot.

DISCUSSION

The activation energy for the conversion from initial to final state is 22.3 kcal mol^{-1}. Brown and co-workers (10) estimated 22.7 kcal mol^{-1} for a process suggested to be the cis-trans isomerism of a proline amide bond. Reeke and co-workers (11) have implicated a similar isomerization based on x-ray studies. Cardin and co-workers (12) have measured a similar activation energy for a process occurring at pH 5.3. We believe that the rate-limiting step in all these processes is the altering of the Mn(II) S1 site.

Conversion can occur at pH 6.5 when only one Mn(II) is bound. Ca(II) is not required but can stoichiometrically promote the conversion to the final state. A catalytic role for contaminating Ca(II) can be ruled out. Because conversion from the initial to final state takes days at 5°C, we were able to

examine saccharide binding to both initial and final conformational states. Both appear to have identical binding characteristics.

Equilibrium dialysis studies in our laboratory have shown that only one Mn(II) binds per Con A monomer in the absence of Ca(II) at either 5°C or higher temperatures near 32°C (5). This stoichiometry is independent of the presence or absence of saccharide. In contrast, other workers have concluded from water proton relaxation measurements that demetalized Con A binds Mn(II) at both sites S1 and S2 in the absence of Ca(II) and, conversely, binds Ca(II) at both sites in the absence of Mn^{2+} (10,13). No direct measurements were made to actually quantitate metal ion content. In light of our findings on Mn(II) binding to demetalized Con A (5) and the data presented here, we suggest that the work of Brown and co-workers (10) did not lend itself well to stoichiometric determination of bound Mn(II) per Con A subunit. Furthermore, we have previously shown that one Ca(II) per Con A monomer is sufficient to activate saccharide binding (4). This suggests that the work of Koenig and co-workers (13), who reported a requirement that Ca(II) be bound at both sites S1 and S2 for saccharide activity to occur, must be reevaluated.

The data presented here along with our earlier reports (4,5) lead us to suggest a simplified model for conformational changes near pH 7 which involve the metal ion site and resulting saccharide binding activity. One Mn(II) binds to a Con A monomer. This induces the saccharide binding activity. A slow conformational change, possibly involving the proline amide cis-trans isomerization and having an activation energy of 22 kcal mol^{-1}, follows Mn(II) binding. This change is detected in water proton relaxation studies as a decrease in the observed relaxation rate. Essentially no change in saccharide binding activity accompanies this conformational change, but a Ca(II) can bind to the new site formed. In the presence of Ca(II) the rate for the conformational change is enhanced for a stoichiometric number of Con A subunits, but no catalytic role for Ca(II) exists. Ca(II) may influence the saccharide binding site as the affinity for sugar appears to be greater when Ca(II) is present (4,5).

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