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Binding of Saccharide to Demetalized Concanavalin A†

Seymour H. Koenig,* Rodney D. Brown, III, and C. Fred Brewer*

ABSTRACT: Demetalized concanavalin A (apo-Con A) exists in two conformational states designated locked (PL) and unlocked (P). Brown et al. [Brown, R. D., III, Koenig, S. H., & Brewer, C. F. (1982) *Biochemistry 21*, 465–469] obtained the value of 0.14 for the equilibrium ratio of [PL]/[P] at 25 °C, pH 6.4. More recently, we have shown that in the presence of 100 mM methyl α-D-mannopyranoside (α-MDM) Mn^{2+} ions bind tightly and pairwise to the S1 and S2 sites of each monomeric unit of PL. This allows measurement of the concentration of PL by titration of a sample with Mn^{2+} ions at 5 °C in the presence of a high concentration of α-MDM, while monitoring the binding of Mn^{2+} by measuring the solvent proton magnetic relaxation rates. We show that equilibration of apo-Con A with α-MDM at 25 °C, pH 6.4, results in an increase of the concentration of locked species due to binding of α-MDM to PL, and we deduce the value 29 mM for K_{SPL}, the dissociation constant of the α-MDM–PL complex at 25 °C, pH 6.4. We find that α-MDM also binds to P, though weakly, and to its binary and ternary complexes with Mn^{2+}. Approximate values for the respective dissociation constants are 2400, 100, and 85 mM, compared to about 100 μM for fully active Con A.

Brown et al. (1977) discovered that the metalloprotein concanavalin A (Con A)[1] can exist in two conformational states, with comparable free energies, that interconvert slowly (minutes to hours) because of a relatively high activation barrier that separates the two conformations. Which of the two states is lower in energy depends on the occupancy of two metal-binding sites per Con A monomer. When both S1 (the "transition metal site") and S2 (the "calcium site") are occupied, the protein at equilibrium is essentially all in the conformation called "locked", so named because of its greater affinity for metal ions than the other, the "unlocked", conformation. Conversely, the demetalized protein at equilibrium is predominantly in the unlocked conformation, the ratio of concentrations of locked to unlocked species being 0.14 at 25 °C, pH 6.4 (Brown et al., 1982; Koenig et al., 1982). Occupancy of only S1 by Mn^{2+} results in an equilibrium ratio of locked to unlocked binary Mn^{2+}–Con A complexes of about 1:2 (Brewer et al., 1983a).

Binding of metal ions to apo-Con A in either conformation is sequential (Kalb & Levitzki, 1968; Brewer et al., 1983a); S1 must be occupied before S2. Addition of appropriate metal ions to unlocked apo-Con A produces a weakly associated ternary complex that then transforms to the locked conformation by a first-order process (Brown et al., 1977). In all cases, the locked ternary complex binds sacchardide with the specificity and strength of "native" Con A[2] (Harrington & Wilkins, 1978). When observed this way, binding of saccharide also appears sequential. The question that arises, and that has been addressed previously (Koenig et al., 1978), is whether binding of saccharide is in fact sequential, requiring the presence of metal ions, or whether it is the conformational change induced by the presence of metal ions that is mainly responsible for the saccharide affinity of Con A. The question if even more meaningful because Brown et al. (1977) also showed that apo-Con A could be produced and maintained, for hours (at least) at 5 °C, in the locked conformation, the conformation of the saccharide-binding metalloforms.

It is also known that Ca^{2+} alone can bind to Con A to produce a locked metalloform that binds saccharide as well as native Con A (Koenig et al., 1978; Harrington & Wilkins, 1978). By inference from these data, this form contains two Ca^{2+} ions per Con A monomer, a point recently reexamined and confirmed by Brewer et al. (1983a). Koenig et al. (1978) studied the kinetics of replacement of Ca^{2+} by Mn^{2+}, to form the more stable Ca^{2+}–Mn^{2+}–Con A (native) complex, as a function of saccharide concentration (α-MDM). They found that the rate saturates for α-MDM concentrations above ~30 mM, indicating that the metal ions could be exchanged with saccharide still bound, from which they inferred that the locked saccharide–apo-Con A complex (SPL) exists. The kinetic experiments of Koenig et al. (1978) only yielded a value for K_{SPL}, the saccharide dissociation constant of SPL, multiplied

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[1] Abbreviations: Con A, concanavalin A with unspecified metal ion content and conformational state; α-MDM, methyl α-D-mannopyranoside; NMRD, nuclear magnetic relaxation dispersion (more specifically, the magnetic field dependence of the spin–lattice relaxation rate of solvent protons in the Con A solutions used here).

[2] Native Con A is taken here to mean the locked Ca^{2+}–Mn^{2+}–Con A ternary complex.

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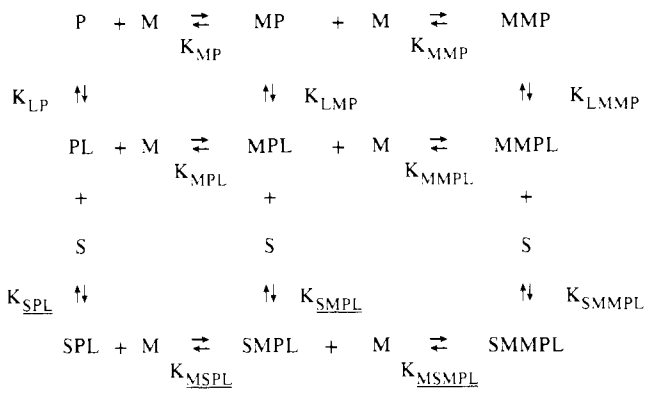
Scheme I



by ratios of unknown kinetic constants. The numerical value of K_{SPL} is not yet known, though a lower limit of ~10 mM may be inferred from the experiments of Sherry et al. (1981).

We have now measured the binding of α-MDM to locked apo-Con A directly, by observing the shift in equilibrium between the unlocked and locked conformations of apo-Con A induced by binding of saccharide to the latter species. The experiments are best understood in terms of the well-documented scheme that describes the multiple equilibria between Mn^{2+} ions and the two conformations of Con A (Scheme I) (Brown et al., 1977; Brewer et al., 1983a). Here P stands for the apoprotein, MP for the binary Mn^{2+}–Con A complex with Mn^{2+} at S1, and MMP for the ternary complex with Mn^{2+} at both S1 and S2. L indicates the locked conformation; its absence indicates the unlocked one. S indicates saccharide, α-MDM throughout the present work except in the indicated cases where (weakly interacting) galactose is used as a control. Values for the equilibrium constants not underlined are known, in some cases at more than one value of pH and temperature, and all the complexes but SMPL have previously been prepared and characterized in terms of their NMRD spectra [cf. Brewer et al. (1983a)].

In the absence of saccharide, the equilibrium ratio [PL]/[P] (=0.14 ± 0.01) (Brown et al., 1982; Koenig et al., 1982) determines the fraction of apo-Con A that is locked. In the presence of α-MDM, the equilibrium concentration of locked species should increase due to the mass-action effect of α-MDM binding to PL. We find that it does, consistent with a value for K_{SPL} of 29 mM at 25 °C, pH 6.4. We also find that addition of a very large concentration (~1 M) of α-MDM does not shift the equilibrium as far as expected, suggesting very weak binding of α-MDM to unlocked apo-Con A, with a dissociation constant of ~2.4 M, indicating that Scheme I must be extended to also include binding of saccharide to the unlocked conformation of Con A. We find that the unlocked binary and ternary Mn^{2+}–Con A complexes also bind α-MDM, with dissociation constants of ≲100 mM.

Materials and Methods

Sample Preparation. Native Con A was obtained from Miles-Yeda (Lot 55L), from which apo-Con A was prepared according to the procedure described by Brown et al. (1977). Manganese chloride tetrahydrate and calcium chloride dihydrate, as well as buffer salts, were obtained from Fisher Chemicals. α-MDM was obtained from Pfanstiehl and D-galactose from Sigma. Distilled, deionized water was used throughout. All samples were prepared and measured in pH 6.4 buffer (0.1 M potassium acetate and 0.9 M potassium chloride). Protein concentrations were determined optically at the beginning and end of each experiment, at pH 5.6, by using an absorbance $A_{280nm}^{1\%,1cm}$ = 12.4 (Yariv et al., 1968), and are reported as the concentration of monomeric units of 27 000 daltons. Saccharide was added as the solid, and Mn^{2+} was added to 0.6-mL samples in microliter amounts from a 0.06 M stock solution. One molar solutions of α-MDM were checked for the possible presence of adventitious Ca^{2+} by using atomic absorption analysis; no significant concentrations were found.

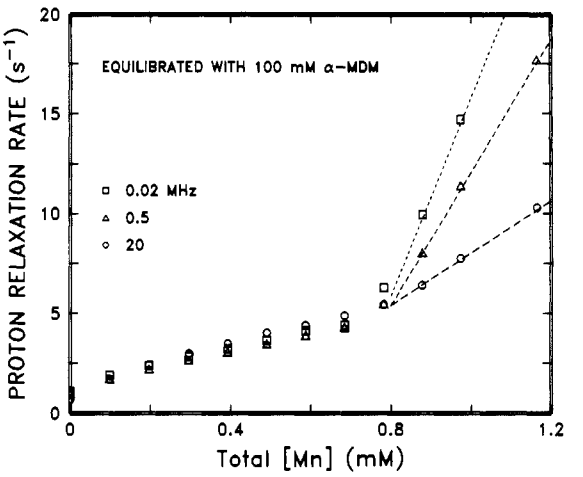


FIGURE 1: Magnetic relaxation rates of solvent protons, at 0.02, 0.5, and 20 MHz, of separate aliquots of a preparation of 0.39 mM apo-Con A in pH 6.4 buffer. The indicated concentrations of Mn^{2+} ions, and 100 mM α-MDM, were added in succession, and the samples were allowed to reach equilibrium at 25 °C. The measurements were at 5 °C. The lines through the data to the right of the break near 0.8 mM total Mn^{2+} concentration indicate the results expected assuming that S1 and S2 of all monomers contain Mn^{2+} ions and that the excess ions are essentially all free in solution.

Solvent Proton Relaxation Measurements. Measurements of the magnetic field[3] dependence of the spin–lattice relaxation rate of solvent protons were made by the field cycling method used previously (Brown et al., 1977; Koenig et al., 1978) and described to a limited extent by Koenig & Schillinger (1969) and Hallenga & Koenig (1976).

Procedures. Samples of apo-Con A (nominally 0.4 mM) were equilibrated at 25 °C for at least 5 days with varying concentrations of α-MDM (30 mM–1 M). The samples were then brought to 5 °C to prevent further transitions between the locked and unlocked conformations [cf. Brewer et al. (1983a)], and Mn^{2+} was titrated into each in 1-μL additions with a microliter syringe to give 0.1 mM incremental increases in the total Mn^{2+} concentration. In one case, the temperature was lowered before addition of saccharide. The magnetic relaxation rates of solvent protons at 0.02, 0.5, and 20 MHz were recorded at 5 °C after each addition of Mn^{2+}, and followed until they become constant in time. At the end of each titration, the total Mn^{2+} concentration was checked by atomic absorption analysis.

Results and Analysis

Equilibrium Titration. Figure 1 shows that relaxation rates of solvent protons at 5 °C as a function of the total Mn^{2+} concentration for 12 aliquots of 0.39 mM apo-Con A that had been equilibrated at 25 °C for a week after addition of Mn^{2+} and 100 mM α-MDM [cf. Figure 5 of Brewer et al. (1983a)]. The lines through the data points to the right of the break in the data, which correspond to two Mn^{2+} ions bound per monomer of Con A, are drawn with slopes calculated from the

[3] Magnetic field strength is given in units of the Larmor precession frequency of protons in that field. The conversion is 4.26 KHz = 1 Oe = 1 G = 10^{-4} T.

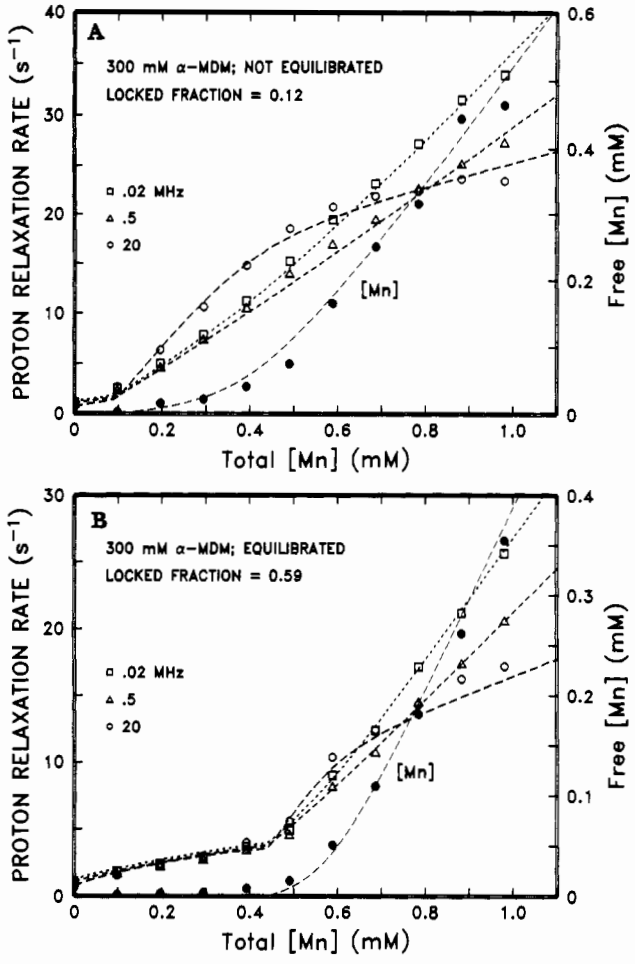


FIGURE 2: (A) Magnetic relaxation rates of solvent protons, at 0.02, 0.5, and 20 MHz, as Mn^{2+} ions are titrated into a sample of 0.37 mM apo-Con A in pH 6.4 buffer. The sample was allowed to equilibrate at 25 °C, after which it was cooled to and maintained at 5 °C. 300 mM α-MDM was then added and the titration performed. The break in the data near 0.1 mM total Mn^{2+} concentation corresponds to 0.12 of the total Con A in the locked conformation. The concentration of free Mn^{2+} ions (filled circles) was computed at each point in the titration from the differences in the relaxation rates at 0.02 and 0.5 MHz (see text). The dashed curves through the data are computed as described in the text and include necessary corrections to account for binding of α-MDM to MP and MMP. (B) Experiment identical with that of (A) except that α-MDM was added to the sample at 25 °C and the complex allowed to equilibrate at 25 °C for 5 days. the sample was then cooled to 5 °C and the titration performed. The break corresponds to 0.59 of the total apo-Con A being in the locked conformation.

known relaxivities of Mn^{2+} aquoions in the presence of 100 mM α-MDM. In terms of Scheme I, these data show that in an equilibrated mixture of apo-Con A, Mn^{2+} ions, and 100 mM α-MDM, essentially all the bound Mn^{2+} is complexed as SMMPL.

Binding of α-MDM to P and PL. Figure 2A shows data for a sample of 0.37 mM apo-Con A, equilibrated at 25 °C, that was cooled to 5 °C; 300 mM α-MDM was then added and Mn^{2+} titrated into the sample. The sample was maintained at 5 °C throughout, so that the added saccharide did not alter [cf. Brewer et al. (1983a)] the initial value of [PL]/[P], which is 0.14 (Brown et al., 1982; Koenig et al., 1982); approximately one-eighth of the total protein was initially locked and remained so throughout the titration. By comparison with the results in Figure 1, the break in the data near 0.1 mM added Mn^{2+} indicates the initial 25 °C equilibrium value of [PL]; this corresponds to about 0.05 mM SMMPL out of 0.37 mM total protein, or very close to the

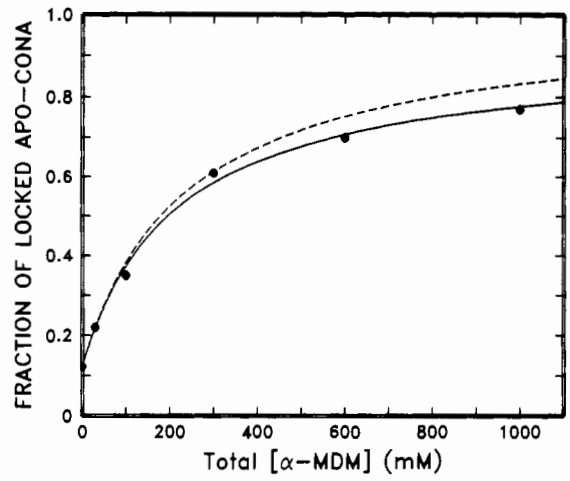


FIGURE 3: Fraction of apo-Con A in the locked conformation after equilibration with a range of concentration of α-MDM, obtained from the break in the titration data for a series of experiments analogous to that relevant to Figure 2B. The solid curve through the data points results from a least-squares comparison of the data and the equilibria indicated in eq 1. The best fit gives K_{SPL} = 29 mM and K_{SP} = 2.4 M. The dashed curve results if K_{SP} is arbitrarily set to infinity; this gives a sense of the effect produced by binding of α-MDM to unlocked apo-Con A. By comparison, refitting the data with the assumption that $K_{SP} = \infty$ gives K_{SPL} = 35 mM and a curve (not shown) that is close to an average of the two shown.

expected one-eighth of the total. These results demonstrate that titration of Mn^{2+} into apo-Con A at 5 °C in the presence of a substantial concentration of α-MDM, and monitoring of the relaxation rate, is a new and quantitative method for determining the concentration of PL present in a sample of apo-Con A.

The concentration of free Mn^{2+}, calculated from the difference in relaxation rates at 0.02 and 0.5 MHz [cf. Brown et al. (1977)], is also shown in Figure 2A. The derivation of the curves through the data will be discussed below, after more data are presented. Only the position of the break in the relaxation data is immediately pertinent.

Figure 2B shows results of an experiment identical in all details with that of Figure 2A except that 0.37 mM apo-Con A with 300 mM α-MDM was allowed to equilibrate at 25 °C for 5 days prior to titration with Mn^{2+}. The fraction of locked protein has increased to almost 0.6, a 5-fold increase, indicating that PL binds saccharide and that the conformational equilibrium had shifted from predominantly P to more than half PL and SPL (the value of [PL]/[P] is, of course, unchanged).

The titration experiment (Figure 2B) was repeated for 30, 100, 600, and 1000 mM α-MDM, and the fraction of locked apo-Con A initially present was computed from the positions of the breaks in the curves, which were measured directly from plots of titration data. The fraction of the locked conformation initially present is plotted as a function of [α-MDM] in Figure 3. The solid line through the data points results from a two-parameter least-squares comparison of the data with the equilibria:

$$SP \overset{K_{SP}}{\rightleftharpoons} S + P \overset{K_{LP}}{\rightleftharpoons} PL + S \overset{K_{SPL}}{\rightleftharpoons} SPL \qquad (1)$$

This set of equilibria is an extension of Scheme I which will be discussed in greater detail below.

The fit gives K_{SPL} = 29 mM. It became clear, however, in comparing the data with eq 1, with the SP complex not included, that the data were not approaching an asymptote of 1.0 for large values of [α-MDM], but something less (0.91 from the fit), indicating weak binding of α-MDM to P. For this reason, Scheme I was augmented as in eq 1; the fit gives

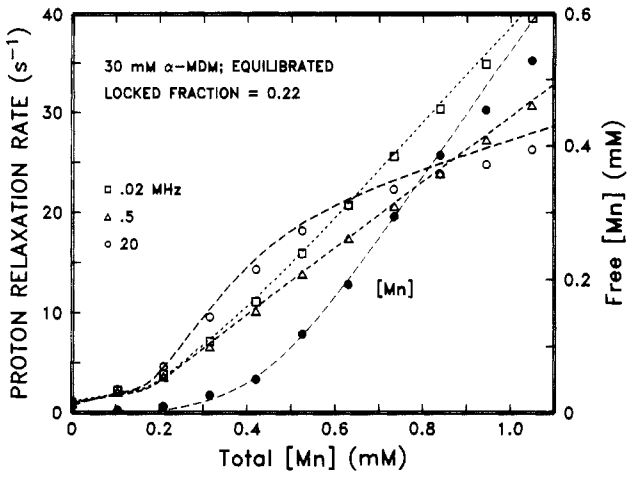


FIGURE 4: Results of an experiment identical in all details with that relevant to Figure 2B, except that 30 mM (rather than 300 mM) α-MDM was added. The dashed curves through the data points indicate the results anticipated, computed by using Scheme I, with no adjustable parameters. The relaxivities of the several species are known from previous work, as are most of the equilibrium dissociation constants. Values for those previously unknown are from the analysis of the data in Figure 3.

$K_{SP} \simeq 2.4$ M. The dashed line in Figure 3 results if K_{SP} is set equal to infinity; this gives an idea of the precision required to be able to say that K_{SP} is about equal to that value quoted, rather than infinity. A one-parameter fit to the data, assuming $K_{SP} = \infty$, gives $K_{SPL} = 35$ mM and a 3-fold greater mean-squared error which, from out experience in analyzing these data and other data not presented here, we regard as significant. The uncertainty in K_{SP}, because of the very weak binding, is rather large, possibly as much as ±1 M.

Binding of α-MDM to MP and MMP. Figure 4 shows titration data for apo-Con A equilibrated at 25 °C with 30 mM α-MDM. The relaxation rates are shown for three fields, and the concentrations of Mn^{2+} aquoions calculated from the 0.02- and 0.5-MHz data at each value of the total Mn^{2+} concentration are also shown. The various dashed curves through the data points are computed, by using Scheme I, with no adjustable parameters as follows. Values of K_{MP}, K_{MMP}, K_{MPL}, and K_{MMPL} are known from previous work (25 μM, 7 mM, 8 μM, and 15 μM, respectively; Brown et al., 1977; Brewer et al., 1983a), as is $1/K_{LP}$ (0.14; Brown et al., 1982; Koenig et al., 1982; Figure 2A). K_{SPL} is known from Figure 3, and [α-MDM] is sufficiently low so that its binding to unlocked forms will be neglected (for now). The value of K_{SMMPL} is not known at 5 °C, but from comparisons of results for binding of α-MDM to the Ca^{2+} analogue at 5 °C (Christie et al., 1978), and the temperature dependence of binding of other saccharides [cf. Christie et al. (1978) and Van Landschoot et al. (1980)], we estimate $K_{SMMPL} = 0.1$ mM. This value is probably good to ±20%; the results are insensitive to its particular value so long as all other dissociation constants are much larger than K_{SMMPL}. K_{SMPL} is not known either except (from Figure 1) that its value is relatively large; this means that [SMPL] and [MPL] are always negligible in our range.

It is then straightforward to compute the expected concentrations of all species for any value of the total Mn^{2+} concentration (Brown et al., 1977; Brewer et al., 1983a). The initial condition, before any Mn^{2+} ions are added, is that [PL] + [SPL] is 0.22 of the total protein, with [PL] ≃ [SPL] (since K_{SPL} is 29 mM and [α-MDM] = 30 mM). Since the sample is maintained at 5 °C, the fraction of locked material remains fixed during the titration. Once the concentrations of all species are known, their contributions to the relaxation rates can be computed since the relaxivities of all the species are also known (Brewer et al., 1983a). The four curves are the results of such a computation. The agreement of data and predictions is excellent with the hint that at the upper right extreme there is slightly more Mn^{2+} bound than expected.

This procedure is confirmed by similar analysis of the titrations of the samples with greater concentrations of α-MDM. Two effects must be considered, and these have been included in the computations relating to the data in Figure 2A,B. They both require that Scheme I be altered to include binding of α-MDM to the unlocked metalloforms. The first effect is that α-MDM binds to MMP, reducing the observed concentration of free Mn^{2+} somewhat from that calculated by using Scheme I unaltered (typically ≃0.05 mM aquoion at the highest concentration of total Mn^{2+}). At 300 mM α-MDM, this effect can be expressed as an apparent decrease of the dissociation constant K_{MMP} from a value of ~7 mM [the uncertainty of this value is rather large; its actual value may be larger because of the nonspecific binding of Mn^{2+}; cf. Brewer et al. (1983a)] to an effective value of 1.9 mM. Taking K_{MMP} as 7 mM, one can calculate $K_{SMMP} \lesssim 85$ mM.

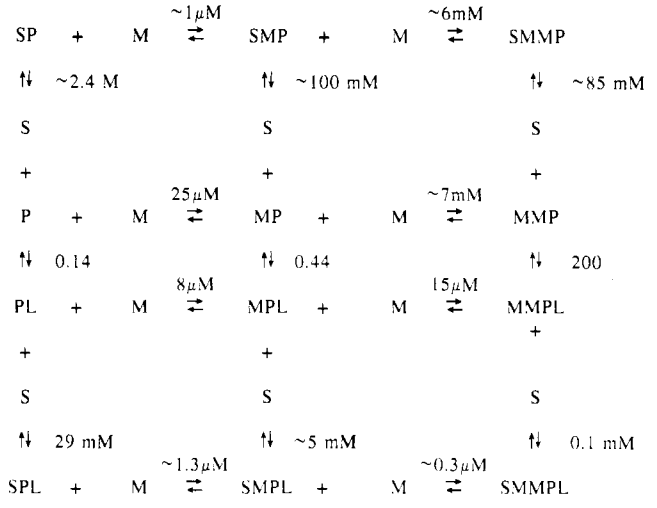
The second effect that had to be considered arose when the relaxation data to the right of the break in Figure 2A were fitted. The presence of 300 mM α-MDM altered the relaxivity of MP (in the midrange of the titration) and MMP (in the right-hand region), whereas at 30 mM α-MDM (Figure 4), it did not. Since α-MDM binds to MMP, a concomitant change in relaxivity is to be expected; the effect is a reduction of the relaxivity at 20 MHz from about 100 to 50 mM^{-1} s^{-1} and no measurable change at the lower fields. Note, however, that the total contribution of MMP to the relaxation rates is never more than ~2 s^{-1} in the figures. The binding of α-MDM to MP does not shift the equilibria because S1 of the unlocked species is saturated with Mn^{2+} in midrange. Nonetheless, the change in relaxivity is saturated at 300 mM α-MDM (from analysis of the 600 and 1000 mM data) and is negligible at 30 mM. Thus, $K_{SMP} \simeq 100$ mM. The reductions in relaxivities are from 35, 35, and 65 mM^{-1} s^{-1} to 27, 27, and 55 mM^{-1} s^{-1} at 0.02, 0.5, and 20 MHz, respectively.

Controls. Three control experiments were carried out. The first was analogous to that relevant to Figure 2A, except that 30 mM (rather than 300 mM) α-MDM was used. The purpose was to make certain that nothing untoward occurred at large concentrations of saccharide. The data (not shown) were quite like those of Figure 2A, except that it was apparent that the small corrections needed in Figure 2A to the relaxivities and to [Mn^{2+}] because of binding of α-MDM to the unlocked forms, discussed above, were not required here. A quantitative analysis was not made.

In the second control, the titration in the presence of 300 mM α-MDM was repeated, this time waiting 11 days for the saccharide–apo-Con A equilibrium. Though the equilibrium constant K_{LP} is known, the rate constant for the P → PL transition has only recently been measured; its value is $^1/_{33}$ h^{-1} at 25 °C, pH 6.4 (Brewer et al., 1983b). Thus, several days may be required to realize equilibrium conditions. The data for the control were indistinguishable in all details from the sample with the shorter (5 days) incubation time.

The third control used galactose, rather than α-MDM; the point was to determine whether the strong specificity that the native Con A metalloform has for α-MDM compared to galactose is preserved in interactions with apo-Con A. The specific experiment was to equilibrate apo-Con A with 300 mM D-galactose for 1 week at 25 °C, pH 6.4. Subsequent

Scheme II



titration of the sample with Mn^{2+} at 5 °C, and analysis of the results, showed that the concentration of locked protein was unaltered by the presence of this essentially nonbinding saccharide.

Time-Dependent Effects. On titrating Mn^{2+} ions into PL in the presence of α-MDM, we found, for all conditons reported here, that the initial relaxivity decays (essentially exponentially) to its ultimate value with a time constant of ~15 min. The magnitude of the effect corresponds to a relaxivity change of ~10 mM^{-1} s^{-1} and is (to first order) independent of the concentration of α-MDM present, and rather insensitive to the concentration of PL remaining to be titrated. The decay of the relaxivity occurs even though no free Mn is present at any point. Once all the PL and SPL are titrated, the change in relaxation rates upon continued titration occurs instantaneously, i.e., within a time to short for us to observe. These time-dependent effects, which in no way influence the foregoing, may be related to monomer–monomer interactions. Though it has not yet been necessary to invoke such interactions in the equilibrium schemes, their influence on some kinetics has been noted previously (Koenig et al., 1978). More experimentation is necessary to elucidate the mechanism that underlies these preliminary observations.

Discussion

We have presented two major findings. The first is a value for the dissociation constant of the α-MDM–PL complex of 29 mM at 25 °C, pH 6.4. The second is that Scheme I must be extended to Scheme II, in which binding of Mn^{2+} to every form of Con A, locked and unlocked, with and without saccharide, is considered. Values for the equilibrium dissociation constants of complexes of α-MDM with a particular form and conformation of Con A are indicated by vertical equilibria in Scheme II. Those for complexes with apo-Con A are for 25 °C; the others are for 5 °C. With the exception of the dissociation of saccharide from SMMPL, all are new results. The dissociation constant of α-MDM from SMPL cannot be obtained from the present experiments directly, since [SMMPL] ≫ [SMPL] for the conditions considered here. However, from the slight curvature of the data near the origin (Figure 1), where SMPL must initially be formed before S2 sites become occupied, and from an estimate of the relative binding of Mn^{2+} to S1 and S2 (Brown et al., 1977), one can estimate $K_{SMPL} \simeq 50K_{SMMPL}$, or ~5 mM.

The numbers indicated with the horizontal equilibria in Scheme II are our values for the dissociation constants of Mn^{2+} from the many Mn^{2+} complexes. Those along the top and bottom rows are computed from the three others to which they relate.

The existence of SPL, specifically the α-MDM complex, has been inferred before by Koenig et al. (1978). They performed experiments in which, by analogy to Scheme I, using Ca^{2+} rather than Mn^{2+}, CCPL and SCCPL were first formed. Then Mn^{2+} was added to displace Ca^{2+} at S1 to form CMPL and SCMPL. The kinetics of formation of SCMPL were observed as a function of increasing concentrations of α-MDM. It was found that the replacement rate saturated as [α-MDM] was increased, indicating that exchange of ions at S1 was occurring with saccharide bound, i.e., that SPL existed (when interpreted within the scheme of sequential binding of metal ions). The replacement rate is related to K_{SPL} multiplied by unknown parameters that depend, among other things, on the relative preference of S1 for Mn^{2+} ions in the absence and presence of saccharide. When it is assumed that no such preference exists, one obtains a value for $K_{SPL} \simeq 15K_{SCMPL}$ or ≃1.5 mM, which is a lower limit to K_{SPL}. Sherry et al. (1981), observing binding of a fluorescent saccharide, concluded that the dissociation of their saccharide from SPL is more than 100-fold greater than from SCCPL, corresponding to $K_{SPL} \geq 10$ mM. The present results show that the equilibrium binding of α-MDM to PL at 25 °C is, in fact, 300-fold weaker than to MMPL at 25 °C. It is interesting to note that, despite the reduced affinity of PL compared to MMPL for α-MDM, the specificity of all complexes for α-MDM, compared to galactose, is maintained.

With Scheme II, and the indicated saccharide dissociation constants, one can address the question of the relation of saccharide binding activity in Con A to the binding of metal ions and protein conformation. More specifically, since it was first shown by Brown et al. (1977) that sequential binding of metal ions to apo-Con A, which induces saccharide binding activity, actually involves the initial formation of a weakly associated ternary intermediate that in turn undergoes a conformational change to produce the native form, it was clear that binding of metals and the change of conformation could both be important determinants of saccharide binding activity in Con A. Koenig et al. (1978), having found that PL binds saccharide, argued that conformation was a significant determinant of activity. We now see (Scheme II) that every step along any pathway from P to MMPL contributes to increased affinity of Con A for α-MDM, but by no means additively. Both conformation and binding of metal ions to S1 and S2 are determinants of the saccharide affinity of Con A.

Registry No. Concanavalin A, 11028-71-0; methyl α-D-mannopyranoside, 617-04-9; manganese, 7439-96-5.

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Association of Blood Coagulation Factors V and X with Phospholipid Monolayers†

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ABSTRACT: Blood coagulation factors X and V adsorbed to phospholipid monolayers and induced surface pressure changes. These proteins also adsorbed to the air-water interface and formed protein surface films. Plots of surface pressure change ($\Delta\pi$) vs. initial monolayer surface pressure (π_0) appeared biphasic for factor X and factor V, indicating two distinct adsorption processes. Surface pressure changes in monolayers spread below the collapse pressure of the respective proteins were characteristic of protein adsorption to the air-water interface while those observed above the protein collapse pressures were consistent with specific protein-acidic phospholipid interactions. Phosphatidylserine-dependent surface pressure changes were very small for both proteins. Factor X induced surface pressure changes required the presence of calcium while factor V induced changes occurred in the presence or absence of calcium. Protein-monolayer binding characteristics were comparable to those obtained by using bilayer vesicles of similar composition and indicated the absence of significant membrane surface curvature effects. The maximum surface concentration corresponded to one bound factor X molecule per 1400 Å^2. Comparison of surface pressure changes induced by factor V with those induced by myelin basic protein suggested that the membrane-binding processes of the two proteins involve similar but small degrees of acyl chain perturbation. Thrombin digestion of factor V had no effect on surface pressure change and the isolated 80 000-dalton peptide of factor Va also showed approximately similar surface pressure effects. The vitamin K dependent proteins caused a smaller surface pressure change per bound protein molecule. The results indicate that the prothrombinase proteins associate primarily, if not exclusively, with the head groups of the phospholipids.

Bilayer vesicles containing acidic phospholipids support clotting activity with efficiencies very similar to activated platelets (Nesheim et al., 1979b). Artificially prepared phospholipid membranes therefore appear to provide a suitable model for the biological membrane surface in coagulation processes. Eventual reconstruction of the prothrombinase complex requires careful measurement of several protein-membrane interactions. The mode of membrane association of the three proteins involved (prothrombin, factor X, and factor V) has been investigated by employing various techniques. Light scattering and fluorescence experiments have indicated that each protein binds to a cluster of acidic phospholipids at the membrane surface (Nelsestuen & Broderius, 1977; Lim et al., 1977; Bloom et al., 1979; Pusey et al., 1982). The prothrombin- and factor X-acidic phospholipid complexes are mediated by calcium (Nelsestuen & Lim, 1977) while the factor V-membrane complex arises from direct protein-acidic phospholipid interactions (Bloom et al., 1979; Pusey et al., 1982). Quasi-elastic light scattering has been used to measure the degree of protrusion of all three proteins from the membrane surface (Lim et al., 1977; Pusey et al., 1982). Stopped-flow light scattering measurements indicated very rapid protein-membrane recognition processes (Wei et al., 1982; Pusey et al., 1982). Recent studies utilized well-defined prothrombin and phospholipid monolayer systems and showed that prothrombin-membrane binding was not dependent on membrane surface curvature (Mayer et al., 1983). Furthermore, these studies indicated that no significant insertion of prothrombin into the hydrophobic region of the membrane occurred. Major hydrophobic interactions between factor Va and the membrane core have been postulated (Nesheim et al., 1980). However, more recent evidence suggested a primarily ionic factor V-membrane interaction (Pusey et al., 1982).

Protein-induced changes in the surface pressure of monomolecular lipid films have been used to deduce the nature of various protein-membrane interactions (Verger & Pattus, 1982; Kimelberg & Papahadjopoulos, 1971). Bougis et al. (1981) demonstrated that the large surface pressure changes caused by cardiotoxin-monolayer adsorption were due to insertion of the peptide into the hydrophobic region of the membrane. These perturbations were dependent on phospholipid head group and acyl chain composition; cardiotoxins effected larger surface pressure changes in monolayers containing acidic phospholipids (PS)[1] than in neutral phospholipid (PC) monolayers. Similar results were obtained for mellittin-phospholipid adsorption (Sessa et al., 1969; Bhakoo et al., 1982). Other proteins, such as δ-lysin, induced surface pressure changes with virtually no phospholipid head-group specificity (Bhakoo et al., 1982).

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[1] Abbreviations: PS, bovine brain phosphatidylserine; PC, egg yolk phosphatidylcholine; π, surface pressure; $\Delta\pi$, surface pressure change; π_0, initial surface pressure; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; SDS, sodium dodecyl sulfate.

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