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INTERACTIONS OF AN OVALBUMIN GLYCOPEPTIDE WITH CONCANAVALIN A

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SUMMARY

The interaction of a highly purified glycopeptide isolated from ovalbumin with Concanavalin A has been investigated by measuring solvent proton relaxation rates over a wide range of magnetic fields. We find that binding of the glycopeptide to Mn-Ca-Concanavalin A uniformly reduces the solvent proton relaxation rates in the same manner as that of simple saccharides such as methyl α-D-mannopyranoside, but that the magnitude of the reduction is not as great. Furthermore, we observe that the glycopeptide is capable of precipitating the lectin, and that the precipitation reaction can be readily reversed by addition of methyl α-D-mannopyranoside. The latter results indicate that the branched chain glycopeptide appears to be bivalent with respect to binding by the lectin.

Interest in the jackbean lectin, Concanavalin A(Con A) is due primarily to its capacity to bind to cell surface membranes of both normal and transformed cells (1). The specificity of these interactions is related to the saccharide binding properties of the protein which has been shown by Goldstein et al., (2) to be directed toward the monosaccharides glucose and mannose. We have been investigating the metal ion and saccharide binding properties of Con A, with the goal of relating the molecular properties of the lectin to its ability to interact with cell surface carbohydrates. Recently we demonstrated that the solvent proton relaxation rates, over a wide range of magnetic fields (corresponding to proton Larmor frequencies from 0.01 to 50 MHz) of solutions of Mn-Ca-Con A (Mn^{2+} in the S1 site, Ca^{2+} in the S2 site), were nearly uniformly reduced by $\sim$20% upon binding of simple saccharides to the protein (3). Using this technique, called nuclear magnetic resonance disperison (NMRD) measurements, we studied the binding of mono- and oligosaccharides to Mn-Ca-Con A. We concluded that the protein possesses a single residue binding site per monomer, and that the greater affinity of certain oligosaccharides for Con A, such as the α(1→2) mannans relative to the

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corresponding monosaccharide, was due to an increase in their probability of binding associated with the presence of more than one saccharide binding residue in the molecules.

This present communication describes our initial observations on the interaction of a glycopeptide isolated from ovalbumin (Fig. 1) with Mn-Ca-Con A as monitored by NMRD measurements. In the course of these studies, we observed that the glycopeptide is capable of precipitating the lectin. This appears to be the first observation of such a reaction between Con A and an isolated glycopeptide.

MATERIALS AND METHODS

Con A was obtained from Miles-Yeda and demetallized to give apo-Con A as previously described (4). Mn^{2+} and Ca^{2+} were added as their chloride salts to solutions of apo-Con A by addition with a microliter syringe, and enough time was allowed from the protein to be converted to the active, "locked" form (4). Protein concentration was determined spectrophotometrically at pH 5.6 using an absorbance $A^{1\%,1cm}_{280Nm} = 12.4$ (5). Methyl α-D-mannopyranoside was obtained from Pfanstiehl Laboratories. The ovalbumin glycopeptide was a gift from Dr. Paul Atkinson; its purity was determined to be greater than 90% as determined by ^1H nmr at 360 MHz (Carver, J. and Atkinson, P., unpublished results). NMRD measurements were made at the IBM T.J. Watson Laboratories, Yorktown Heights, N.Y. Details of the technique and theory can be found elsewhere (6)

RESULTS AND DISCUSSION

The top data (●) in Fig. 2 shows the NMRD profile of Mn-Ca-Con A in the absence of saccharide at pH 5.6, 25°. A quantitative analysis of the profile in terms of the parameters that enter into the theory of magnetic relaxation dispersion has been previously published (6). The important features are that all of the Mn^{2+} ions in the Mn-Ca-Con A complex are tightly bound to the protein, and that a water ligand(s) of the Mn^{2+} is exchanging fairly rapidly with bulk solvent to give the observed relaxation profile. There is a significant contribution of the residence time (τ_M) of the exchanging water molecule(s) to the observed profile. The diamagnetic contribution of the protein to the observed rates under these conditions is less than 1 sec^{-1} (for example, apo-Con A) and is therefore small compared to the paragmagnetic contribution.

Addition of sufficient amounts of methyl α-D-mannopyranoside to saturate the binding sites of the protein result in a nearly uniform reduction in the rates at all fields (Fig. 2,(X)), as we previously reported (3,6). Analysis

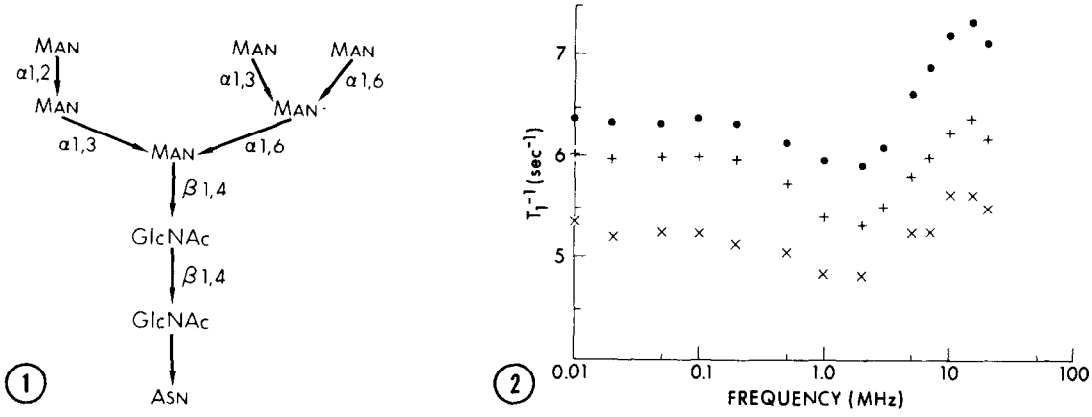

Fig. 1. Glycopeptide isolated from ovalbumin. Man indicates mannose; GlcNAc indicates N-acetyl-glucosamine; Asn indicates asparagine.

Fig. 2. NMRD spectrum of a pH 5.60 solution (buffered with 0.1 N potassium acetate, 0.9 M potassium chloride) containing 0.46 mM Mn-Ca-Con A (•); the same solution in the presence of 1.82 mM methyl α-D-mannopyranoside (+); and the protein solution in the presence of 1.7 mM ovalbumin glycopeptide (X). The Mn-Ca-Con A was prepared by adding 0.46 mM $MnCl_2$ and 1.3 mM $CaCl_2$ to a solution of 0.55 mM apo-Con A and allowing the solution to stand until Mn-Ca-Con A formation was complete. Under these conditions, 0.09 mM Ca-Ca-Con A was also formed. The temperature for all of the measurements was 25°.

indicates that the most significant change in the parameters which give rise to the reduction of the profile is an increase in τ_M of the exchanging water ligand(s) of the Mn^{2+}. The lifetime of the exchanging water ligands on the metal ion is thus increased in the presence of this monosaccharide bound to the protein. This change appears to be a result of a conformational transition in Con A upon saccharide binding, and not to steric hindrance of the exchanging water ligand(s) by the monosaccharides (3,6). We have further shown that the change in the observed relaxation rate is sensitive to the affinity constants of the saccharide used, in that the effect was proportional to the amount of saccharide bound, and thus the NMRD profile is a sensitive monitor of saccharide interaction with the lectin.

We have recently investigated the effects of adding a variety of mono- and oligosaccharides to Mn-Ca-Con A on the NMRD of solutions of the protein (3). We observed essentially the same change in the dispersion profile for all of the saccharides tested that bind to Con A, and we suggested from these results as well as considering other data in the literature that the protein most likely possesses

a single residue binding site. The enhanced binding of certain oligosaccharides such as the α(1→2) mannose oligomers was ascribed to be due to the presence of more than one saccharide binding residue in these molecules, which increases the probability of their binding, and not to an extended binding site on the protein.

In this regard, we suggested that the interaction of the lectin with more complex carbohydrate structures such as glycoproteins and glycolipids might also involve a similar binding mechanism in cases where multiple mannose or glucose residues were present. Such a mechanism could begin to account for the range of apparent affinities constants for Con A that branched chain molecules of these types have exhibited as well as their enhanced affinity constants relative to monosaccharides (cf. 8). We have thus begun to examine the interactions of complex glycopeptides with Con A using NMRD techniques.

When the highly purified glycopeptide (Fig. 1) from ovalbumin was added in excess to a solution of Mn-Ca-Con A, the observed NMRD profile was reduced uniformly as shown in Fig. 2(+). This reduction was qualitatively similar to that observed when methyl α-D-mannopyranoside was added to the protein, however, the magnitude of the reduction was not as great (~40%). It appears, therefore, that the lifetime of the exchanging water ligand(s) on the Mn^{2+} ion of the protein is increased in the glycoprotein complex, but that the increase is not as great as that found for simple mono- and oligosaccharides (3). Addition of large amounts of methyl α-D-mannopyranoside to the glycopeptide-Mn-Ca-Con A solution reduced the observed rates to those characteristic of the monosaccharide binding to the protein, thus demonstrating that the glycopeptide could be competitively displaced from the lectin.

A series of dilution experiments were performed on the glycopeptide-Mn-Ca-Con A complex to check for concentration effects that might alter the observed NMRD data. The glycopeptide concentration was reduced from 1.7 mM to 1.1 mM, to 0.55 mM, and to 0.28 mM while the Mn-Ca-Con A concentration was maintained at 0.46 mM by addition of a stock protein solution. In each instance, the observed relaxation rates were unchanged. Since the ~1:2 ratio of glycopeptide to protein solution possesses the same rates, this suggests that perhaps two molecules of Con A can

interact with a single glycopeptide molecule. Furthermore, a small cloudiness was initially observed in the solution which disappeared upon stirring. An even more interesting phenomenon occurred as the concentration of glycopeptide was further reduced to $\sim$ 0.14 mM in the presence of 0.46 mM Mn-Ca-Con A: a large precipitate formed in the solution which was stable upon standing. Addition of methyl α-D-mannopyranoside quickly resulted in dissolving the precipitate. Thus, in the presence of a sufficient excess of Con A, the ovalbumin glycopeptide precipitates the protein. The precise ratio of glycopeptide to Con A required for precipitation will be carefully investigated in future work.

To our knowledge, this is the first observation of the capacity of a glycopeptide to precipitate Con A. At pH 5.6 the protein is a dimer (7). From the structure of the glycopeptide, the two branched chains containing multiple mannose residues are evidently spacially far enough from each other to bind and crosslink two Con A molecules. Thus, a lattice can be formed resulting in the precipitate. This observation may well explain the observations of Kobata's group (8) and Schachter's group (9) that at least two mannose residues on branched chain glycopeptides must be present in order to bind well to Sepharose-Con A, since such molecules may be able to bind to two lattice-attached Con A molecules simultaneously. The highest affinity molecules observed by both groups may be a result of this effect plus a statistical enhancement effect on binding that we have posited (3) for molecules containing multiple individual binding residues, as is the case for the ovalbumin glycopeptide. The results also suggest that one function of branched chain glycopeptides as a whole may be to provide the capacity to crosslink exogeneous binding proteins. These type of molecules may be involved in the well known phenomenon of "patching" and "capping" that takes place on the surface of cells in the presence of multivalent proteins such as Con A. In addition, branched chain molecules of this type may play an important role in signal transduction on cell membranes since small crosslinked populations of complexed glycoprotein receptors may be involved.

In summary, the NMRD results for the interaction of a branced chain glycopeptide from ovalbumin with Mn-Ca-Con A are similar to those found for simple

saccharides binding to the protein, but are different in that the magnitude of the changes in the NMRD spectrum are not as great. Secondly, the precipitation of Con A by the glycopeptide may provide a clue to the functional aspects of the structure of branched chain glycopeptides.

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