Concanavalin A Interactions with Asparagine-linked Glycopeptides

BIVALENCY OF HIGH MANNOSE AND BISECTED HYBRID TYPE GLYCOPEPTIDES*

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We have previously reported that concanavalin A (ConA) is precipitated by a high mannose type glycopeptide (Brewer, C. F. (1979) Biochem. Biophys. Res. Commun. 90, 117–122; Bhattacharyya, L., and Brewer, C. F. (1986) Biochem. Biophys. Res. Commun. 137, 670–674). In the present study, we have investigated the ability of a series of high mannose and bisected hybrid type glycopeptides to bind and precipitate the lectin. The modes of binding of the glycopeptides were studied by nuclear magnetic relaxation dispersion (NMRD) techniques, and their affinities were determined by hemagglutination inhibition measurements. The stoichiometries of the precipitation reactions were investigated by quantitative precipitation analysis. The equivalence zones (regions of maximum precipitation) of the precipitin curves indicate that certain high mannose and bisected hybrid type glycopeptides are bivalent for lectin binding. From the NMRD and precipitation data, we have identified two protein binding sites on each glycopeptide: one site on the α(1–6) arm of the core β-mannose residue involving a trimannosyl moiety which binds with high affinity (primary site); and the other site on the α(1–3) arm of the core β-mannose residue involving an α-mannose residue(s), which binds with lower affinity (secondary site). These two types of sites bind to ConA by different mechanisms.

Certain bisected hybrid type glycopeptides were found to possess only the primary ConA binding sites, but not the secondary sites, and hence were able to bind but not precipitate the lectin. Other related glycopeptides have only the secondary type sites and thus exhibit low affinity and are unable to precipitate the protein. The results are related to the possible structure-function properties of cell-surface glycopeptides.

Carbohydrates that function as receptors on the surface of normal and transformed cells are predominantly found as glycopeptides and glycolipids (1). The oligosaccharide chains of many of the glycoproteins appear to be involved in a variety of biological functions, including cell-cell recognition, and migration of cells to particular organs, including the metastasis of tumor cells (2, 3). In addition, their compositions and numbers are often correlated with cell transformation (2). Oligosaccharides that are linked to the side chain amide group of asparagine residues in glycoproteins are designated as N-linked glyclosidic chains and include high mannose, hybrid, and complex type carbohydrates (3, 4). Although the structures of many of these type of oligosaccharides and glycopeptides are known (3, 4), little information has been obtained on their molecular binding properties, other than their interactions with glycosylases (5), which regulate their biosynthesis, and their binding specificities to lectins (6–9).

Lectins are cell-agglutinatig proteins of nonimmune origin that bind to specific carbohydrate determinants without chemically modifying them (10). They are found in plants, animal tissues, and invertebrates (11). A number of mammalian lectins are able to mediate receptor-specific endocytosis of glycoproteins (12, 13). However, the majority of the best characterized lectins are those from plants, and these lectins have been most widely used to explore the membrane structures and properties of both normal and transformed cells (2, 11). The cell surface receptors for lectins in many cases are the N-linked glycopeptides (11). Thus, elucidating their binding interactions provides a means of examining the molecular recognition determinants of these cell-surface carbohydrates.

Among the most widely used plant lectins is the jack bean protein concanavalin A (ConA). For some time, ConA was defined as specific toward monosaccharides containing D-glucose or D-mannose residues (14). However, using solvent proton nuclear magnetic relaxation dispersion (NMRD) techniques, we have recently provided direct evidence that the high affinity binding of certain N-linked glycopeptides and related oligosaccharides is primarily due to extended site interactions of the lectin with the 3,6-di-O-(α-D-mannopyrano- nyl)-D-mannosyl moiety present in these molecules (8, 9). These findings are supported by structure-activity studies of structurally diverse N-linked glycopeptides binding to the lectin (7). The NMRD results also provided direct evidence that the lectin binds primarily to the outer trimannosyl group of high mannose and bisected hybrid type glycopeptides, and to the central trimannosyl region in complex type glycopeptides.

We have previously observed (15, 16) that ConA is precipi-

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1 The abbreviations used are: ConA, concanavalin A with unspecified metal ion content; CMPL, ConA with Mn2+ and Ca2+ at the S1 and S2 sites, respectively, in the locked conformation (18); t, 3,6-di-O-(α-D-mannopyranosyl)-D-mannose; α-MDM, methyl α-D-mannopyranoside; NMRD, nuclear magnetic relaxation dispersion, the magnetic field dependence of nuclear magnetic relaxation rates, in the present case, the longitudinal relaxation rate, 1/T1, of solvent protons.

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It isated by the high mannose type glycopeptide, D3 (Fig. 1). In the present study, we have investigated the ability of a series of high mannose and bisected hybrid type glycopeptides to selectively bind and precipitate the protein. The stoichiometries of the precipitation reactions were investigated by quantitative precipitation analyses. The modes of binding of the glycopeptides were studied by NMARD (17, 18), and their relative affinities were determined by hemagglutination inhibition assays. The results indicate that certain high mannose and bisected hybrid type glycopeptides are bivalent and capable of precipitating the lectin. We have identified two protein binding sites on the glycopeptides and have shown that these sites have different affinities and mechanisms of binding. Certain bisected hybrid type glycopeptides are also shown to be univalent for the lectin. The findings in the present study are related to the possible structure-function properties of the glycopeptides on the surface of cells.

The results in the second paper (19) indicate that certain bisected complex type carbohydrates are also bivalent and capable of precipitating the lectin, although their mechanisms of binding differ from those of high mannose and hybrid type glycopeptides.

MATERIALS AND METHODS AND RESULTS

The principal finding in this study is that the high mannose type glycopeptides AC-CB, D3, and E3 (Fig. 1) and the bisected hybrid type glycopeptide C3b (Fig. 2) are capable of specifically binding and precipitating ConA. Analyses of the stoichiometries of the glycopeptides and protein at the equivalence zones of the precipitin curves indicates that the glycopeptides are divalent with respect to ConA binding. The nature of the two ConA binding sites in each glycopeptide has been identified, and their affinities have been assigned. Details of these findings are discussed below.

NMARD Studies—Our recent NMARD studies have provided direct evidence that certain high mannose and bisected hybrid type glycopeptides primarily bind to ConA by extended site interactions of the trimannosyl moiety located on the outer region of the α(1-6) arms of the glycopeptides (9). These conclusions were based largely on the observations that the high mannose type glycopeptides D3 and E3 (Fig. 1), and the bisected hybrid type glycopeptide C3b (Fig. 2), all produced changes in the NMARD profile of CMPL similar to that induced by the binding of the synthetic trisaccharide, 3,6-di-O-(α-D-mannopyranosyl)-D-mannose (I), which, in turn, differed from that induced by monosaccharide binding (17, 18). Trisaccharide I produced a smaller change in the NMARD profile of CMPL compared to the larger change induced by α-MDM (8, 9). Furthermore, it has 130-fold greater affinity for the lectin, compared to α-MDM (8, 9), which is comparable to the affinities observed for D3, E3, and C3b (Table I) (9). These results indicated that the trimannosyl oligosaccharide and the glycopeptides induced a different conformational change in the protein compared to the binding of α-MDM and other mono- and oligosaccharides, including α(1-2) mannosyl oligosaccharides, as indicated by changes in the NMARD

FIG. 1. Structures of high mannose type glycopeptides. Man, GlcNAc, and Asn indicate D-mannose, N-acetyl-D-glucosamine, and asparagine residues, respectively.

2 Portions of this paper (including “Materials and Methods,” “Results,” and Figs. 3–7) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 96M-1661, cite the authors, and include a check or money order for $3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

Profiles (8, 9, 15) and ultraviolet circular dichroism spectra of the protein (30).

The results of the present study show that the disaccharides, 3-O-(α-D-mannopyranosyl)-D-mannose and 6-O-(α-D-mannopyranosyl)-D-mannose, which are part of the structure of I, do not possess its high affinity binding (Table I). Furthermore, both disaccharides induce changes in the NMARD profile of CMPL (not shown) similar to α-MDM. These results strongly argue for extended site binding of the two nonreducing mannose residues of I, in agreement with our previous conclusions (8, 9).

The NMARD profiles of CMPL in the presence of the high mannose type glycopeptide AC-CB (Fig. 3), and the bisected hybrid type glycopeptides AC-BA (Fig. 4) and AC-A (Fig. 5) to CMPL, are all similar to those observed for I (8, 9). Similar findings have been reported for D3, E3, and C3b (9, 15). These results and others (7) indicate that all of these glycopeptides bind primarily by the trimannosyl moiety on their α(1-6) arms. The fact that the bisected hybrid type glycopeptides AC-CC (Fig. 2) and a related glycopeptide, AC-BB, bind only weakly to ConA (7) is consistent with the lack of an outer trimannosyl moiety on their α(1-6) arms. NMARD measurements indicate that their mechanism of binding resembles α-MDM, which appears to be due to the remaining terminal α(1-3) mannose residue on their α(1-6) arms.

Precipitation Studies—Fig. 6 shows that high mannose type glycopeptides AC-CB and E3 are capable of precipitating the lectin under appropriate conditions. Similar results have recently been reported for D3 (16). The data are similar to antigen-antibody and lectin-polyasaccharide precipitin profiles (31, 32) and suggest multivalent interactions between the
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**FIG. 2. Structures of bisected hybrid type glycopeptides.** Man, Gal, GlcNAc, and Asn stand for D-mannose, D-galactose, N-acetyl-D-glucosamine, and asparagine residues, respectively. The structure of AC-CC is not shown. It is similar to AC-CC, but has an additional β(1-4) Gal on the β(1-2) GlcNAc of the α(1-3) arm of core β-mannose.

Glycopeptides and ConA. The data for D3 (16) showed that the ConA tetramer, which exists at pH 7.2, results in the formation of more precipitate than the ConA dimer which exists at pH 5.6 (33). These results are consistent with the greater valency of the tetramer (31). The shapes of the curves also indicate tight binding of the glycopeptides to ConA (31), with the 3-, 4-, and 6-hydroxyl groups of mannose (and glucose) residues remaining free for ConA binding (14). Thus, the GlcNAcβ(1-2)Man disaccharide moiety of C3b is capable of binding. However, the β(1-4) substituted mannose residues in the α(1-3) arms of AC-BA and AC-A are no longer able to inhibit ConA binding. NMRD data for C3b show that the trimannosyl moiety on the α(1-6) arm is the primary binding site for ConA, under conditions of excess glycopeptide (9). The NMRD profiles of CMPL in the presence of AC-BA and AC-A (Figs. 4 and 5, respectively) show that these bisected hybrid type glycopeptides also bind by the trimannosyl determinant. However, AC-BA and AC-A fail to precipitate the protein under the same conditions as C3b and the high mannose type glycopeptides. These results indicate that AC-BA and AC-A have the primary binding sites, but not the second sites.

**TABLE I**

<table>
<thead>
<tr>
<th>Glycopeptides or oligosaccharides</th>
<th>Minimum concentrations required for complete inhibition of hemagglutination</th>
<th>Relative inhibitory potency*</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-MDM</td>
<td>3.1 mM</td>
<td>1</td>
</tr>
<tr>
<td>Manαββββ(1,6)Man</td>
<td>1.6 mM</td>
<td>1.9</td>
</tr>
<tr>
<td>Manαββββ(1,3)Man</td>
<td>1.3 mM</td>
<td>2.2</td>
</tr>
<tr>
<td>D3</td>
<td>23.8 μM</td>
<td>130*</td>
</tr>
<tr>
<td>E3</td>
<td>44.2 μM</td>
<td>70*</td>
</tr>
<tr>
<td>C3b</td>
<td>61.0 μM</td>
<td>50*</td>
</tr>
<tr>
<td>AC-A</td>
<td>26.0 μM</td>
<td>120*</td>
</tr>
<tr>
<td>AC-CB</td>
<td>40.0 μM</td>
<td>75</td>
</tr>
<tr>
<td>AC-BA</td>
<td>6.3 μM</td>
<td>500</td>
</tr>
<tr>
<td>AC-BB</td>
<td>1.3 mM</td>
<td>94*</td>
</tr>
<tr>
<td>AC-CC</td>
<td>&gt;1.2 mM</td>
<td>(0.1')</td>
</tr>
</tbody>
</table>

* All data normalized to that of α-MDM. Higher values indicate greater inhibitory potency.

**TABLE II**

<table>
<thead>
<tr>
<th>Glycopeptide</th>
<th>Concentration of glycopeptide at equivalence point</th>
<th>Protein concentration</th>
<th>Ratio of concentration of glycopeptide to protein monomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.2</td>
<td>pH 5.6</td>
<td>pH 7.2</td>
<td>pH 5.6</td>
</tr>
<tr>
<td>mM</td>
<td>mM</td>
<td>mM</td>
<td></td>
</tr>
<tr>
<td>AC-CB</td>
<td>0.11</td>
<td>0.21</td>
<td>1.19</td>
</tr>
<tr>
<td>D3*</td>
<td>0.11</td>
<td>0.10</td>
<td>0.21</td>
</tr>
<tr>
<td>E3</td>
<td>0.09</td>
<td>0.18</td>
<td>1.18</td>
</tr>
<tr>
<td>C3b</td>
<td>0.10</td>
<td>0.19</td>
<td>1.19</td>
</tr>
</tbody>
</table>

* Data taken from Bhattacharyya and Brewer (16).
bind. On the other hand, examination of the structures of the high mannose type glycopeptides (Fig. 1) show that all of their α(1-3) arms are capable of binding to ConA. Therefore, the secondary binding sites in AC-CB, D3, E3, and C3b involve the mannose residue(s) on their α(1-3) arms.

Construction of a Corey-Pauling-Koltun space-filling model of E3 (Fig. 8), with the bond angles of the α(1-3) arms in agreement with recent NMR data (34), and the rotation angle of the inner α(1-6) arm set to \( \omega = 180^\circ \) (35) and the outer α(1-6) arm of the trimannosyl moiety set to 180° (19), shows that the mannose residue on the α(1-3) arm and the outer trimannosyl group on the α(1-6) arm face in opposite directions, and are thus capable of binding two separate ConA molecules simultaneously. Interestingly, it appears that the inner α(1-6) mannose residue which is connected to the core β-mannose residue (Man6 in Fig. 8) can exist with \( \omega = 180^\circ \) or -60° and still allow E3 to bind separate ConA molecules at the outer trimannosyl moiety and the inner α(1-3) arm.) This is also true for AC-CB, D3, and C3b. Thus, the branched chain structures of these glycopeptides permit cross-linking and precipitation of ConA dimers and tetramers, under appropriate conditions.

Affinities of the Primary and Secondary ConA Binding Sites of the Glycopeptides—The primary binding sites in AC-CB, D3, E3, C3b, AC-BA, and AC-A have been identified as the trimannosyl moiety on the α(1-6) arms. This trisaccharide determinant is chiefly responsible for their high affinity for the lectin, and it binds by an extended site mechanism. (The higher affinity of AC-CB relative to the other glycopeptides in Table I will be discussed elsewhere.) The dissociation constant of \( \mathbf{I} \) is approximately 1.2 \( \mu \text{M} \) at 25 °C (the inhibitory activity of \( \mathbf{I} \) is 130 times greater than that of \( \alpha-\text{MDM} \), for which the dissociation constant is 150 \(\mu \text{M} \) at 25 °C (36)). Therefore, as a first approximation, the dissociation constant of the trimannosyl group at the primary binding site in these glycopeptides can be assigned a value of 1.2 \(\mu \text{M} \).

The secondary binding sites of ConA in these glycopeptides are on their α(1-3) arms. These sites involve either a single β-mannose residue, as in E3, the α(1-2) rhamnogloboio moiety, as in AC-CB and D3, or the GlcNAc(1-2)-Man moiety in C3b. For E3, the dissociation constant of α-DM, 150 \( \mu \text{M} \) at 25 °C (36), can be used as an estimate for the affinity of the α-mannosyl residue at this position. Therefore, for E3, the primary site binds with approximately 100-fold higher affinity than the secondary site. Furthermore, the mechanisms of binding at the two sites are different.

The α(1-2) mannobiosyl moiety of the α(1-3) arm of D3 (and AC-CB) is expected to bind with higher affinity than the single mannose residue on E3. α(1-2) Mannobiose binds approximately 5 times better than α-DM (36), which corresponds to a \( K_d \sim 30 \mu \text{M} \) at 25 °C. Therefore, for D3, the primary site binds with approximately 20-fold higher affinity than the secondary site. The mechanism of binding of the α(1-2) mannobiosyl moiety is also different from that of the trimannosyl moiety (9). The enhanced affinity of the α(1-2) mannobiose has been shown to be due to a statistical increase in the probability of binding due to the presence of 2 mannose residues with free 3-, 4-, and 6-hydroxyl groups in the same molecule, which alternately bind to the monosaccharide binding site of ConA, and not due to extended binding site interactions of the protein (18, 37).

The GlcNAc(1-2)-Man moiety on the α(1-3) arm of C3b is expected to bind somewhat better than α-DM, but not as strong as α(1-2) mannobiose (38). Thus, the difference in affinities between the primary and secondary binding sites on C3b would be between 20- and 100-fold.

These results explain why the NMRD studies, done in the presence of excess glycopeptides, reveal only binding of the higher affinity trimannosyl moiety in AC-CB, D3, E3, and C3b. In addition, the lower amount of precipitate formed by E3 with ConA compared to the other three glycopeptides appears to relate to the lower affinity of the secondary binding site in E3.

Biological Implications of the Bivalency of High Mannose and Bisected Hybrid Type Glycopeptides—The present finding that certain high mannose and bisected hybrid type glycopeptides are bivalent for ConA may relate to the ability of so-called "ConA receptors" on the surface of cells to undergo "patching and capping" when exposed to the lectin (39). Evidence suggests that microaggregation of cell-surface glycocconjugates is a key step in signal transduction effects associated with lectin binding. This, in turn, leads to a variety of different biological responses such as mitogenesis (39). Furthermore, since the structures and therefore affinities of the primary and secondary sites of high mannose and bisected hybrid type glycocconjugates are regulated by glycosylases (5), this may offer a mechanism of controlling the distribution and function of receptors that are specific for carbohydrate binding proteins.

The observation that the bisected hybrid type glycopeptides AC-BA and AC-A can bind well to ConA, but do not precipitate the protein, may offer another mechanism of controlling microaggregation of receptors on the surface of cells. In any case, the intrinsic ability of high mannose and bisected hybrid type glycopeptides to function as bivalent cross-linking molecules for specific binding proteins such as ConA may relate to the role of these carbohydrates as specific receptors on the surface of cells.

Acknowledgments—We wish to thank Dr. William Chaney of Albert Einstein College of Medicine for generous gifts of the ovalbumin glycopeptides D3, E3, and C3b, Dr. Jorgen Lonngren of Pharmacia Fine Chemicals, Uppsala, Sweden, and Martin Haraldsson of the University of Stockholm, Sweden, for oligosaccharide I, and Dr. F. W. R. Adey of the Royal College of Surgeons in Ireland for the ovalbumin.
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Khushi Mata of Rosewell Park Memorial Institute, Buffalo, NY, for the mannobiosides. We also wish to thank Drs. Seymour H. Koenig and Rodney D. Brown, III of IBM Thomas J. Watson Research Center, Yorktown Heights, NY, for helpful discussions and the use of the NMRD facilities.

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SUPPLEMENTARY MATERIAL TO CONCANAVALIN A INTERACTIONS WITH ASPARAGINE-LINKED GLOYPESIDES. RELEVANCE OF HIGH MANNOSE AND BETA-HIBATED STRUTY GLOYPESIDES
by
Lokesh Bhattacharyya, Costante Ceccarini, Patrizia Lorenzoni, and C. Fred Brown

MATERIALS AND METHODS
Materials: Con A was purchased from Miles-Yeda, CMPL was prepared and characterized as previously described (26). Protein concentrations were determined spectrophotometrically at 280 nm using an absorption A280 of 12.4 at 280° and 230° (22). Glycoproteins AC-A, AC-CA, AC-BA, AC-BB, AC-CB, and AC-CC were purified from ovalbumin according to described procedures (27,28). To 25, 27, and 265 (22), the solutions were generated from Dr. William Chaney. The AC-A, AC-BA, and AC-CA mannoses, and 3,4-di-O-mannopyranosyl-β-mannose (11) were generous gifts from Dr. Edward C. Brown, R. D., III, and Brewer, C. F. (1979) Biochem. Biophys. Res. Commun. 90, 117-122

Inhibition Assays: These were performed at 21°C by 2-fold serial dilution in 30 mM sodium phosphate buffer, pH 7.2, containing 0.15 M NaCl, using 76 suspensions of rabbit erythrocytes (21).

NMR Measurements: Measurements of the magnetic field dependence of the solvent water proton relaxation rates (17), i.e., nuclear magnetic relaxation dispersion (NMRD), corresponding to proton (14) to 50 MHz, were made at 25°C using improved field cyclic device previously described (26,27). The bulk of the data was generally better than 1%. Several solutions (10-16) contained known concentrations of CMPL together with sufficient amounts of carbohydrates to saturate the protein binding sites, in pH 5.6 buffer 10 M potassium acetate, 0.9 M NaCl.

RESULTS
Protein NMR Measurements: Figures 3-5 show protein (14) and mα-CH, and the bisected hybrid type glycopeptides, AC-A and AC-CA, in pH 5.6 buffer. The profiles of CMPL in the absence and presence of α-CH were included for comparison. In each case, the solvent water proton relaxation rates at various magnetic field values as expressed in proton (14) frequency undergo a similar pattern drop compared to the lower decrease in the presence of α-CH. These results show that although the glycopeptides differ widely in their structures (Figures 1 and 2) and have different affinities for Con A (Table 1, Table 2), their binding interactions produce similar perturbations in the NMR profiles, which are distinct from those induced by α-manoschic acid binding.

Homopolarization Inhibition Assays: Table 1 shows the results of inhibition of homopolarization by Con A using high mannose and bisected hybrid type glycopeptides and the α-1,3- and α-1,6-mannose. The relative inhibition activity of trisconchial 1 (9) is also included for comparison.
The high mannose type glycopeptide AC-CB binds 500 times better than α-MDM. This is the highest affinity of any glycopeptide in Table 1. The relative affinities of the other high mannose type glycopeptides, D3 and E3, are nearly 6- and 10-fold lower than that of AC-CB, respectively, but D3 and E3 exhibit much higher affinities than α-MDM (Table I), as has been noted by Ohyama et al. (7).

The relative affinities of the bisected hybrid type glycopeptides C1b, AC-BA, and AC-A are also listed in Table 1. Their affinities for the protein are nearly equal, in agreement with Ohyama et al. (7). AC-CC shows much lower affinity for Con A, as previously reported (7). AC-B8, which is similar in structure to AC-CC but has an additional α1(3)-linked α-Man in the (1→2) linkage of the (1→3) arm of core 6-mannose (not shown), also shows much lower affinity for the lectin (7).

Quantitative Precipitation Analyses: We have recently reported the quantitative precipitation curves for D3 (16) at pH 5.4 and 7.2, which showed that the percentage of precipitated protein was greater at pH 7.2 than at pH 5.4. Therefore, most of the data for the glycopeptides were obtained at pH 7.2. Figure 6 shows quantitative precipitation curves for Con A in the presence of the high mannose type glycopeptide AC-CB and E3. The latter induced approximately 0.4% precipitation of the protein (Figure 6a) compared to AC-CB (Figure 6a) and E2 (16). In all of the cases, the precipitates were prevented from forming in the presence of 0.1 M α-MDM, and dissolved upon addition of the monosaccharide, which demonstrated specific binding interactions between the glycopeptides and the lectin.

The concentration of the glycopeptide at the equivalence zone (maxima) precipitation of each precipitin and the concentration of protein monomer are listed in Table II. The ratios of the concentrations of the glycopeptides to Con A are also listed in Table II.

Figure 6. Precipitation curves for precipitation Con A by high mannose type glycopeptides AC-CB (a) and E3 (b) in pH 7.2 buffer at 21°C.

Figure 7 shows the quantitative precipitation curve for Con A in the presence of the bisected hybrid type glycopeptide C1b at pH 7.2. The curve is similar to those obtained with the high mannose type glycopeptides at pH 7.2 (Figure 6). The ratio of the concentrations of C1b and protein at the equivalence zone in Table II, which is similar to those found for the high mannose type glycopeptides. Addition of α-MDM also prevented formation of precipitates.

The bisected hybrid type glycopeptides AC-A and AC-B8, which bind well to Con A (Table I), did not precipitate the protein at either pH 5.6 or 7.2. Manosaccharide also did not precipitate the protein under similar conditions.

Figure 7. Precipitation curve for precipitation Con A by bisected hybrid type glycopeptide C1b in pH 7.2 buffer at 21°C.