# Differences in the Binding Affinities of Dimeric Concanavalin A (Including Acetyl and Succinyl Derivatives) and Tetrameric Concanavalin A with Large Oligomannose-Type Glycopeptides†

Dipak K. Mandal and C. Fred Brewer*

*Department of Molecular Pharmacology and Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461*

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ABSTRACT: Dimeric derivatives of concanavalin A (Con A) such as acetyl- and succinyl-Con A have been used for years as probes of cellular membranes. The altered binding and biological activities of these derivatives relative to native tetrameric Con A have generally been attributed to their reduced valence. However, the present study shows that acetyl- and succinyl-Con A possess lower affinities than tetrameric Con A toward certain oligomannose-type glycopeptides which are found on the surface of cells. It has previously been shown that native tetrameric Con A possesses 5–30-fold enhanced affinities toward Man7–Man9 oligomannose-type glycopeptides, respectively, relative to Man5 and Man6 oligomannose-type glycopeptides [Bhattacharyya, L., & Brewer, C. F. (1989) *Eur. J. Biochem. 178*, 721–726]. Using titration microcalorimetry and hemagglutination inhibition measurements, methyl α-D-mannopyranoside, methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (which binds with about 60-fold higher affinity than methyl α-D-mannopyranoside and is the major Con A binding epitope on oligomannose-type carbohydrates), and a Man5 oligomannose-type oligosaccharide are shown to bind to underivatized dimeric Con A at pH 5.2 and acetyl- and succinyl-Con A at pH 7.2 with affinities equal to those of native tetrameric Con A. However, a mixture of Man7 and Man8 glycopeptides and a Man9 oligomannose-type glycopeptide were shown to bind to underivatized dimeric Con A and acetyl- and succinyl-Con A with affinities only about 2-fold higher than the Man5 oligosaccharide, in contrast to the higher affinities of native tetrameric Con A for these carbohydrates. Thus, Man7–Man9 oligomannose-type glycopeptides bind with approximately 4- and 10-fold lower affinities, respectively, to dimeric Con A and its derivatives relative to tetrameric Con A. Differences in the affinities of dimeric and tetrameric Con A for the larger oligomannose-type glycopeptides are ascribed to the ability of the longer α(1–3) and α(1–6) arms of the Man7–Man9 glycopeptides to "jump" between adjacent monomer binding sites of the tetramer before dissociating from the protein and the absence of this effect in the dimer where the binding sites are further separated. The present findings indicate that acetyl- and succinyl-Con A can not be used as mere "divalent" derivatives of the lectin in studies of cell membranes which possess Man7–Man9 oligomannose-type carbohydrates.

Concanavalin A (Con A)[1] is one of the most widely utilized lectins in biology. Its uses include probing the dynamics and structures of normal and tumor cell membranes (Ben-Bassat & Goldblum, 1975; Brown & Hunt, 1978), establishing glycosylation mutants in transformed cells (Stanley, 1982), and yielding preparations of polysaccharides, glycopeptides, and glycoproteins from Con A affinity columns (Bittiger & Schnebli, 1976). Early studies showed that Con A recognized α-Glc[2] and α-Man residues with free 3-, 4-, and 6-hydroxyl groups (Goldstein & Poretz, 1986). The lectin is a tetramer above pH 7 and a dimer below pH 6. Each monomer ($M_r$ 26 500) possesses one saccharide binding site as well as a transition metal ion site (S1) and a $Ca^{2+}$ site (S2) (Brewer et al., 1983; Goldstein & Poretz, 1986).

Succinyl- and acetyl-Con A are pH-independent dimeric derivatives of the lectin which have been widely used to determine the effects of Con A valency on its biological activities (Gunther et al., 1973; Wang et al., 1976). Such studies include a comparison of thymidine-induced incorporation and mitogenic effects of succinyl-Con A and native tetrameric Con A in normal and transformed cells (Trowbridge & Hilborn, 1974), investigations of the "capping" behavior of cell receptors for dimeric and tetrameric Con A (Gunther et al., 1973), and the cell agglutination properties of dimeric versus tetrameric Con A (Gunther et al., 1973). In view of the similar affinities of acetyl- and succinyl-Con A and the native lectin for monosaccharides such as methyl α-D-glucopyranoside, differences in their biological activities have been attributed to differences in their valencies (Gunther et al., 1973).

The receptors on the surface of normal and transformed cells that bind lectins are generally found as glycoproteins and glycolipids [cf. Lennarz (1980)]. The oligosaccharide chains of many of the glycoproteins appear to function as receptors for lectins in a variety of biological recognition processes (Brandley & Schnaar, 1986). Asparagine-linked oligomannose-type carbohydrates constitute one class of oligosaccharide chains associated with cellular glycoproteins (Kobata, 1984). The trimannosyl moiety 3,6-di-*O*-(α-D-

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* To whom correspondence should be addressed.

[1] Abbreviations: Con A, concanavalin A (lectin from jack bean); MeαMan, methyl α-D-mannopyranoside; FPLC, fast-protein liquid chromatography; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; NMR, nuclear magnetic resonance.

[2] All sugars are in the D-configuration.

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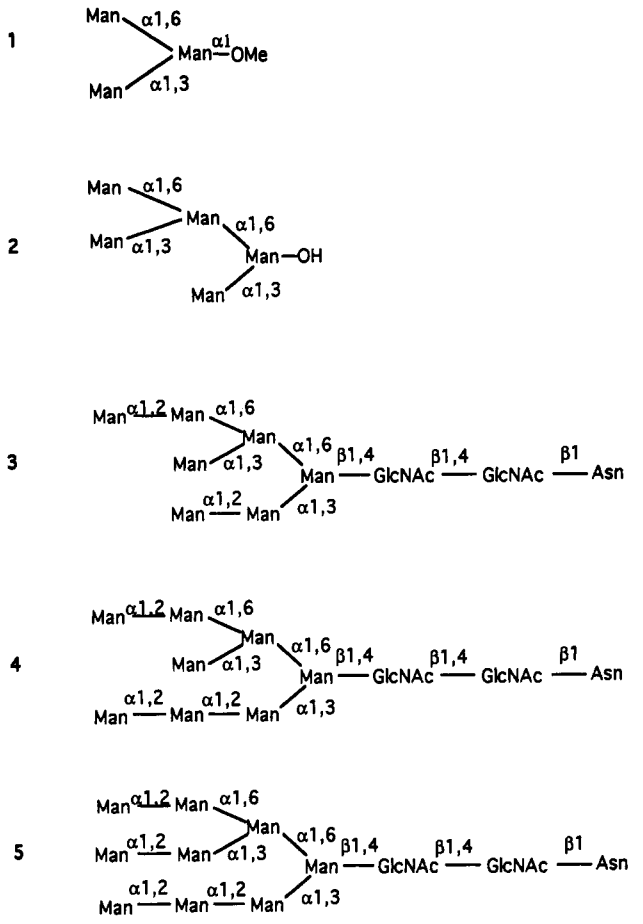


FIGURE 1: Structures of trimannoside **1**, Man5 oligosaccharide **2**, and Man7–Man9 glycopeptides **3**–**5**. The structures of the Man7 and Man8 oligomannose-type glycopeptides isolated from quail ovalbumin were determined by $^1$H NMR (Vliegenthart et al., 1983) in the present study. Man, GlcNAc, and Asn represent mannose, *N*-acetylglucosamine, and asparagine residues, respectively.

mannopyranosyl)-α-D-mannopyranose (**1** is the methyl α-anomer in Figure 1), which is located on the outer α(1–6) arms of oligomannose-type carbohydrates, has been demonstrated to be the major epitope recognized for high-affinity binding by Con A [cf. Brewer and Bhattacharyya (1986)]. The trimannoside possesses nearly 60-fold higher affinity than methyl α-D-mannopyranoside (MeαMan) for the lectin. Thus, the Man5 oligomannose-type oligosaccharide (**2**) in Figure 1 which possesses the trimannosyl moiety on its α(1–6) arm binds to native Con A with the same affinity of **1** (Brewer & Bhattacharyya, 1986). However, certain larger oligomannose-type carbohydrates such as Man7, Man8, and Man9 glycopeptides (**3**, **4**, and **5**, respectively, in Figure 1) show increased affinities of 5–30-fold for tetrameric Con A relative to the Man5 carbohydrate (Bhattacharyya & Brewer, 1989). [Differences in the affinities of the glycopeptides were also manifested in their precipitation activities with tetrameric Con A since the glycopeptides are divalent due to binding of their α(1–3) arms in addition to the higher affinity binding of their corresponding α(1–6) arms (Bhattacharyya & Brewer, 1989; Bhattacharyya et al., 1988b).] The enhanced affinities of these larger glycopeptides have been ascribed to the presence of the added α(1–2) residues in the molecules which provide additional lower affinity sites of binding for the lectin (Bhattacharyya & Brewer, 1989).

Titration microcalorimetry is a powerful tool for investigating the thermodynamics of carbohydrate–protein interactions [cf. Bains et al. (1992)]. Such measurements directly determine the association constant of binding ($K_a$), as well as the enthalpy and stoichiometry of binding. In the course of investigating the thermodynamics of binding of simple mono- and oligosaccharides as well as oligomannose-type glycopeptides to Con A, we observed that the affinities of dimeric Con A as well as acetyl- and succinyl-Con A, which are pH-independent dimeric derivatives of the lectin, for Man7–Man9 oligomannose type glycopeptides were lower than those reported for native tetrameric Con A (Bhattacharyya & Brewer, 1989). In this study, we present microcalorimetric and hemagglutination inhibition data which show that Man7–Man9 glycopeptides bind to dimeric Con A and its acetyl and succinyl derivatives with 4–10-fold lower affinities than to native tetrameric Con A. These results have important consequences for the use of dimeric derivatives of Con A for comparative studies with native tetrameric Con A in biological systems.

## MATERIALS AND METHODS

*Materials.* Native Con A was prepared from jack bean seeds (Sigma) as previously described (Agrawal & Goldstein, 1967). The concentration of Con A was determined spectrophotometrically at 280 nm using $A^{1\%,1cm} = 13.7$ at pH 7.2 (Goldstein & Poretz, 1986) and 12.4 at pH 5.2 (Yariv et al., 1968) and expressed in terms of the monomer ($M_r = 26\,500$) (Goldstein & Poretz, 1986). Acetyl- and succinyl-Con A were prepared as described (Khan et al., 1991) and were separated from tetrameric Con A by FPLC on Superdex 75. The concentrations of acetyl- and succinyl-Con A were measured spectrophotometrically at 280 nm using $A^{1\%,1cm} = 13.7$ at pH 7.2 and expressed in terms of the monomer ($M_r = 28\,000$) (Gunther et al., 1973).

MeαMan and **1** in Figure 1 were purchased from Sigma Chemical Co. Man5 oligosaccharide **2** was obtained from Dextra Laboratories Ltd., U.K. Man9 glycopeptide **5** was prepared from the Pronase digest of soybean agglutinin (SBA) according to a previously described procedure (Lis & Sharon, 1978). A mixture of Man7 (**3**) and Man8 (**4**) glycopeptides (~1:1 by $^1$H NMR) was isolated from the Pronase digest of quail ovalbumin as described (Mandal & Brewer, 1992). The concentrations of carbohydrates were determined by the phenol–sulfuric acid method (Dubois et al., 1956) using Man as standard. The structures and purities of the carbohydrates were established by $^1$H NMR spectroscopy at 500 MHz (Vliegenthart et al., 1983).

*Hemagglutination Inhibition Assays.* Assays were performed at room temperature by a 2-fold serial dilution technique (Osawa & Matsumoto, 1972) in 0.01 M sodium phosphate buffer, pH 7.2, containing 0.15 M NaCl, 0.1 mM $Mn^{2+}$, and 0.1 mM $Ca^{2+}$, using a 3% (v/v) suspension of rabbit erythrocytes.

*Titration Calorimetry.* Isothermal titration calorimetry was performed using an OMEGA titration microcalorimeter from Microcal, Inc. (Northampton, MA). Briefly, in the individual titrations, injections of 3 μL of saccharide were added from the computer-controlled 100-μL microsyringe at an interval of 4 min into the lectin solution (cell volume = 1.3424 mL) dissolved in the same buffer as the saccharide, while stirring at 350 rpm. Control experiments performed by making identical injections of saccharide into a cell-containing buffer with no protein showed negligible ligand heat of dilution. The experimental data were fitted to a theoretical titration curve using software supplied by Microcal to yield directly the values for $K_a$ (the association constant in $M^{-1}$), as well as $\Delta H$ (the enthalpy change in cal $mol^{-1}$) and $n$ (stoichiometry

Table I: Association Constants Derived from the Titration of Con A and Its Derivatives with Various Oligosaccharides and Glycopeptides at 25 °C

| carbohydrate | carbohydrate concn (mM) | lectin concn (mM) | $K_a$[a] ($M^{-1}$) |
|---|---|---|---|
| | Tetrameric Con A at pH 7.2[b] | | |
| MeαMan | 46.0 | 0.483 | 0.85 (±0.03) ×10⁴ |
| **1** | 7.04 | 0.132 | 4.90 (±0.15) ×10⁵ |
| | Succinyl-Con A at pH 7.2[b] | | |
| MeαMan | 37.0 | 0.620 | 0.99 (±0.03) ×10⁴ |
| **1** | 7.66 | 0.155 | 5.13 (±0.22) ×10⁵ |
| **2** | 1.60 | 0.031 | 6.63 (±0.40) ×10⁵ |
| **3**/**4** mixture[e] | 1.40 | 0.030 | 3.03 (±0.41) ×10⁵ |
| **5** | 0.93 | 0.031 | 8.96 (±0.14) ×10⁵ |
| | Acetyl-Con A at pH 7.2[c] | | |
| **1** | 1.40 | 0.028 | 3.10 (±0.43) ×10⁵ |
| **2** | 1.10 | 0.031 | 3.51 (±0.27) ×10⁵ |
| **5** | 1.10 | 0.032 | 5.45 (±0.43) ×10⁵ |
| | Dimeric Con A at pH 5.2[d] | | |
| **1** | 11.0 | 0.195 | 5.10 (±0.21) ×10⁵ |
| **5** | 1.10 | 0.025 | 1.10 (±0.10) ×10⁶ |

[a] Values in parentheses indicate the standard deviation of fit between the experimental binding curve and the calculated curve obtained with the fitted thermodynamic parameters. [b] The buffer was 0.1 M HEPES containing 0.9 M NaCl, 1 mM $Mn^{2+}$, and 1 mM $Ca^{2+}$ at pH 7.2. [c] The buffer was 0.02 M HEPES containing 0.15 M NaCl, 1 mM $Mn^{2+}$, and 1 mM $Ca^{2+}$ at pH 7.2. [d] The buffer was 0.05 M dimethyl glutarate containing 0.25 M NaCl, 1 mM $Mn^{2+}$, and 1 mM $Ca^{2+}$ at pH 5.2. [e] Man7 and Man8 glycopeptides were isolated from the Pronase digest of quail ovalbumin (Mandal & Brewer, 1992).

of binding). The quantity $c = K_a M_t(0)$, where $M_t(0)$ is the initial macromolecule concentration, is of importance in titration microcalorimetry (Wiseman et al., 1989). All experiments were performed with $c$ values in the range 10–100, which optimizes measurements of $K_a$. The instrument was calibrated by using the calibration kit containing ribonuclease A (RNase A) and cytidine 2′-monophosphate (2′-CMP) supplied by the manufacturer.

## RESULTS AND DISCUSSION

The present study describes the determination of the absolute and relative $K_a$ values of a series of oligomannose-type carbohydrates with respect to smaller mono- and oligosaccharides by titration microcalorimetry and hemagglutination inhibition. The results demonstrate that certain large oligomannose-type glycopeptides (Man7–Man9) bind to dimeric forms of Con A, including acetyl- and succinyl-Con A, with lower affinities than to tetrameric Con A. The thermodynamic parameters of binding of some of the oligosaccharides and glycopeptides to dimeric and tetrameric Con A determined from the titration microcalorimetry will be presented elsewhere.

*Titration Microcalorimetry Measurements.* Using titration microcalorimetry, the $K_a$ values obtained for binding of MeαMan to dimeric succinyl-Con A and tetrameric native lectin at pH 7.2 are shown in Table I. These results show that dimeric succinyl-Con A binds the monosaccharide MeαMan with essentially the same affinity (~9 × $10^3$ $M^{-1}$) as the native tetrameric lectin, which is consistent with previous reports [cf. Wang et al. (1976)] which show similar $K_a$ values (2 × $10^3$ $M^{-1}$) for binding of methyl α-D-glucopyranoside to acetyl- and succinyl-Con A and to the native lectin obtained by equilibrium dialysis techniques.

The value of $K_a$ determined for trimannoside **1** binding to succinyl-Con A in high salt buffer at pH 7.2 is 5.1 × $10^5$ $M^{-1}$ (Table I). This value is the same for underivatized dimeric Con A in the presence of low salt buffer at pH 5.2 and is essentially identical to that determined for the tetrameric native lectin at pH 7.2 in high salt buffer (Table I). The affinity of **1** for acetyl-Con A in low salt buffer at pH 7.2 is also similar to that observed for tetrameric Con A in high salt buffer at pH 7.2 (Table I). These results demonstrate that the binding affinities of dimeric and dimeric derivatives of Con A and tetrameric Con A for the trimannosyl oligosaccharide are identical and that variations in pH between 5.2 and 7.2 and salt concentration between 0.15 and 0.9 M NaCl have no significant effects on the affinity of the lectin. The nearly 60-fold increase in affinity of **1** relative to MeαMan for dimeric and tetrameric Con A agrees well with their relative affinities for the native lectin determined by hemagglutination inhibition (Brewer & Bhattacharyya, 1986) (see also Table II).

Table II: Inhibitory Power of Oligomannose-Type Oligosaccharides and Glycopeptides for Hemagglutination of Rabbit Erythrocytes by Native Con A, Acetyl-Con A, and Succinyl-Con A

| oligosaccharide or glycopeptide | relative inhibitory potency[a] | | |
|---|---|---|---|
| | native Con A | acetyl-Con A | succinyl-Con A |
| MeαMan | 1 | 1 | 1 |
| **1** | 105 | 105 | 105 |
| **2** | 115 | 115 | 115 |
| mixture of **3** and **4**[b] | 740 | 180 | 240 |
| **5** | 2100 | 205 | 260 |

[a] All data were normalized to MeαMan. Higher values indicate greater inhibitory potency. The minimum concentration of MeαMan required for complete inhibition of agglutination by native Con A, acetyl-Con A, and succinyl-Con A at four hemagglutinating doses (Osawa & Matsumoto, 1972) is 3.1 mM while the minimum hemagglutinating activities of native Con A, acetyl-Con A, and succinyl-Con A are 3.5, 27, and 26 μg/mL, respectively. [b] Man7 and Man8 glycopeptides were isolated from the Pronase digest of quail ovalbumin (Mandal & Brewer, 1992).

Microcalorimetry studies of the binding of oligomannose-type carbohydrates **2**–**5** could not be performed with tetrameric Con A since they have been shown to bind and precipitate as divalent ligands with the native lectin (Bhattacharyya & Brewer, 1989). However, these carbohydrates form soluble complexes with dimeric Con A and its derivatives since divalent lectins do not precipitate with divalent carbohydrates [cf. Bhattacharyya et al. (1988a)]. Therefore, only dimeric Con A and acetyl- and succinyl-Con A were used in titration microcalorimetry experiments to determine the $K_a$ values for binding of the oligomannose-type carbohydrates to the lectin.

The $K_a$ values for binding of the Man5 oligosaccharide (**2**), a ~1:1 mixture of Man7 (**3**) and Man8 (**4**) glycopeptides, and Man9 glycopeptide (**5**) (Figure 1) determined from titration microcalorimetry with dimeric acetyl-Con A at pH 7.2 in low salt buffer, succinyl-Con A at pH 7.2 in high salt buffer, or underivatized dimeric Con A at pH 5.2 in low salt buffer are shown in Table I. Typical titration data are shown in Figure 2 for binding of **2** with dimeric succinyl-Con A (31 μM). The theoretical curve passes closely through the experimental points and resembles the high-affinity binding curve obtained for **1**. Indeed, the $K_a$ value of 6.6 × $10^5$ $M^{-1}$ for **2** is similar to the value of 5.1 × $10^5$ $M^{-1}$ for **1**. The affinity of **2** for acetyl-Con A is also essentially the same as that for succinyl-Con A (Table I). These results confirm previous studies that suggest that the trimannosyl moiety (**1**) located on the outer α(1–6) arm of **2** is the primary epitope for high-affinity binding to Con A (Brewer & Bhattacharyya, 1986). Since the Man5 oligomannose-type glycopeptide has been reported to bind to native Con A with nearly the same affinity as **1** (Bhattacharyya & Brewer, 1989), the results also indicate that the Man5 oligomannose-type oligosaccharide

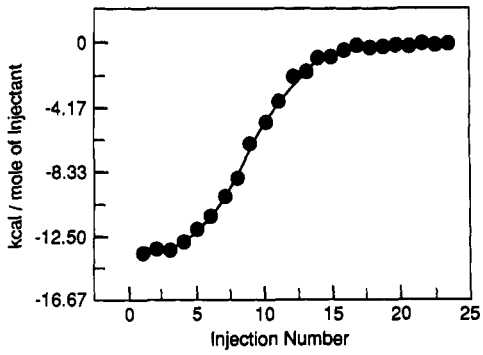


FIGURE 2: Calorimetric integrated titration curve showing experimental points (●) and the best fit (—) for titration of succinyl-Con A (31 μM) with **2** (1.6 mM) at 25 °C, obtained for 24 injections of **2**, each of 3 μL. The buffer was 0.1 M HEPES containing 0.9 M NaCl, 1 mM $Mn^{2+}$, and 1 mM $Ca^{2+}$ at pH 7.2.

binds to acetyl- and succinyl-Con A with essentially the same affinity as native tetrameric Con A.

The apparent $K_a$ for binding of the mixture of Man7 and Man8 glycopeptides from quail ovalbumin to succinyl-Con A at pH 7.2 in high salt buffer is $3 \times 10^5$ $M^{-1}$ at 25 °C (Table I). This is similar to the $K_a$ values for **1** and **2**. The $K_a$ values obtained by titration microcalorimetry for binding of the Man9 oligomannose-type glycopeptide to succinyl-Con A at pH 7.2 in high salt buffer, acetyl-Con A at pH 7.2 in low salt buffer, and underivatized Con A at pH 5.2 in low salt buffer are shown in Table I. All three values of $K_a$ are about 2 times higher than those for the **1** and **2**, which indicates that the Man9 glycopeptide binds with only slightly higher affinity to dimeric Con A and its derivatives relative to **1** and **2**.

The Man7 and Man8 oligomannose-type glycopeptides have been previously reported to bind to tetrameric Con A with approximately 5- and 9-fold higher affinities, respectively, than trimannoside **1**, as determined by hemagglutination inhibition (Bhattacharyya & Brewer, 1989). The Man9 glycopeptide, in the same study, was reported to bind to tetrameric Con A with nearly 30-fold higher affinity than **1**. Since the $K_a$ values for **1** binding to dimeric Con A and its derivatives as well as to tetrameric Con A are essentially the same (Table I), it appears that the affinities of the Man7, Man8, and Man9 glycopeptides for dimeric Con A and its derivatives determined by titration calorimetry are lower than those reported for the glycopeptides's binding to native tetrameric Con A determined by hemagglutination inhibition.

In order to obtain further data on the relative affinities of the Man7–Man9 glycopeptides for dimeric and tetrameric Con A, hemagglutination inhibition experiments were carried out with acetyl- and succinyl-Con A and compared with the results for native Con A.

*Inhibition Studies.* Table II shows the relative affinities (inhibitory potencies) of Meα-Man, **1**, **2**, the mixture of **3** and **4**, and **5** in the hemagglutination inhibition assays of native tetrameric Con A, acetyl-Con A, and succinyl-Con A with rabbit erythrocytes. Trimannoside **1** shows equal affinity for all three forms of the lectin and a nearly 100-fold increase in affinity relative to Meα-Man as previously reported (Brewer & Bhattacharyya, 1986). The Man5 oligosaccharide (**2**) also binds to the three preparations of Con A with affinities similar to that for **1**, which is similar to the affinity of the corresponding Man5 glycopeptide for native Con A (Bhattacharyya & Brewer, 1989). The mixture of Man7 and Man8 glycopeptides possesses a 2-fold increase in affinity for acetyl- and succinyl-Con A but approximately a 7-fold increase in affinity for native tetrameric Con A relative to **1** and **2**. The Man9 glycopeptide (**5**) also shows a 2-fold increase in affinity toward acetyl- and succinyl-Con A but a nearly 20-fold increase in affinity for native tetrameric Con A relative to **1** and **2**. The results for the Man7–Man8 glycopeptide mixture and Man9 glycopeptide binding to native Con A are similar to those previously reported by hemagglutination inhibition measurements (Bhattacharyya & Brewer, 1989).

The hemagglutination inhibition data for the relative affinities of the Man7–Man8 glycopeptide mixture and Man9 glycopeptide binding to acetyl- and succinyl-Con A (Table II), with respect to **1**, are consistent with their relative $K_a$ values obtained by titration microcalorimetry (Table I). These findings confirm that the Man7–Man8 mixture and the Man9 glycopeptide possess lower binding affinities, approximately 4- and 10-fold, respectively, to acetyl- and succinyl-Con A, and to underivatized dimeric Con A, than to native tetrameric Con A. These results suggest that the binding affinities of the larger Man7–Man9 glycopeptides are influenced by the quaternary structure of Con A, while the smaller carbohydrates such as Meα-Man, **1**, and **2** are not.

X-ray diffraction studies have shown that the saccharide binding sites of Con A are located at the far ends of peanut-shaped dimers and that two dimers are arranged nearly perpendicular in the tetramer (Becker et al., 1976; Reeke et al., 1975). The enhanced affinity of the larger Man7–Man9 oligomannose carbohydrates for tetrameric Con A can be attributed to the closer proximity of saccharide binding sites in the tetramer relative to the dimer. This could lead to "jumping" of the relatively long α(1–6) and α(1–3) arms of these glycopeptides between saccharide binding sites located on adjacent monomers of the two dimers of a tetramer. This would have the effect of slowing up the off-rate of the carbohydrate before complete dissociation of the complex. This type of recapture effect has been described for the binding of α(1–2) mannosyl oligosaccharides (Brewer & Brown, 1979). Smaller oligosaccharides would be unable to "jump" between adjacent binding sites of the Con A tetramer and thus would have the same affinities for dimeric and tetrameric Con A, as is observed. Thus, the affinities of certain large oligomannose-type glycopeptides depend on the quaternary structure of the lectin.

*Summary.* Dimeric acetyl- and succinyl-Con A have been used for nearly 2 decades as "divalent" derivatives of the lectin in numerous studies with cells. The altered biological effects of these derivatives with respect to native tetrameric Con A have been attributed to their reduced valencies [cf. Gunther et al. (1973) and Trowbridge and Hilborn (1974)]. However, the present study shows that Man7–Man9 oligomannose-type glycopeptides have approximately 4- and 10-fold reduced affinities, respectively, for dimeric Con A and its acetyl- and succinyl-derivatives relative to tetrameric Con A. Since these oligomannose-type glycopeptides are often found on the surface of normal and transformed cells as "Con A receptors", use of such dimeric derivatives of Con A to explore the effects of reduced valency of the lectin on the membrane properties of such cells must be made with great caution.

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