Quantitative analysis of multivalent interactions of carbohydrate-encapsulated gold nanoparticles with concanavalin A†

Chun-Cheng Lin,a Yi-Chun Yeh,b Chan-Yi Yang,b Gee-Fong Chen,b Yi-Chen Chen,b Yi-Chun Wu* and Chia-Chun Chen*a

a Institute of Chemistry, Academia Sinica, Taipei 115, Taiwan
b Department of Chemistry, National Taiwan Normal University and Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei 116, Taiwan. E-mail: cjchen@cc.ntnu.edu.tw; Fax: 886-289316383; Tel: 866-289316151
c Institute of Molecular and Cellular Biology, National Taiwan University, Taipei 106, Taiwan

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Multivalent interactions between carbohydrate-encapsulated gold nanoparticles and Con A are found with high affinity and specificity.

Multivalent interactions between cell surface receptors and carbohydrates have been discovered as a number of biological processes including fertilization, proliferation, viral/bacterial infection and the inflammatory response.1 Because of the simultaneous binding of multiple ligands on one biological entity to multiple receptors on another, the multivalent interactions are often with high binding affinity and specificity. A number of diverse scaffolds have been generated for multivalent carbohydrate ligand presentation.2 Low-molecular weight polysaccharides,3 copolymers,4 dendrimers,5 nanoparticles,6 and liposomes,7 for examples, have been employed as powerful carriers to present carbohydrate ligands in polydisperse, linear or globular architectures. In particular, LewisX (Lex, a trisaccharide antigen) encapsulated on gold nanoparticles,6 mimicking glycosphingolipid clusters, exhibited significantly enhanced affinity and selectivity as compared with Lex monomers. However, the multivalent binding between carbohydrate-based nanoparticles and lectins has not been demonstrated thus far.

Concanavalin A (Con A), a plant lectin, can form a tetramer under appropriate conditions and bind specifically to manno- and glucopyranosides.8 The binding between Con A and carbohydrates has long been recognized as an excellent system to study multivalent effects. Multivalent interactions have been studied using various techniques such as electron microscopy,9 calorimetry,10 X-ray crystallography11 and surface plasmon resonance (SPR).12 These studies have provided information on the Con A and saccharide binding model and interaction kinetics, which are useful for the development of potent inhibitors and biological effectors.

Recently, we have demonstrated that mannose-encapsulated gold nanoparticles (m-AuNP) can be used as a probe to target specific proteins in living bacteria.13 In this paper, we explore the multivalent interactions between Con A and mannose-, glucose- and galactose-encapsulated gold nanoparticles (abbreviated as m-AuNP, g-AuNP and t-AuNP, respectively, see Fig. 1). The SPR technique is applied to quantitatively analyze the interactions. We found that the binding of m-AuNP to Con A exhibited a strong multivalent effect and that the binding specificity of Con A for the multivalent carbohydrate-encapsulated gold nanoparticles (carbohydrate-AuNP) was similar to that of the monovalent counterparts. We also show that the affinity of m-AuNP for Con A can be adjusted by altering the nanoparticle size or sugar moiety. Our results demonstrate that nanoparticle-based multivalent carbohydrates for lectins and that carbohydrate-AuNP have great potential as effective inhibitors of protein–carbohydrate interactions in biological system.

Various carbohydrate-AuNP as described in Fig. 1 were synthesized following similar methods as previously reported.13 The detailed procedures of synthesis and characterization of carbohydrate-AuNP are described in ESI.† In brief, nearly mono-dispersed gold nanoparticles with average diameters of 6 or 20 nm were prepared, and their sizes were confirmed using transmission electron microscopy.14 Various carbohydrate ligands with different linker lengths were synthesized with an S–H group at one side, which was then linked to the nanoparticle through the formation of a strong thiol bond. The amount of carbohydrates attached on each gold nanoparticle was quantitatively determined by H2SO4/phenol assay and elemental analysis.15

To assess the binding affinity of α-α-methylmannopyranoside (αMeMan) and various carbohydrate-AuNP for Con A, we utilized the SPR competition binding assay based on the previous report12,16 with some modifications. A self-assembled monolayer composed of 20% mannopyranoside ligand and 80% thioctan-1-ol mixture was generated on a J1 biosensor chip (Fig. 1).17 The affinity of Con A for this chip was determined by titration with series of Con A concentrations to generate multiple SPR response curves. Using the rectangular hyperbolic equation18 the association constant Ks of Con A for this chip was obtained, and the value was 7.95 × 106 M−1. In competition assays, inhibition curves were generated by measuring the binding responses for 0.5 μM Con A tetramer in

![Fig. 1](image-url)
the presence of different concentrations of αMeMan or carbohydrate-AuNP as competitive inhibitors. Fig. 2 presents a set of SPR response curves for Con A in the presence of 6-m-AuNP. From the inhibition curves of each carbohydrate-AuNP, its inhibition constant (K_i) is obtained using the equations derived by Attie et al.20 (Table 1). To compare the inhibition potencies of the individual mannose ligand on three different m-AuNP (6-m-AuNP, s-20-m-AuNP or l-20-m-AuNP) with respect to monovalent αMeMan, we calculated the relative inhibition potency (RIP).20

The RIP values for the mannose ligands of three m-AuNP are from 11 to 128 (Table 1), indicating that the multivalent mannose ligands of these m-AuNP have one to two orders higher affinities to Con A than monovalent mannose ligands. In addition, all three m-AuNP exhibited a stronger inhibition effect than 6-g-AuNP and 6-t-AuNP. 6-t-AuNP displayed no detectable inhibition effect. This is consistent with the previous studies that Con A binds to mannose better than glucose but does not bind to galactose.21,12 Therefore, no switch of Con A specificity for carbohydrates clustered on nanoparticles was observed in our system. Taken together, our results demonstrate that Con A tetramer has a stronger inhibition effect on 6-t-AuNP and 6-g-AuNP, 6-t-AuNP displayed no detectable inhibition effect. This is consistent with the previous studies that Con A binds to mannose better than glucose but does not bind to galactose.21,12 Therefore, no switch of Con A specificity for carbohydrates clustered on nanoparticles was observed in our system. Taken together, our results demonstrate that clustering of carbohydrate ligands on a nanoparticle significantly enhances the ligand binding affinity for lectins, with no change in lectin binding specificity.

It has been studied that the Con A tetramer presents two saccharide binding sites on each face, and the distance between them is 6.5 nm.22 We compared the inhibition potencies of 6-m-AuNP and 20-m-AuNP in the SPR competition assays (Table 1). As the particle diameters of 6-m-AuNP and 20-m-AuNP are comparable to or significantly larger than the distance between two relevant binding sites on Con A, respectively, the mannose ligands of 6-m-AuNP are less favorable to engage in the divalent binding of a Con A tetramer than those of 20-m-AuNP.23 Our system showed that the carbohydrate ligands with the ability to span the requisite distance to occupy two Con A saccharide binding sites are more effective multivalent inhibitors than those which fail to engage divalent binding. We further investigated the effects of different linker lengths of s- and l-20-m-AuNP on Con A binding affinity (Table 1). The two-times difference in the RIP values of s- and l-20-m-AuNP might be attributed to the differences in their intrinsic properties, for example, the orientation of mannose groups on the surface and/or the rigidity of the linkers.

In conclusion, we have demonstrated that a nanoparticle can be a good multivalent ligand carrier. The multivalent interactions between m-AuNP and Con A are affected by nanoparticle size and the linker of mannose ligands. Our approaches may be also applicable to other types of nanoparticles such as quantum dots24 and magnetic nanoparticles.

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Notes and references
15 6-m-AuNP, s- and l-20-m-AuNP have on average 200, 680 and 840 mannose ligands, respectively, clustered on the nanoparticle surface.
20 The RIP of each ligand of m-AuNP is calculated as: K_i(αMeMan)/[K_i(m-AuNP)] × the average number of mannose ligands on the m-AuNP.
23 The distances between two neighboring mannose groups of m-AuNP are estimated to be < 6.5 nm, far closer than that of two binding sites of Con A tetramer.

Fig. 2 Inhibition of 0.5 μM Con A binding to the chip by 6-m-AuNP. A set of inhibition curves for 0, 0.175, 0.5 and 1 μM 6-m-AuNP (top to bottom) are shown.

Table 1 The data of dissociation constants (K_i) and relative inhibition potency (RIP) of carbohydrate-AuNP to Con A (no inhibition; / not determined)

<table>
<thead>
<tr>
<th>Compound</th>
<th>K_i</th>
<th>RIP</th>
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<tbody>
<tr>
<td>αMeMan</td>
<td>20 × 10^{-4}</td>
<td>1.0</td>
</tr>
<tr>
<td>6-m-AuNP</td>
<td>8.8 × 10^{-4}</td>
<td>11.2</td>
</tr>
<tr>
<td>s-20-m-AuNP</td>
<td>2.3 × 10^{-9}</td>
<td>127.8</td>
</tr>
<tr>
<td>l-20-m-AuNP</td>
<td>3.5 × 10^{-9}</td>
<td>67.5</td>
</tr>
<tr>
<td>6-γ-AuNP</td>
<td>1.6 × 10^{-7}</td>
<td>/</td>
</tr>
<tr>
<td>6-α-AuNP</td>
<td>—</td>
<td>—</td>
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