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

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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 in 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 displays,[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.

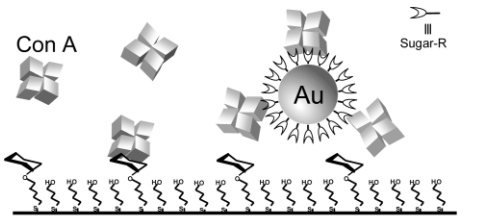
Concanavalin A (Con A), a plant lectin, can form a tetramer under appropriate conditions and bind specifically to manno- and glucopyranosides.[8] The binding between Con A and carbohydrates has long been recognized as an excellent system to study multivalent effects. Multivalent interactions have been studied using various techniques such as electron microscopy,[9] calorimetry,[10] X-ray crystallography[11] 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 nanoparticles can be excellent multivalent carbohydrate carriers for lectins and that carbohydrate-AuNP has 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 H_2SO_4/phenol assay and elemental analysis.[15]

To assess the binding affinity of α-D-methyl-mannopyranoside (αMeMan) and various carbohydrate-AuNP for Con A, we utilized the SPR competition binding assay based on the previous report[12,16] with some modifications. A self-assembled monolayer composed of 20% mannopyranoside ligand and 80% thiobutanol 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 equation[18] the association constant K_a of Con A for this chip was obtained, and the value was 7.95×10^6 M^{-1}. In competition assays, inhibition curves were generated by measuring the binding responses for 0.5 μM Con A tetramer in

(a)



(b)

Sugar	R	Particle size	Abbreviation
Mannose		6 nm	6-*m*-AuNP
Mannose		20 nm	*s*-20-*m*-AuNP
Mannose		20 nm	*l*-20-*m*-AuNP
Glucose		6 nm	6-*g*-AuNP
Galactose		6 nm	6-*t*-AuNP

Fig. 1 (a) Schematic illustration of the interactions of carbohydrate-AuNP and Con A on the biosensor chip used in the competition assays. (b) Five different carbohydrates (sugar-R) encapsulated on two different sizes of gold nanoparticles are summarized. Specifically, gluco- and galactopyranoside are encapsulated on nanoparticles of 6 nm and abbreviated as 6-*g*-AuNP and 6-*t*-AuNP, respectively. Mannopyranoside clustered on nanoparticles of 6 nm and 20 nm in diameter are abbreviated as 6-*m*-AuNP and 20-*m*-AuNP, respectively. Mannopyranoside with short or long linker lengths are encapsulated on 20 nm nanoparticles to form *s*-20-*m*-AuNP and *l*-20-*m*-AuNP, respectively.

† Electronic supplementary information (ESI) available: detailed experimental procedures, SPR response curves and compound characterization data. See http://www.rsc.org/suppdata/cc/b3/b308995a/

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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.*[19] (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 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.

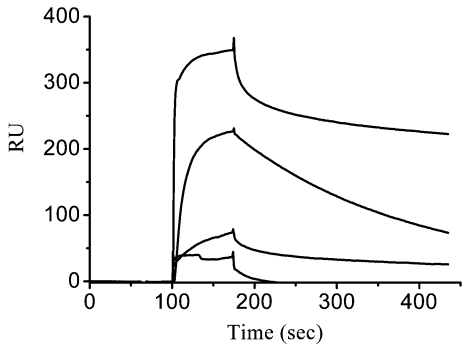

Fig. 2 Inhibition of 0.5 μM Con A binding to the chip by 6-*m*-AuNP. A set of inhibition curves for 0, 0.175, 0.5 and 1 μM 6-*m*-AuNP (top to bottom) are shown.

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

Compound	K_i	RIP
αMeMan	20×10^{-4}	1.0
6-*m*-AuNP	8.8×10^{-8}	11.2
s-20-*m*-AuNP	2.3×10^{-9}	127.8
l-20-*m*-AuNP	3.5×10^{-9}	67.5
6-*g*-AuNP	1.6×10^{-7}	/
6-*t*-AuNP	—	—

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 dots[24] and magnetic nanoparticles.

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