Communication

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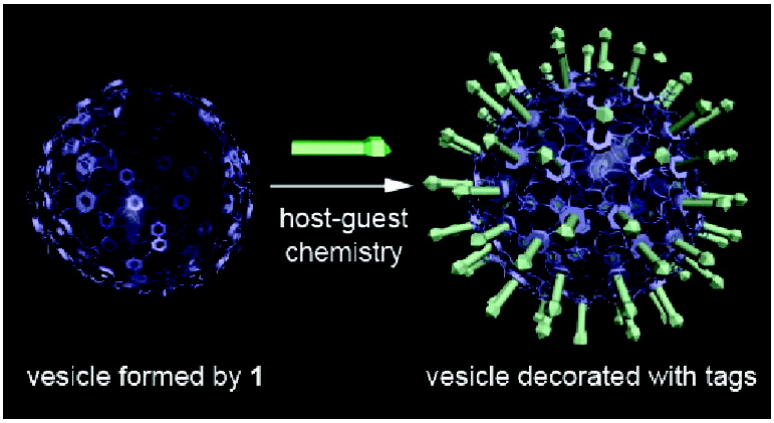
J. Am. Chem. Soc., **2005**, 127 (14), 5006-5007• DOI: 10.1021/ja042172s • Publication Date (Web): 19 March 2005



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Published on Web 03/19/2005

Vesicle Formed by Amphiphilc Cucurbit[6]uril: Versatile, Noncovalent Modification of the Vesicle Surface, and Multivalent Binding of Sugar-Decorated Vesicles to Lectin

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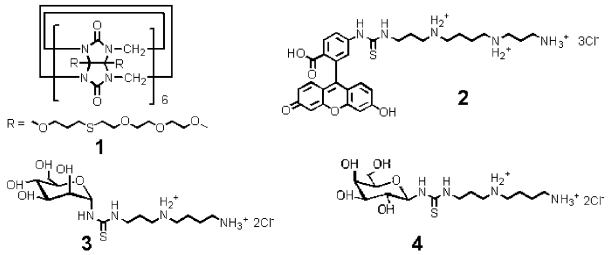
Vesicles have been a subject of intense research[1−4] for the last three decades because of their potential applications in various areas such as the development of biomimetic systems,[2] drug/gene delivery systems,[3] and nanostructured materials.[4] In all such applications, the incorporation of functional moieties on the surface of vesicles has been extremely important, particularly in the design of targeted drug delivery.[3] However, the modification of vesicle surfaces has been mostly achieved by attaching modifier moieties to the vesicle component via covalent bonds, which requires laborious, often low-yield, multistep syntheses. A noncovalent alternative, which would provide a more versatile method for creating vesicles with new properties and functions, receives increasing attention lately.[5]

Cucurbit[*n*]uril (CB[*n*] $n = 5-8$), a family of macrocyclic compounds comprising *n* glycoluril units, has a hydrophobic cavity that is accessible through two identical carbony-fringed portals.[6] Supramolecular chemistry of CB[*n*], including their host−guest chemistry, has been studied extensively by Mock,[6a] us,[6b−d,7] and others.[8] In particular, CB[6] and its derivatives have been found to form exceptionally stable host−guest complexes with polyamines in aqueous solution (typically $K > 10^5$ M^{-1}).[6] Recently, we have developed a direct functionalization method of CB[*n*][9] that allows us to synthesize a wide variety of derivatives and to explore their applications.[9,10] Herein we report a new amphiphilic CB[6] derivative that forms a vesicle, the surface of which can be easily modified through host−guest interactions by taking advantage of molecular cavities, readily accessible at the vesicle surface, and their strong affinity toward polyamines. We also demonstrate that the surface-modified vesicle can bind to a specific protein in a multivalent manner.

Chart 1



Amphiphilic CB[6] derivative **1** (Chart 1) was synthesized by reaction between $(allyloxy)_{12}$CB[6][9] and 2-[2-(2-methoxyethoxy)-ethoxy]-ethanethiol (see Supporting Information). Vesicles were prepared by adding water to a film of **1** and sonicating the mixture for 30 min. In the concentration range of 10^{-3} to 10^{-4} M, spherical vesicles of ∼30−1000 nm diameter were observed by TEM. The high-resolution TEM image in Figure 1a shows hollow spheres with a diameter of 170 ± 50 nm and a membrane thickness of 6 ± 1 nm. Similar to conventional lipid molecules, monodisperse vesicle can be obtained by repeated extrusion through syringe filters with a defined pore size. Light scattering studies on a monodisperse vesicle sample of **1** revealed that the radius of gyration ($R_g = 54.7$ nm) and hydrodynamic radius ($R_h = 50.9$ nm) are almost identical (ρ (R_g/R_h) = 1.06), which is characteristic of vesicles.[11] The confocal microscope images of vesicles that had been prepared in sulforhodamine G solution and purified by size-exclusion chromatography using Sephadex G-50 showed bright fluorescent signals corresponding to the entrapped dye molecules (see Supporting Information). Taken together, these experiments confirmed that amphiphilic CB[6] **1** forms vesicles though it does not belong to a common class of vesicle-forming lipids.[5,12] At the moment, it is not clear how **1** forms vesicles, and the mechanism remains under investigation.[13]

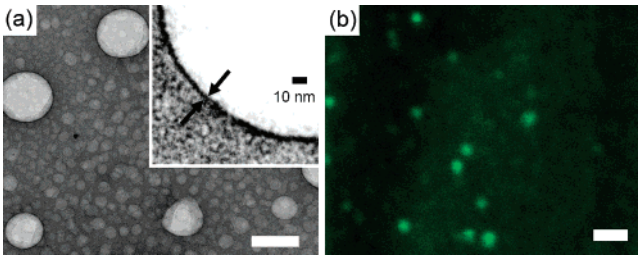


Figure 1. (a) High-resolution TEM image of vesicle **1** (0.4 mM), which was obtained by extrusion through a membrane with 200-nm pores (scale bar = 200 nm). The small grains in the background are due to the staining agent uranyl acetate. The inset shows the membrane thickness, indicated by arrows (scale bar = 10 nm). (b) Confocal microscope image of vesicle **1** (0.4 mM), the surface of which is decorated with **2** (scale bar = 2 μm).

Since CB[6] is known to form stable host−guest complexes with polyamines such as spermidine ($K > 10^6$ M^{-1}) or spermine ($K > 10^7$ M^{-1}),[6a] we anticipated that the surface of the vesicle can be easily modified using host−guest chemistry, that is, noncovalent interactions between the accessible CB[6] derivative in the vesicle membrane and polyamine derivatives. Treatment of vesicle **1** with FITC (fluorescein isothiocyanate)−spermine conjugate ligand **2** (Chart 1),[9] in which spermine serves as a binding motif to CB[6] and FITC as a fluorescent tag, followed by purification by size-exclusion chromatography using Sephadex G-50 produced surface-modified vesicle **1b**. The signals of the spermine part, now located inside the CB[6] cavity in **1b**, were shifted to higher field in ^{1}H NMR spectroscopy. In addition, green fluorescent spheres were

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10.1021/ja042172s CCC: $30.25

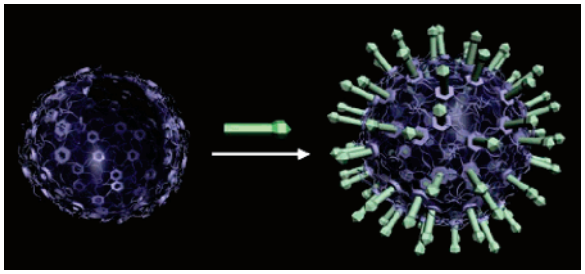

Figure 2. Pictorial illustration of the facile surface modification of the vesicle through host−guest chemistry.

observed under a confocal microscope (Figure 1b), which confirmed the accessibility of the host molecule in the vesicle membrane toward the spermine-based ligand that resulted in a facile noncovalent modification of the vesicle surface with the fluorescent tag.

This result provides us with a new noncovalent, modular approach to the modification of vesicle surfaces. By treating the vesicle derived from the amphiphilic CB[6] with a tag-attached polyamine, we can easily decorate the surface of the vesicle with the specific tag (Figure 2). Because there are many accessible CB-[6] molecules in the vesicle membrane, numerous tag moieties can be easily introduced on the surface of the vesicle, which can interact with specific receptors in a multivalent manner.

To demonstrate the multivalent interactions between the surface-modified vesicles and receptors, we synthesized sugar-decorated vesicles and investigated their interactions with concanavalin A (ConA),[14] a lectin with specificity toward α-mannose. Thiourea-linked α-mannose−spermidine conjugate **3** (Chart 1) was incorporated onto the surface of the vesicle **1** as described above. When the vesicle decorated with **3** was mixed with a solution of ConA, aggregation occurred immediately.[15] In contrast, neither free ligand **3** nor the vesicle decorated with **4**, a galactose−spermidine conjugate (Chart 1), formed aggregates with ConA. This observation illustrates the specific and multivalent interactions between the mannose-decorated vesicle and ConA, which does not interact with the galactose-decorated vesicle or free ligand **3** strongly enough to form aggregates.

The binding affinities of the sugar-decorated vesicles and **3** to ConA were quantified by surface plasmon resonance (SPR) experiments using a ConA immobilized SPR chip.[16] The binding constant of the vesicle decorated with **3** to ConA was measured to be $\sim 3 \times 10^4$ M^{-1}, which is almost 3 orders of magnitude higher than that of free ligand **3** to ConA (~ 50 M^{-1}) (see Supporting Information). The binding constant of the vesicle coated with **4** to ConA, on the other hand, was too small to be measured.

In conclusion, we have demonstrated the formation of a novel vesicle from amphiphilic CB[6], which possesses several remarkable features. Most notable is its ability for easy modification of its surface by host−guest chemistry. This is possible through the exceptionally high binding affinity of CB[6] toward polyamines in aqueous solution, which ensures the tight binding of tags on the vesicle surface. The multivalency achieved by this modification retains the specificity of the tag but also increases its binding affinity. Furthermore, several different types of tags can be anchored on the vesicle surface at the same time. The ability for facile surface modification suggests many practical applications, including its use in targeted drug delivery and immunization. Further work along this line is in progress.

Acknowledgment. We gratefully acknowledge the Creative Research Initiative Program and International Joint R&D Projects (Korean Ministry of Science and Technology) and the BK 21 Program (Korean Ministry of Education) for support of this work and Professor T. Chang and S. Park for light scattering experiments.

Supporting Information Available: Experimental details for synthesis of **1**, **3**, and **4**, and for TEM, SPR, and dye entrapment experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA042172S