Dynamic multivalent recognition of cyclodextrin vesicles†

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Received (in Cambridge, UK) 27th July 2005, Accepted 7th October 2005
First published as an Advance Article on the web 21st October 2005
DOI: 10.1039/b510540d

Cyclodextrin bilayer vesicles have dynamic membranes that recognize guest molecules through efficient multivalent host–guest interaction reminiscent of multivalent binding of a ligand with receptors in a biological membrane.

Receptor clustering is a powerful tool that cells and bacteria use to tune affinity and select competing ligands on their membrane surface.[1] In chemical terms, this phenomenon is an example of dynamic molecular recognition through multivalent interaction.[2] Here we describe an artificial membrane with embedded receptor molecules that recognizes and binds a suitable ligand *via* multivalent interaction with a small cluster of receptors. Previously, we have reported the preparation of amphiphilic cyclodextrins (CDs) and the corresponding CD bilayer vesicles, which have the ability to recognize and bind specific guests.[3] In this communication we report that a vesicle membrane composed of β-CD host molecules **1b** has specific, multivalent interactions with a dye-labeled, divalent guest **3**. Fluorescence resonance energy transfer (FRET)[4] was used to monitor the complexation of fluorescent guest molecules to the CD vesicles. In vesicles composed of a minority of β-CD host **1b** in a majority of "inert" α-CD **1a**, clustering of β-CD host **1b** leads to efficient multivalent interaction. We use a quantitative model to interpret the clustering of receptors as an increased effective concentration of receptor molecules at the membrane surface.

Fig. 1 shows a schematic illustration of a bilayer vesicle composed of amphiphilic CDs and the structure of amphiphilic CDs **1a** and **1b**. Fig. 1 also shows *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-labeled cholesterol (NBD-Chol) **2**, which is well known as a hydrophobic membrane probe,[5] and *N*-(lissamine-rhodamine B)-labeled divalent adamantyl guest (LRB-Ad_2) **3**.[6] NBD and LRB dyes are well known as a FRET donor–acceptor pair in studies of biological and model membranes.[4]

Using conventional extrusion methods, we prepared unilamellar CD vesicles (diameter *ca.* 160 nm) containing 1 mol% NBD-Chol **2**. The formation of bilayer vesicles from amphiphilic CDs **1a** and **1b** in water was observed by transmission electron microscopy and dynamic light scattering consistent with previous reports.[3]

Fig. 2 shows the steady-state fluorescence changes upon adding guest LRB-Ad_2 **3** (dissolved in 5 mM phosphate buffer, pH 7.5, 0.5% DMSO) to the host vesicle solution (10 μM CD **1b** in 5 mM phosphate buffer and pH 7.5) containing 1 mol% NBD-Chol **2**. The fluorescence intensity of donor **2** (λ_{ex} = 450 nm, λ_{em} = 530 nm)

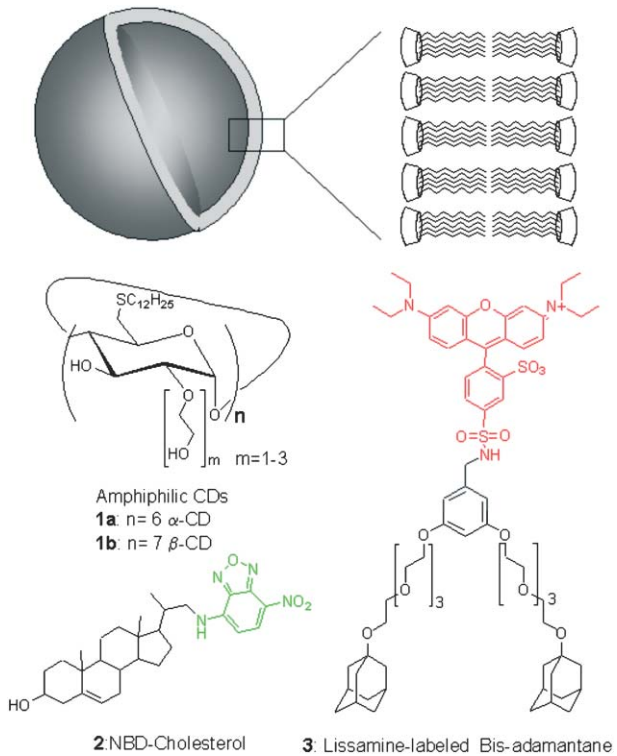

Fig. 1 Bilayer vesicle composed of amphiphilic CDs and molecular structure of amphiphilic CDs **1a** and **1b**, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-labeled cholesterol (NBD-Chol) **2** and *N*-(lissamine-rhodamine B)-labeled divalent adamantane guest (LRB-Ad_2) **3**.

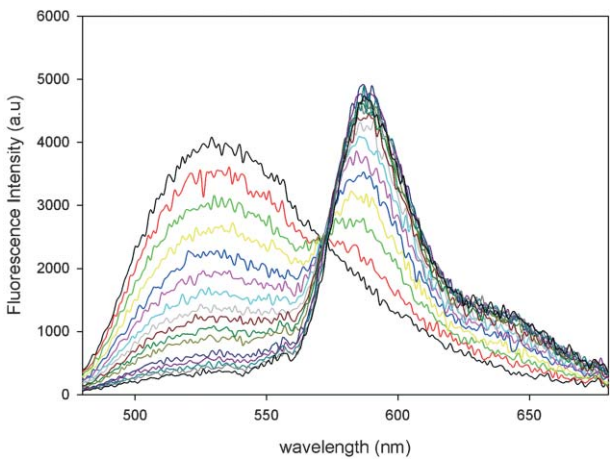

Fig. 2 Fluorescence emission spectra (λ_{ex} = 450 nm) of vesicles of β-CD **1b** (10 μM) containing 1 mol% NBD-Chol **2** (0.1 μM) upon adding divalent guest LRB-Ad_2 **3**. [**3**] = 0–0.9 μM. All measurements were carried out in 5 mM phosphate buffer at pH = 7.5 and T = 25 °C.

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† Electronic supplementary information (ESI) available: control experiments and calculation of the effective concentration C_{eff} of CD in mixed vesicles. See DOI: 10.1039/b510540d

decreased while the intensity of acceptor **3** (λ_{em} = 585 nm) increased upon addition of **3**. The emission of **2** was nearly completely quenched upon addition of < 1 μM guest **3**, indicating that efficient FRET occurred as a result of the interaction between guest **3** and β-CD host **1b**, which brings acceptor **3** in close proximity to donor **2**. The Förster distance (R_0) for NBD and LRB ranges from 50 to 70 Å.[4] According to the dimensions of the CD bilayer provided by X-ray diffraction data and Langmuir Blodgett isotherms,[3] acceptor guest molecule **3** and donor membrane probe **2** will approach within the Förster distance upon complexation of **3** at the surface of vesicles of CD host **1b** containing **2**. Since the concentration of acceptor **3** was very low ($[\mathbf{3}]_{max}$ = 0.9 μM), inner filter effects and collisional quenching will be negligible. Note also that in all experiments the concentration of guest **3** was much lower than host **1b**.

To visualize the interaction between guest **3** and vesicles of host **1b**, confocal microscopy images of CD vesicles (10 μM) containing 1 mol% NBD-Chol **2** (0.1 μM) in the absence and presence of guest **3** were collected on a Carl Zeiss LSM510 microscope (Fig. 3). Fluorescence images were obtained by recording the red emission (above 560 nm) and the green emission (between 500 nm and 550 nm), while exciting at 458 nm. Hydrophobic probe **2** is exclusively localized in the CD vesicles (see ESI for additional evidence). In the absence of guest **3**, the vesicles are observed in both emission channels as a result of the broad emission spectrum of NBD-Chol **2**. However, when 1 μM guest **3** was added to the NBD-Chol **2** containing vesicle solution (10 μM), fluorescent vesicles were observed only in the red emission channel, indicating that the green fluorescence of **2** embedded in the vesicles was quenched *via* a FRET mechanism induced by binding guest **3** to β-CD **1b** on the surface of the vesicles. The intense red fluorescence observed results from acceptor **3**.

In a negative control experiment, LRB without a divalent adamantane anchor was added to vesicles containing donor **2**. Emission spectra showed that there was no significant FRET between **2** in the β-CD vesicle and LRB in bulk solution (see ESI).

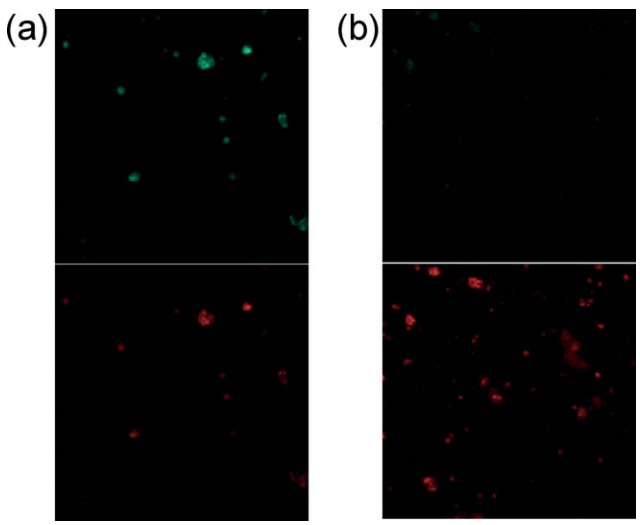

Fig. 3 Confocal microscopy images of vesicles of β-CD **1b**. Scale: 207 × 207 μm for each window. (a) Vesicles of β-CD **1b** containing NBD-Chol **2**. (b) LRB-Ad_2 **3** bound to vesicles of β-CD **1b** containing NBD-Chol **2**. Top: λ_{em} = 500–550 nm. Bottom: λ_{em} > 560 nm. See text for details.

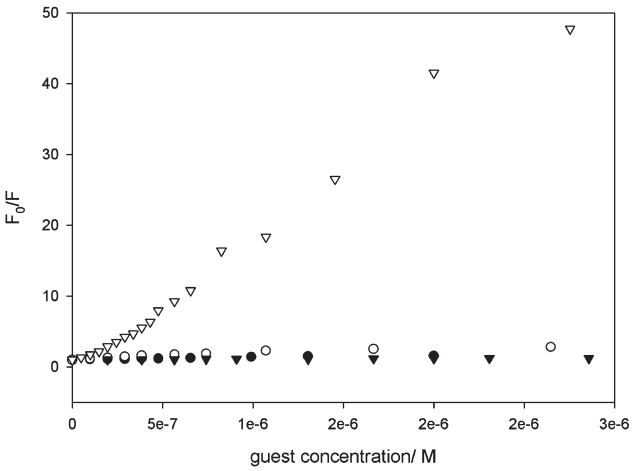

Fig. 4 NBD-Chol **2** fluorescence intensity (F_0/F) *versus* guest concentration: (∇) Vesicles of β-CD **1b** and LRB-Ad_2 **3**. Negative controls: (▼) Vesicles of β-CD **1b** and LRB; (●) Vesicles of $C_{12}EO_3/C_{14}EO_3$ and LRB-Ad_2 **3**; (○) Vesicles of α-CD **1a** and LRB-Ad_2 **3**. [NBD-Chol **2**] = 0.1 μM and [**1a**] = [**1b**] = 10 μM. [$C_{12}EO_3/C_{14}EO_3$] = 100 μM. All measurements were carried out in 5 mM phosphate buffer at pH = 7.5 and T = 25 °C.

LRB-Ad_2 **3** was also added to vesicles of α-CD **1a** and to non-CD vesicles[3] composed of *n*-dodecyl triethyleneglycol ($C_{12}EO_3$, 90%) and *n*-tetradecyl triethyleneglycol ($C_{14}EO_3$, 10%), both containing NBD-Chol **2** (0.1 μM). Neither the vesicles of α-CD **1a** (which is too small to fit adamantane) nor the non-CD vesicles (which do not contain host molecules) show any change of fluorescence of NBD-Chol **2** upon addition of divalent guest **3**. The Stern–Volmer plot (Fig. 4) demonstrates that the recognition of guest **3** by the vesicles is specifically mediated by the host–guest complexation of adamantane guest **3** and CD host **1b** at the vesicle surface.

The slope of the Stern–Volmer plot can be employed to estimate the apparent association constant $K_a = 1.5 \times 10^7$ M^{-1} between host **1b** and divalent guest **3**.‡ The magnitude of this association constant is diagnostic for a divalent interaction of one guest molecule with two host molecules. The efficient divalent interaction of guest **3** with host **1b** is characterised by the equilibrium constant $K_{a2} = C_{eff} \times K_{a1}^2$ where K_{a1} is the monovalent association constant and C_{eff} is the effective concentration of host molecules **1b** at the surface of the CD vesicle.[7] C_{eff} reflects the high number of β-CD hosts on the membrane surface accessible to the second adamantyl group, after the first adamantyl group of guest **3** binds to the vesicle. C_{eff} was calculated from a straightforward geometrical model taking into account the experimental molecular surface area of the CD host molecule **1b**[3] and the distance between the two adamantyl groups at guest **3**[7] (see ESI). According to this model, C_{eff} = 0.17 M, which is consistent with the value of C_{eff} of CD host molecules at the surface of densely packed CD monolayers on gold and glass.[6,7] Assuming that the divalent interaction K_{a2} is the result of two equal and independent monovalent interactions K_{a1}, it follows that $K_{a1} = (K_{a2}/C_{eff})^{0.5} = 9.4 \times 10^3$ M^{-1}, which is consistent with the experimental value for the monovalent interaction of **1b** with adamantane carboxylate ($K_{a1} = 7.0 \times 10^3$ M^{-1}).[3] We conclude that the recognition of guest **3** by host **1b** is amplified by the formation of a divalent host–guest complex at the membrane surface.§

Table 1 Apparent association constant (K_a) and effective concentration (C_{eff}) of CD experienced by guest LRB-Ad_2 **3** binding to vesicles of β-CD **1b** mixed with α-CD **1a**

Vesicle composition[a]	100	70	50	30	10
K_a (M^{-1})	1.5×10^7	1.2×10^7	1.2×10^7	1.1×10^7	3.5×10^6
C_{eff} (M)[b]	0.17	0.14	0.14	0.12	0.04
C_{eff} (M)[c]	0.17	0.11	0.08	0.04	0.002

[a] Percentage of β-CD **1b** in mixed vesicles with α-CD **1a**. [b] Effective concentration C_{eff} derived from K_a. Error ±10%. See text for details. [c] Calculated effective concentration C_{eff} for a statistical mixture of β-CD **1b** and α-CD **1a**. Error ±10%. See ESI for details.

Furthermore, we measured the apparent association constant K_a of guest **3** with CD vesicles composed of mixtures of "good" host CD **1b** and "inert" CD **1a** (Table 1). CDs **1a** and **1b** differ in ring size only and can be readily mixed in all proportions. Since the alkyl chains of **1a** and **1b** are relatively short (C_{12}), the bilayer membranes are in a liquid crystalline-like L_α phase with rapid lateral diffusion of the cyclodextrins on the vesicle surface. We therefore assume that the vesicle surface is a dynamic, homogeneous, two-dimensional solution with fractions of **1a** and **1b** directly proportional to their ratio in the mixture and a random distribution of **1a** and **1b** across the vesicle surface.

Although K_a decreases with the mole percentage of **1b**, K_a is still $> 1 \times 10^6$ M^{-1} at a low percentage of **1b**, indicative of divalent rather than monovalent interaction. Table 1 lists the value of C_{eff} calculated from $K_a = K_{a2}$ and $K_{a1} = 9.4 \times 10^3$ M^{-1} according to $C_{eff} = K_{a2}/(K_{a1})^2$. Finally, Table 1 lists the values of C_{eff} calculated from the experimental molecular surface area of CDs **1a** and **1b**[3] and the distance between the two adamantane groups at guest **3** (see ESI). The values of C_{eff} as a function of the percentage of **1b** in the CD vesicles are also shown in Fig. 5. It can be seen from Fig. 5 that C_{eff} as calculated from the apparent binding constant K_a deviates *strongly and positively* from C_{eff} as calculated for a statistical mixture of **1a** and **1b**. In other words, the effective concentration C_{eff} of host **1b** in a mixture with inert **1a** experienced by a suitable divalent guest molecule is much higher than expected for a random, statistical mixture of **1a** and **1b**. A plausible and appealing explanation for the high C_{eff} of **1b** is that divalent guest **3** induces clustering of **1b** in an excess of **1a**.[8] The small entropy cost of clustering will be easily offset by the large free energy gain of forming a divalent inclusion complex. However, we can not rule out (neither *a priori* nor by experiment) that some cluster formation occurs in the mixed CD vesicles even in the absence of divalent guest **3**.

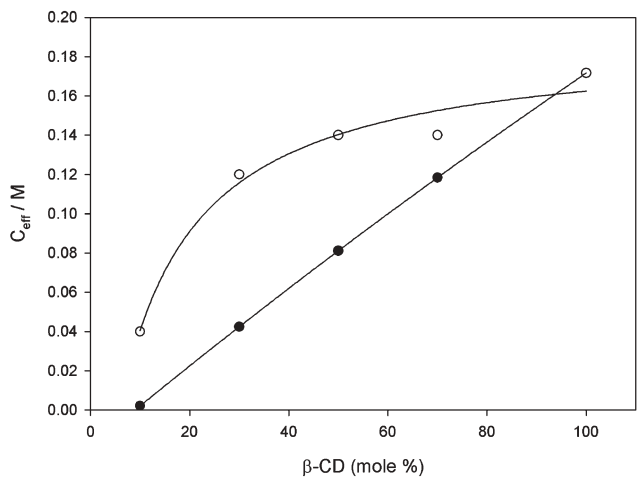

Fig. 5 Experimental (○) and calculated (●) effective concentration (C_{eff}) of β-CD **1b** experienced by guest **3** *versus* percentage of β-CD **1b** in vesicles composed of mixtures of CDs **1a** and **1b**. See text for details.

In conclusion, we have demonstrated dynamic multivalent molecular recognition in an artificial membrane, reminiscent of the way that cells and bacteria select ligands on their membrane surface.

We thank Alart Mulder for the synthesis of **3** and Jurriaan Huskens for helpful discussions.

Notes and references

‡ The efficient divalent interaction of LRB-Ad_2 **3** with the CD vesicles is in marked contrast to inclusion interaction of polyanionic guest polymers with the CD vesicles.[3] These guest polymers bind to the vesicles in a brush- or mushroom-type conformation with a $K_a = 2 \times 10^6$ M^{-1} at most. It is likely that the oligo(ethylene glycol) residues protruding from the surface of the vesicles prevent optimal multivalent interaction with the guest polymers. This type of steric repulsion is well known for colloids and surfaces decorated with poly(ethylene glycol). Also there is a degree of electrostatic repulsion between the polyanion and the vesicles, which have a negative surface potential.[3]

§ The apparent association constant K_a is determined from the equation: $F_0/F = 1 + K_a[\mathbf{3}]$, where F and F_0 are the fluorescence intensity of donor NBD-Chol **2** in the presence and the absence of acceptor LRB-Ad_2 **3** and [**3**] is the concentration of **3**.

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