Polymer Recognition by Vesicles

Molecular Recognition of Polymers by Cyclodextrin Vesicles**

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Dedicated to Professor H. Ringsdorf

In biology, processes such as cell–cell recognition and the initiation of signal transduction depend on the formation of multiple noncovalent complexes between substrates or ligands and membrane-bound receptors. Multiple, instead of isolated, host–guest interactions enhance binding affinity as well as selectivity.[1] Here we have investigated the role of multiple noncovalent interactions in molecular recognition at a membrane surface by using a model system consisting of bilayer vesicles of hydrophobically modified cyclodextrins[2] (CDs; the membrane receptors) in combination with monomer and polymer guest molecules[3] (the substrates). Molecular recognition and multiple host–guest interactions have been described for CD dimers[4] (including CD dimers in the presence of liposomes[5]) and polymers[3,6] as well as CD micelles[7] and self-assembled CD monolayers.[8] The anchoring of hydrophobically modified polymers into liposomes has also been studied.[9] We demonstrate that poly(isobutene-*alt*-maleic acid) substituted with hydrophobic *p-tert*-butylphenyl groups binds very strongly and selectively to the surface of CD bilayer vesicles, without affecting the integrity of the vesicles. No interaction is observed when the membrane does not contain host molecules or when the polymer does not contain hydrophobic guest substituents.

The CD vesicles used in this study were composed of **1**, which is β-CD substituted with S-dodecyl groups on the primary side and oligo(ethylene glycol) groups on the

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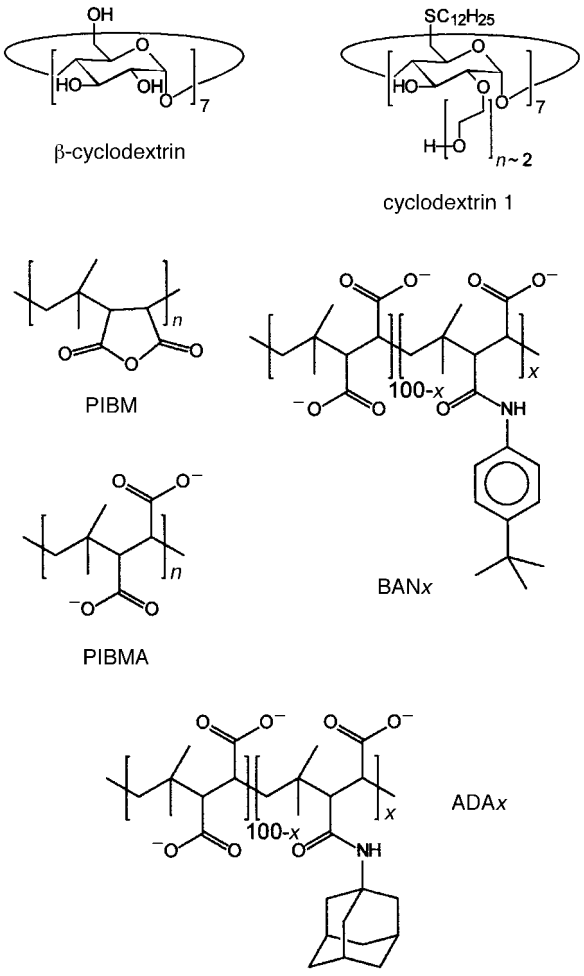
[**] B.J.R. acknowledges a Schering-Plough Co. Newman Scholarship at UCD.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

  DOI: 10.1002/ange.200350923

secondary side (Scheme 1).[10] The formation of bilayer vesicles from this CD was described previously.[2] Cyclodextrin vesicles consist of bilayers of cyclodextrins (in which the hydrophobic "tails" are directed inwards and hydrophilic macrocycle "head groups" are facing water) enclosing an



Scheme 1. Chemical structures of CDs and guest polymers (x = molar percentage of substitution). The molecular weight of each guest-substituted polymer repeat unit (namely, equivalents of hydrophobic guest) was taken from ref. [3b].

aqueous interior. In this work, we used unilamellar vesicles prepared from multilamellar vesicles by repeated extrusion through a polycarbonate membrane (pore size 0.1 μm) which according to dynamic light scattering (DLS) studies have an average hydrodynamic diameter of approximately 160 nm.[11] The guest polymers BAN and ADA (Scheme 1) were prepared from poly(isobutene-*alt*-maleic anhydride) (PIBM) of $M_w = 60\ \mathrm{kg\,mol^{-1}}$ (namely, all the polymers have the same chain length) by amidation with *p-tert*-butylaniline or adamantanamine, respectively, followed by hydrolysis of the remaining anhydride groups.[3] Poly(isobutene-*alt*-maleic acid) (PIBMA) was obtained by hydrolysis of PIBM. Both the *p-tert*-butylphenyl and the adamantyl group are known to form stable inclusion complexes with β-CD in water. The binding constant $K_a = 1.0\text{–}4.0 \times 10^4\ \mathrm{M^{-1}}$, depends on the nature of the hydrophobic derivative, the substitution on the CD, and the ionic strength of the medium.[12] By using isothermal titration microcalorimetry (ITC)[3] and affinity capillary electrophoresis[13] we found that the K_a values for β-CD with the *p-tert*-butylphenyl groups on the polymers is of the same order of magnitude as that for *p-tert*-butylbenzoate, but that the K_a values are almost one order of magnitude lower for adamantyl-containing polymers than for adamantanecarboxylate. We demonstrated that binding of the polymers to β-CD leads to a transition of the polymer from a compact globular unimer to an extended random coil, which is energetically more costly for ADA than for BAN polymers.[13] The results of these studies are summarized in Table 1.

Table 1: Apparent binding constants determined by capillary electrophoresis for the complexation of polymer guests, *p-tert*-butylbenzoate and adamantanecarboxylate, to β-CD and to vesicles of **1**.

Guest	β-CD K_a [10^3 L mol^{-1}]	Vesicles of **1** K_a [10^3 L mol^{-1}]
BAN09	39 ± 3	2200 ± 400
BAN42	28 ± 1	450 ± 60
p-tert-butylbenzoate	12.7 ± 0.3	6 ± 1
ADA10	3.5 ± 0.9	2.4 ± 0.5
ADA20	2.0 ± 0.3	20 ± 2
adamantanecarboxylate	n.d.[a]	4 ± 1

[a] Not determined.

Dynamic light scattering was used to study the interaction of vesicles of **1** and liposomes of egg phosphatidyl choline (egg PC)[11] with BAN, ADA, and PIBMA polymers in dilute aqueous solution (Figure 1). An increase in the average hydrodynamic diameter of the vesicles was observed upon addition of BAN42 to vesicles of **1** (Figure 1a). If more than approximately one equivalent of polymer (by weight) was added, no further increase of diameter was observed. Overall, the diameter of the vesicles increased from about 160 nm to about 240 nm. No such increase was observed if PIBMA instead of BAN42 was added.[14] Also, when BAN42, ADA20, or PIBMA were added to liposomes of egg PC, the average liposome diameter was constant, even when more than seven equivalents of polymer (by weight) were added. Figure 1b shows the average diameter of the vesicles of **1** in the presence of each polymer as a function of the molar ratio of the polymer-bound guest to accessible CD. We assume that approximately 50% of the CD molecules reside on the inner bilayer surface and are inaccessible to the polymer. The ratio of guest-bound polymer to accessible CD required for saturation is much higher than 1:1 (see below).

These results indicate that the polymer guests, but not PIBMA, interact with vesicles of **1**, but not with egg PC liposomes. We propose that the *p-tert*-butylphenyl and the adamantyl groups, respectively, on the BAN and ADA polymers form inclusion complexes with the CD cavities on the surface of the vesicles of **1**, thus leading to a coating of the vesicles by the polymer guest. Since no interaction with the egg PC liposomes is observed, random hydrophobic anchoring[9] instead of inclusion of the *p-tert*-butylphenyl and the adamantyl groups into the bilayer must be negligibly weak.

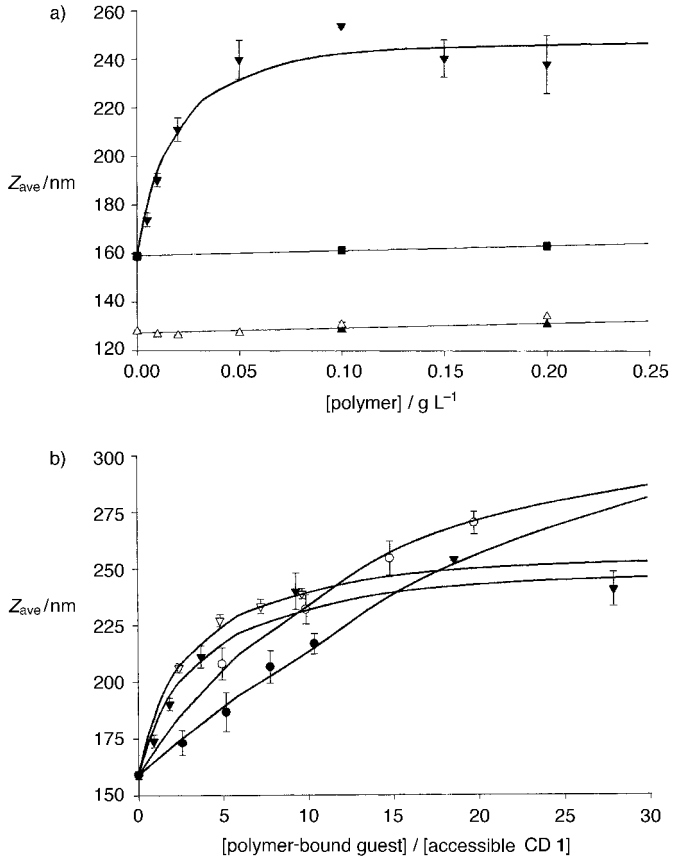


Figure 1. Average hydrodynamic diameter Z_{ave} of vesicles of **1** and egg PC liposomes in the presence of BAN, ADA, and PIBMA polymers. [**1**] = 0.05 g L^{-1} = 17 μM and [egg PC] = 0.05 g L^{-1}. a) Plot of the vesicle (liposome) diameter as a function of polymer concentration; ▼: **1** with BAN42, ■: **1** with PIBMA, △: egg PC with BAN42, ▲: egg PC with PIBMA. b) Plot of the vesicle diameter as a function of the [polymer bound guest]/[accessible CD **1**] molar ratio. ▽: BAN09, ▼: BAN42, ○: ADA20, and ●: ADA10.

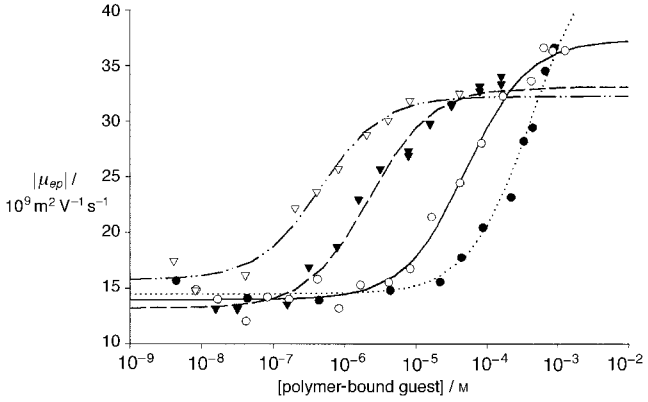


Figure 2. Absolute electrophoretic mobility μ_{ep} of vesicles of **1** as a function of the concentration of polymer-bound guests. ▽: BAN09, ▼: BAN42, ○: ADA20, and ●: ADA10. Note that the measured electrophoretic mobility is negative.

Since no interaction of PIBMA with either the vesicles of **1** or the egg PC liposomes is observed, surface absorption of the polymers can also be excluded. Furthermore, any effects relating to osmotic shock, depletion interaction, aggregation, or solubilization of either the CD vesicles or the liposomes can be excluded at these concentrations.

Affinity capillary electrophoresis (CE) was used to obtain quantitative information about the binding of guest polymers to the vesicles of **1** in dilute aqueous solution. The use of this technique to study the formation of host–guest complexes between β-CD and the polymer guests has been described in detail previously.[13] Here, the electrophoretic mobility of the vesicles of **1** was measured in the presence of increasing concentration of guest polymers in the background electrolyte (Figure 2). The increase of electrophoretic mobility of the vesicle in the presence of increasing concentration of polymer guest was analyzed in terms of the formation of a 1:1 inclusion complex between **1** and the substituent on the guest polymer and characterized by the apparent binding constant K_a. The results are summarized in Table 1.[15]

The binding constants collected in Table 1 allow for a number of interesting comparisons to be made regarding hydrophobic host–guest interactions between independent host and guest molecules in solution; between multiple, membrane-bound hosts and single guests; between single hosts and polymer guests; and finally between multiple, membrane-bound hosts and polymer guests. First of all, the interaction of both *p-tert*-butylbenzoate and adamantanecarboxylate is stronger with β-CD ($K_a = 10–30 \times 10^3\,M^{-1}$) than with vesicles of **1** ($K_a = 4–6 \times 10^3\,M^{-1}$). The difference might be attributed to some hindrance to inclusion into the cavity of **1** arising from the presence of hydrophilic oligo(ethylene glycol) residues or a degree of anticooperativity arising from the increasing presence of anionic guests on the vesicle surface. However, a Scatchard plot constructed in the case of *p-tert*-butylbenzoate had a linear slope and an abscissa intercept of 0.98, thus proving the presence of identical and independent binding sites on the vesicle surface. We can therefore ascribe the inferior binding constant for **1** to some steric hindrance and some reduction in the hydrophobicity of the host because of the presence of oligo(ethylene glycol) residues.

Secondly, the interaction between β-CD and *p-tert*-butylbenzoate and BAN polymers is quite similar ($K_a = 10–40 \times 10^3\,M^{-1}$) but the interaction between β-CD and adamantanecarboxylate ($K_a = 33 \times 10^3\,M^{-1}$)[3] is much stronger than the interaction between β-CD and ADA polymers ($K_a = 2.0–3.5 \times 10^3\,M^{-1}$). As discussed previously,[13] this difference may be attributed to increased intramolecular hydrophobic interaction of the adamantyl groups in the ADA polymers relative to that of the *p-tert*-butylphenyl groups in the BAN polymers, which competes with inclusion in the CD cavity.

Finally, while the interaction between ADA polymers and vesicles of **1** ($K_a = 2–20 \times 10^3\,M^{-1}$) is significantly stronger than the interaction between ADA polymers and β-CD ($K_a = 2.0–3.5 \times 10^3\,M^{-1}$), the interaction between BAN polymers and vesicles of **1** ($K_a = 450–2200 \times 10^3\,M^{-1}$) is almost two orders of magnitude stronger than that between BAN polymers and β-CD ($K_a = 28–39 \times 10^3\,M^{-1}$). Clearly, the presence of multiple, weakly self-associating guest substituents in the BAN poly-

mer and multiple CD cavities on the CD vesicle surface leads to a very high binding affinity. Not all the *p-tert*-butylphenyl groups on the polymer are necessarily involved in bonding to the vesicle surface. Complete binding at the vesicle surface would severely restrict the conformational freedom of the polymer, as well as the lateral diffusion of the CDs in the bilayer, and would impose a very high entropic penalty. Also, inclusion will be in competition with intramolecular association, and only a minority of the *p-tert*-butylphenyl groups will be bound at any given time. This situation may explain why BAN09 binds to the CD vesicles even more efficiently than BAN42: the lower degree of hydrophobic substitution will decrease the tendency for intramolecular association and favor inclusion at the vesicle surface. This view is supported by the DLS experiments (Figure 1b), where one can see that up to ten hydrophobic substituents per accessible CD **1** are required for both BAN polymers to bind the vesicles fully. When the number of hydrophobic units per polymer strand is taken into account (ca. 164 and 35 for BAN42 and BAN09, respectively)[13] the degree of binding of BAN42 and BAN09 per accessible CD cavity is found to be 6 and 29%, respectively. In the case of both ADA polymers, saturation is not obtained even at high polymer concentration (Figures 1b and 2). Both the hydrodynamic diameter and the electrophoretic mobility of fully coated vesicles extrapolate to higher values than in the case of the BAN polymers, which indicates that the bound polymers have a somewhat different structure. As shown by the DLS experiments (Figure 1b) saturation is not obtained even in the presence of 30 hydrophobic substituents per accessible CD **1** for both ADA polymers. When the number of hydrophobic units per polymer strand is taken into account (ca. 78 and 39 for ADA20 and ADA10, respectively)[13] a degree of binding per accessible CD cavity of at least 40 and 80% is found for ADA20 and ADA10, respectively. This result would indicate a departure from a "mushroomlike" conformation towards a more "brushlike" configuration,[16] with the ADA polymer chains more fully extended (Figure 3). This interpretation is corroborated by the CE measurements, which show a significantly higher extrapolated electrophoretic mobility for ADA-bound vesicles than for the BAN-bound vesicles. The apparent increase in electrophoretic mobility despite the increase in the hydrodynamic diameter of the fully bound vesicles indicates a higher surface potential which probably arises from higher polymer coverage. A change in the polymer conformation from a globular mushroom to an extended, rodlike brush for both ADA polymers could be enthalpically stabilized by intermolecular interactions but would be entropically costly, and could explain the striking difference in binding affinity of the vesicles of **1** for the two polymer types.

The following conclusions can be drawn from combining the qualitative results from DLS and the quantitative information from CE. BAN polymers bind very strongly and selectively to the surface of CD bilayer vesicles. No interaction is observed when the membrane is composed of egg PC and does not contain CD host molecules or when the polymer does not contain hydrophobic substituents. Much weaker interactions are observed with ADA polymers because of competition from intramolecular interactions and possible rearrangement of this polymer type to give brushlike covered vesicles. In summary, these findings demonstrate the high affinity molecular recognition of a membrane-bound host by a water-soluble guest through multiple hydrophobic interactions. The thermodynamics of multiple complexation, as well as the kinetics of association and dissociation will be the subject of further study. Ultimately, the relevance of these coated vesicles may rest in their potential as drug-delivery systems.

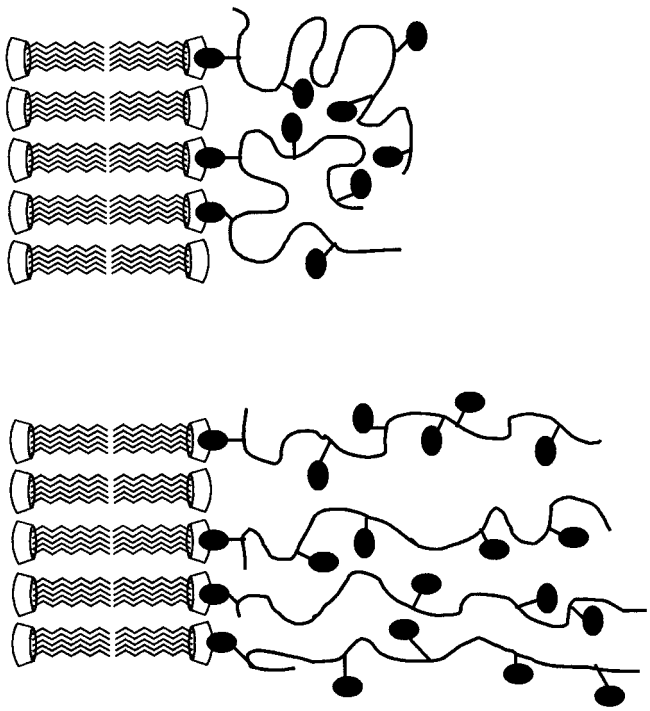


Figure 3. Coating of cyclodextrin bilayer vesicles by hydrophobically modified polyelectrolytes through multiple noncovalent interactions. Top: a BAN-covered bilayer of **1** with a low percentage of **1** bound to the polymer and a mushroomlike polymer coating. Bottom: an ADA-covered bilayer of **1** with a high percentage of **1** bound to the polymer and a brushlike polymer coating.

Received: January 13, 2003 [Z50923]

Keywords: cyclodextrins · host–guest systems · molecular recognition · polymers · vesicles

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