Tetrahedron 73 (2017) 4972–4978

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Dynamic multivalent interaction of phenylboronic acid functionalized dendrimers with vesicles

Tobias Otremba, Bart Jan Ravoo*

Organic Chemistry Institute and Center for Soft Nanoscience, Westfälische Wilhelms-Universtät Münster, Corrensstrasse 40, 48149, Münster, Germany

ARTICLE INFO

Article history:
Received 10 February 2017
Received in revised form
16 March 2017
Accepted 19 April 2017
Available online 20 April 2017

Keywords:
Boronic acid
Cyclodextrin
Dendrimer
Multivalency
Vesicle

ABSTRACT

Over the past decade cyclodextrin vesicles were established as a versatile model for biological membranes since they can be easily modified with functional groups due to the spontaneous formation of host-guest complexes. In this article we report the interaction of phenylboronic acid functionalized polyamidoamine (PAMAM) dendrimers and cyclodextrin vesicles decorated with a catechol-adamantane conjugate to investigate dynamic multivalent recognition processes on membrane surfaces. The orthogonality and the multivalency in the ternary system of host vesicle, guest catechol conjugate and functionalized dendrimers were studied by isothermal titration calorimetry (ITC), dynamic light scattering (DLS) and time-depended measurements of optical density at 400 nm (OD400). It was shown that the aggregation of vesicles is highly dependent on the concentration of boronic acids as well as the generation, i.e. valency, of the dendrimers.

1. Introduction

Multivalent interactions take part in almost all biological recognition processes, for example in cell-cell interactions, the formation of the DNA-double helix and infections of cells by virus and bacteria. The characteristics of multivalent interactions are that these interactions together are much stronger in comparison to the corresponding monovalent interaction. An important biological example of multivalency is the interaction of proteins (called "lectins") and carbohydrates presented on the surfaces of membranes (called "glycocalix"). The association constant K_a for a monovalent interaction between a carbohydrate and a lectin is rather weak (10^3 - 10^4 M^{-1}). Hence most of the lectins have multiple binding sites leading to multivalent interactions and an effective recognition and adhesion of cells.[1,2]

It has been known since the middle of the 20th century that phenylboronic acids can form complexes with 1,2- and 1,3-diols by formation of boronic esters and hence can act as "synthetic lectins" i.e. biomimetic carbohydrate receptors.[3,4] Since then these selective interactions have enabled application of boronic acids in carbohydrate recognition[5–10] and functional materials.[11–13] A highly versatile systems to investigate the recognition of diols by boronic acids was developed by Wang and Springsteen. The dye Alizarin S (ARS) was used in an indicator displacement assay and showed a drastic change in fluorescence intensity and maximum absorbance upon binding to a boronic acid, so that the boronic acid-diol interaction can be analyzed by fluorescence and UV/Vis spectroscopy.[14–16] This assay contributed to the development of many different sensors, such as dendrimer,[17,18] peptide[19,20] and vesicle[21] based systems. Moreover, the functionalization of dendrimers with boronic acid derivatives has led to functional films[22] and magnetic nanoparticles that can be used to sequester glycoproteins.[23] In general, functionalized dendrimers have been frequently used to bind a variety of biomolecules such as heparin,[24] DNA[25] and proteins.[26] Furthermore, dendrimers represent an easily accessible platform to induce multivalent interactions.[27,28]

In recent years the dynamic multivalent recognition on the outer membrane surface of vesicles and liposomes has become an increasingly popular topic in supramolecular chemistry. Synthetic vesicles represent a versatile model for biological membranes.[29] In particular, cyclodextrin vesicles contributed to this area since they can be easily modified with functional groups due to the spontaneous formation of host-guest complexes. In aqueous media, unilamellar cyclodextrin vesicles can be obtained from amphiphilic cyclodextrins functionalized with alkyl chains on the primary face of the cyclodextrin and oligoethylene glycol units on the secondary face.[30] The cyclodextrin cavities on the surface of the vesicles are available for complexation of hydrophobic guests, such as

* Corresponding author.
E-mail address: b.j.ravoo@uni-muenster.de (B.J. Ravoo).

http://dx.doi.org/10.1016/j.tet.2017.04.043
0040-4020/

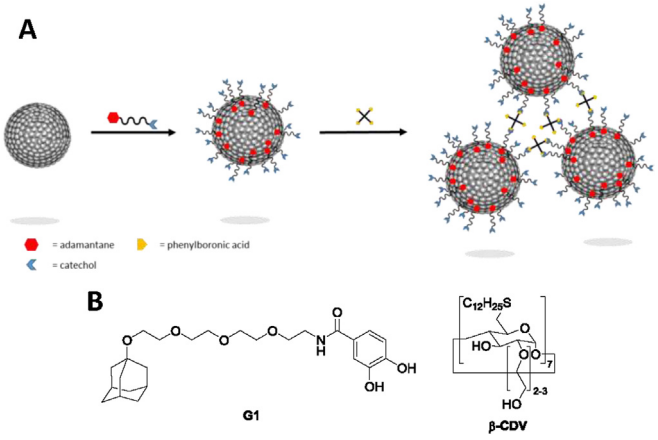
adamantyl and *tert*-butylbenzyl derivatives.[31] In this way, it was possible to investigate the lectin-carbohydrate recognition on the surface of cyclodextrin vesicles[32] and to gain a deeper understanding of multivalent interactions.[33,34] Recently, we reported a self-assembled carbohydrate sensor system based on cyclodextrin vesicles decorated with boronic acids which exhibits a significant improvement in detection sensitivity.[21]

Herein we report the functionalization of polyamidoamine (PAMAM) dendrimers with 2, 4, 8, or 16 boronic acid end groups as well as a reference compound with only one boronic acid function. These molecules can interact with a bifunctional catechol-adamantane conjugate **G1** that binds to amphiphilic β-cyclodextrin with the adamantane group due to host-guest chemistry and to boronic acids with the catechol group via reversible ester formation (Scheme 1). In this way, a model glycocalix was prepared on the membrane surface of amphiphilic cyclodextrin vesicles and the interaction with the dendrimers ("synthetic lectins") was systematically studied. The orthogonal multivalent interactions in this ternary system of host vesicles, guest catechol-adamantane conjugate **G1** and dendrimers were investigated by isothermal titration calorimetry (ITC), dynamic light scattering (DLS) and time dependent measurements of optical density at 400 nm (OD400). In particular, the influence of the generation and hence valency of the dendrimers was evaluated in these measurements.

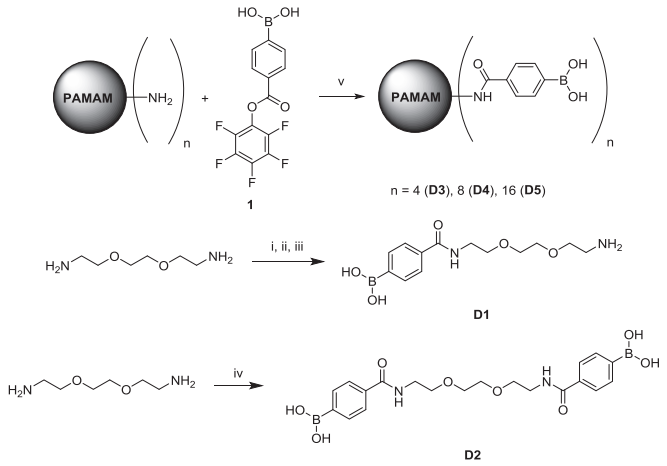
2. Results and discussion

For the synthesis of **D1** and **D2**, 2,2-(ethylenedioxy)bis(ethylamine) was used as a core. **D1** was obtained in a three-step synthesis and amide coupling between the amine function and the activated carbonyl group of boronic acid **1**. In the case of **D2**, both amine groups were functionalized. The dendrimers **D3**, **D4** and **D5** were obtained by functionalization of PAMAM dendrimers of generation 0 (G0), generation 1 (G1) and generation 2 (G2) with phenylboronic acids via amide coupling between **1** and the amine groups of the PAMAM dendrimers (Scheme 2).

The catechol-adamantane conjugate **G1** was synthesized in five steps with an overall yield of 17%. Amphiphilic β-cyclodextrin (β-CD) was synthesized as described[31] and unilamellar bilayer vesicles of amphiphilic β-cyclodextrin (CDV) with a diameter around 100 nm were prepared by extrusion in 20 mM HEPES buffer (pH = 7.4, 75 mM NaCl). Details of the synthesis, purification and analysis of the dendrimers **D1** – **D5** and **G1** can be found in the



Scheme 1. A) Schematic illustration of cyclodextrin vesicle aggregation induced by phenylboronic acid functionalized dendrimers in presence of catechol-adamantane conjugate **G1**; **B)** Molecular structures of catechol-adamantane conjugate **G1** and amphiphilic β-cyclodextrin.



Scheme 2. Synthesis of dendrimers **D1** - **D5**: i) Boc_2O, DCM, 18 h, rt, 99%; ii) 1, DMF, DIPEA, 18 h, rt, 85%; iii) DCM/TFA (1:1), 5 h, rt, quant.; iv) 1, DMF, DIPEA, 18 h, rt, 66%; v) 1, DMF/MeOH, 18 h, rt, **D3**: quant., **D4**: 75%, **D5**: quant..

experimental section as well as in the Supporting Information (SI). The analytical data of **D1** – **D5** and **G1** are consistent with the molecular structures shown in Schemes 1 and 2.

ITC measurements were carried out with **G1**, unmodified β-CD and **D1** to determine the binding constants and thermodynamic parameters of the individual interactions (Fig. 1). The adamantane unit of **G1** is known to be an excellent guest for the host β-CD and a slightly weaker binding guest for amphiphilic β-CD.[31] However, the interaction of β-CD can be used to obtain a reliable approximation of the interaction with CDV. A 10 mM solution of β-CD was titrated to a 1 mM solution of **G1** (Fig. 1A). The thermodynamic parameters as well as the binding constant $K_a = 3 \times 10^4\ M^{-1}$ are characteristic for inclusion complexes of adamantane and β-CD. The enthalpy change is negative, the change in entropy is positive and the stoichiometry of the inclusion complex is close to 1, indicating the formation of a 1:1 complex. The reversible ester formation between the catechol group of **G1** and the boronic acid function of **D1** was studied by titration of a 10 mM solution **D1** to a 1 mM **G1** solution (Fig. 1B). The stoichiometry is close to 1 and indicates the expected 1:1 interaction between the boronic acid and the diol. The interaction exhibits a negative change in enthalpy and positive change in entropy which is comparable to previous studies.[35,36] The calculated binding constant $K_a = 1.2 \times 10^4\ M^{-1}$ is significant higher than in previous studies. This can be attributed to the amide function in **D1**, which lowers the pK_a of the boronic acid and leads to an increase diol affinity. Additionally, the amide function in **G1** has a similar influence on the pK_a of the catechol group and assists the increase in binding affinity. To the best of our knowledge, this is the first time that the interaction of a functionalized phenylboronic acid and a functionalized catechol was studied in detail by ITC. Furthermore, the interaction between dopamine and β-CD was investigated to prove that the catechol moiety cannot form an inclusion complex with β-CD. The results can be found in the SI (Fig. SI1) and show no significant binding of dopamine towards β-CD. Previously it was described that phenylboronic acids exhibit no significant affinity to bind in the cavity of β-CD.[21] The combined binding studies verify that the interactions between the host β-CD, guest **G1** and the dendrimers **D1** – **D5** are highly selective and orthogonal.

To prove our hypothesis that the dendrimers **D2** – **D5** have a similar binding affinity towards **G1** as **D1**, the interaction of the dendrimers **D1** – **D5** with diols was investigated by titration of a 10 mM dopamine solution to a 1 mM solution of the respective