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Cyclization of peptoids by formation of boronate esters

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Abstract

Introduction of conformational constraints into peptoids (N-substituted oligoglycines) will enable new applications in molecular recognition and self-assembly. Peptoids that contain both a phenylboronic acid side chain and a vicinal diol cyclize by intramolecular condensation to form boronate esters. A fluorescent indicator of free boronic acid was used to assay esterification. A galactose moiety 2 to 5 monomer units away from a boronic acid side chain in a peptoid reacts with the boronic acid in competition with the indicator. The intramolecular reaction predominates in each case, with 80–90% of the peptoid cyclized. When the diol is a simple 2,3-dihydroxypropyl group, esterification is less favored but still appreciable.

Keywords

peptoid; boronic acid; peptidomimetic; boronate ester; β -D-galactose

1. Introduction

Peptoids (N-substituted oligoglycines) are being vigorously investigated for their potential as high specificity ligands^{1–3}, biological probes⁴, pharmaceutical agents⁵, and biomimetic materials^{6–8}. These peptidomimetic compounds are easily synthesized^{9,10}, protease resistant¹¹, and cell permeable¹². Furthermore, a wide range of chemical functionality is readily displayed on the peptoid backbone^{9,13–16}, and the synthesis and screening of high diversity peptoid libraries in a variety of formats is facile^{1–3,17}.

A widely appreciated challenge in the design of peptoids is the incorporation of conformational restraints into the molecular architecture¹⁸. Structural elements that restrain the otherwise inherently flexible peptoid scaffold will enhance its ability to present a spatially defined array of chemical functionality, reducing the entropic penalty associated with organizing the peptoid into its active conformation. Several approaches have been investigated for constraining the peptoid backbone. Peptoid sequences rich in bulky chiral side chains have been shown to adopt a stable helical conformation¹⁹, and helical peptoid units have been further constrained into two-helix bundles by incorporation of high-affinity zinc binding sites²⁰. Conformationally restricted cyclic peptoids have been created by macrocyclization using amide coupling chemistry²¹, cycloaddition²², ring-closing metathesis²³, or a triazine bridge²⁴.

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A prominent feature of boronic acids is their reversible formation of esters with diols in aqueous solution²⁵. This reaction suggests a potential structural feature that could be used to encode conformational restraints into a peptoid's monomer sequence: intramolecular esterification of a boronate side chain with a diol side chain will cyclize the peptoid. Therefore, we have investigated the potential for peptoids that bear both boronic acid side chains and groups with vicinal hydroxyls to undergo intramolecular esterification.

2. Results

To investigate the cyclization of peptoids by formation of boronate esters, we synthesized a series of model compounds, **1–11** (Figure 1). Standard methods of peptoid synthesis were employed⁹, and the phenyl boronic acid moiety was incorporated by copper catalyzed 1,3 dipolar cycloaddition²⁶ of 4-azidomethyl phenylboronic acid with a propargyl side chain on the peptoid backbone. To assess the formation of boronate esters, we have monitored free boronic acid concentrations using the indicator Alizirin Red S (ARS)²⁷. Esterification of a boronic acid with ARS changes the absorbance and fluorescence spectra of the indicator, and the dissociation constant for this bimolecular interaction can be derived from a plot of fluorescence vs. log(boronic acid concentration).

Data in this form are shown for the interaction of peptoid boronic acid **1** with ARS in Figure 2. The EC₅₀ derived from this curve, 0.14 mM, is taken as the dissociation constant for the interaction. This value is substantially lower than we observe for the interaction of phenylboronic acid with ARS, $0.57 \pm .04$ mM (data not shown), presumably in large part due to electrostatic interaction of the anionic indicator with the cationic peptoid. For the interaction of ARS with compound **2**, which includes both galactose and boronic acid side chains, the binding curve is shifted to a higher apparent dissociation constant ($K_d=0.90$ mM), indicating that the galactose competes with ARS for esterification with the boronic acid. When the concentration of boronic acid **1** is held constant (1 mM), addition of peptoid **3** up to 15 mM does not diminish ARS fluorescence, indicating that, at the relevant concentrations of peptoid and indicator, the sugar unit must be in the same molecule as the boronic acid to compete effectively with ARS. This result also rules out direct interaction of the carbohydrate moiety with the indicator as the cause of decreased binding.

Two structures that could account for the competing interaction are shown in Figure 3. If the “head-to-tail” dimer structure of Figure 3b were predominant, the fraction of free boronic acid would depend on the total peptoid concentration. In that case, the binding curve for interaction with ARS would be less steep, a smaller fraction of the boronic acid being free to react with the indicator as higher peptoid concentration favored the dimer. This change in the shape of the binding curve is not observed. The Hill slopes of the plots for compounds **1** (0.82 ± 0.10) and **2** (0.87 ± 0.13) are within experimental uncertainty of each other. Furthermore, they are significantly higher than the value predicted (0.62) for a dimer interaction producing the same apparent dissociation constant. Therefore, we conclude that the cyclic structure of Figure 3a accounts for most of the diminished binding to ARS. If the observed increase in dissociation constant between **1** and **2** is entirely due to formation of the monomeric cyclic species, it corresponds to an equilibrium constant for cyclization of 5.4, with 84% of the peptoid cyclized.

The binding parameters for the series of compounds in Figure 1 are listed in Table 1. Each of the compounds with the galactose side chain and boronic acid in the same molecule (**2**, **4–6**) has a higher dissociation constant for interaction with ARS than compound **1**. Of these compounds, only **4** has a Hill slope for its binding curve with ARS less than that for **1**, and in that case, the difference is not statistically significant ($P>.05$). Therefore, we conclude that each of these compounds forms an intramolecular boronate ester. The extent of this

cyclization does not depend on the number of peptoid monomer units between the boronic acid and the galactose for spacings of one to four residues. In each case, 80–90% of the molecules are cyclized under the conditions of the experiment.

The effect on association with ARS is smaller, but still significant, for the compounds listed in Table 1 that include an acyclic diol and boronic acid (**7**, **9–11**). Compound **8** does not affect association of **1** with ARS, confirming that the diol must be in the same molecule as the boronic acid to diminish complexation with ARS. The differences between the Hill slopes for these compounds and **1** are not statistically significant. However, the extent to which the slope is decreased when a bimolecular interaction competes for association with the indicator depends upon the extent of the competition. When the effect on the EC₅₀ of the binding curve is small, the anticipated effect on the slope is also small, because most of the boronic acid is unaffected by the competing interaction. For the observed shift in apparent binding constant, a decrease of approximately 5% in the slope is expected from dimerization, a decrease smaller than the limits of precision of our measurement. Thus, we cannot rule out substantial bimolecular, head-to-tail esterification for these compounds. However, comparison to the galactose-containing peptoids suggests that these compounds also cyclize as monomers. As with the galactose-containing compounds, the extent of condensation does not depend on the spacing between the boronic acid and the diol, with 40–50% of the boronic acid esterified in each case.

3. Discussion

The ARS competition system for assaying boronate esterification was developed by Springsteen and Wang to overcome well-known challenges in assessing the reaction of boronic acids with non-fluorescent diols²⁸. For example, direct physical observation by boron NMR is prohibited, because the boron linewidth is large compared to the chemical shift difference between boronic acids and their esters²⁹. Direct analysis of peptoid conformation in solution by NMR is also difficult, because the proton NMR spectra of peptoids are complicated by heterogeneity of backbone torsion angles that exchange slowly on the NMR time scale³⁰. Fortunately, ARS competition has proved effective for assaying esterification of boronate-bearing peptoids by their diminished propensity to condense with the indicator.

The observed requirement for the diol moiety to be in the same molecule as the boronic acid, affording an entropic benefit to the esterification reaction, is consistent with previously measured bimolecular binding constants for galactose and propylene glycol with phenylboronic acid. The dissociation constant for galactose and phenylboronic acid under comparable conditions is 67 mM²⁸, well above the concentrations relevant to our experiments.

The less effective esterification of the 1,2-dihydroxypropyl side chain compared to the galactose side chain is consistent with the intrinsic relative stabilities of these boronate esters. The association constant of free propylene glycol with phenylboronic acid is 70-fold lower than that of galactose²⁵. Though the resulting smaller shift in the binding curve of compounds **7**, **9**, **10**, and **11** with ARS precludes distinguishing between the head-to-tail dimer and the cyclized monomer as the products of esterification, the balance of thermodynamic factors that favors intramolecular cyclization for **2**, **4**, **5**, and **6** is likely to favor the same process for the lower affinity diol. The dependence of the extent of esterification on the intrinsic propensity of the diol to form an ester with phenylboronic acid suggests that the extent of cyclization can be tuned as desired by the choice of diol incorporated as a peptoid submonomer. The reversibility of the reaction is a unique, and potentially useful, aspect of the boronate ester as a linkage to constrain peptoids.

The insensitivity of the extent of cyclization to spacing between the diol and boronic acid is at least partly attributable to the inherent flexibility of the peptoid backbone. If the backbone were rigid or conformationally constrained prior to boronate ester formation, condensation would be favored for some spacings of boronic acid and diol and disfavored for others. From the standpoint of molecular design, a potential benefit of the observed flexibility is that a range of macrocycle sizes can be created with near equal efficiency. An important application of the peptoid scaffold is in the creation of molecular libraries by combinatorial synthesis. Because cyclization occurs spontaneously in aqueous solution at positions determined by the peptoid sequence (i.e., the positions of diol and boronic acid), the boronic ester crosslink, its presence and its position, can serve as a diversifiable element of a combinatorial library.

Peptide boronic acids have been identified as potent inhibitors of serine³¹ and threonine³² proteases, including at least one therapeutic inhibitor of the proteasome³³. These substrate mimics bind to the proteolytic active site by formation of a dative bond with a lone pair from the serine or threonine hydroxyl. The boron center enables a unique, covalent mode of molecular recognition. This type of interaction remains available to boronate esters such as the cyclized peptoids described here. Esterification of the boronic acid with a diol preserves the ability of the boronic acid to form a dative bond with another nucleophile, one position on the boron either occupied by a coordinatively labile water or an empty p orbital accessible for overlap with a lone pair of electrons. Thus, the boron center in peptoids such as those studied here might concurrently enable both backbone constraint and covalent interaction with nucleophilic groups of host molecules.

4. Conclusions

The peptoid backbone can be constrained by spontaneous, reversible formation of a boronate ester between boronic acid and diol-bearing side-chains. When the diol is a galactose derivative, esterification proceeds primarily through intramolecular cyclization, and the equilibrium favors condensation over hydrolysis. This equilibrium is insensitive to the size of the macrocycle formed from three to six monomer units. Ester formation also occurs when the diol is a simple 1,2-dihydroxypropyl group, though in that case, the equilibrium favors hydrolysis. The formation of boronate esters provides an approach to covalent constraint of the peptoid backbone in a reaction that occurs spontaneously in aqueous solution directed by the side chain sequence.

5. Experimental

Peptoids were synthesized using standard methods⁹. The galactose submonomer was incorporated as 6-amino-6-deoxy-D-galactopyranose diacetone¹⁴. The 1,2-dihydroxypropyl submonomer was incorporated as 2,2-dimethyl-1,3-dioxalane-4-methanamine (Acros). Azidomethyl phenyl boronic acid was prepared by previously described methods³⁴. To prepare boronic acid peptoids, a five-fold excess of the azidomethyl phenyl boronic acid was reacted with propargyl-bearing peptoids on solid support at 50°C overnight in DMSO in the presence of CuSO₄, tris(2-carboxyethyl)phosphine, and Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl] amine (TBTA) as catalyst³⁵. Peptoids were cleaved from support, purified by HPLC, and characterized by MALDI-MS (m/z (M+H⁺); **1**: 922.85 **2**: 1045.16 **3**: 867.93 **4**: 1045.20 **5**: 1028.73 **6**: 1028.73 **7**: 957.36 **8**: 779.87 **9**: 956.94 **10**: 940.82 **11**: 940.79).

Binding assays were carried out in potassium phosphate buffer, pH 7.2, with Alizirin Red S at a final concentration of 25 μM. Fluorescence was measured with excitation at 468 nm, emission at 572 nm, and a cutoff filter at 495 nm. Measurements were made 1.5 hours after

preparation of solutions and did not change subsequently, indicating equilibrium. Concentration of boronic acid-containing peptoids was measured by UV absorbance, with $\epsilon=409 \text{ M}^{-1}\text{cm}^{-1}$. Complexation with galactose does not affect the UV absorbance spectrum of phenylboronic acid under conditions of the measurement. Binding data were fit to a logistic dose-response curve.

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Figure 1.
Peptoids used to study boronic acid esterification.

Figure 2. Binding curves for peptoids with ARS. Each point is the mean of three independent determinations. Error bars, generally smaller than the data markers, are the standard deviation in the mean.

Figure 3. Structures of boronate esters of compound **2** that are consistent with requirement for galactose and boronic acid components in the same molecule. One of multiple possible regioisomers is shown for each. **a.** Product of intramolecular condensation. **b.** Head-to-tail dimer.

Table 1

Binding parameters for peptoids associating with ARS.

Compound	K_d^a	Hill Slope ^b	K_{cyc}^c	Fraction Cyclized ^d
1	0.14 ± .04	0.82 ± .10	-	-
2	0.90 ± .19	0.87 ± .13	5.4	0.84
4	1.00 ± .18	0.67 ± .10	6.1	0.86
5	1.10 ± .10	1.09 ± .12	6.8	0.87
6	0.92 ± .10	0.91 ± .07	5.6	0.85
7	0.23 ± .04	0.93 ± .03	0.65	0.39
9	0.26 ± .05	0.72 ± .09	0.86	0.46
10	0.23 ± .04	0.73 ± .02	0.67	0.40
11	0.27 ± .05	0.65 ± .07	0.98	0.49

^aDissociation constant for binding of peptoid with ARS derived as the EC₅₀ for the interaction from the plot of fluorescence vs log(peptoid concentration). Mean ± s.d. is reported. Eight independent determinations were made for **1**. Three independent determinations were made for all other compounds.

^bHill slope for best logistic dose response curve fit to plot of fluorescence vs log(peptoid concentration).

^cEquilibrium constant for cyclization assuming decreased association with ARS is entirely due to intramolecular condensation. $K_{cyc} = [K_d / (K_d \text{ for cpd.1})] - 1$.

^d $K_{cyc} / (1 + K_{cyc})$