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Improved Carbohydrate Recognition in Water with an Electrostatically Enhanced β-Peptide Bundle

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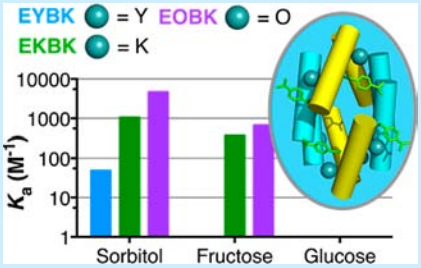
Supporting Information

ABSTRACT: The selective recruitment of oligosaccharides, or even simple sugars, in water solvent is an unsolved molecular recognition problem. Structure-guided, electrostatic redesign led to a significant increase in the affinity of a β-peptide "borono-bundle" for simple sugars in neutral aqueous solution. The affinity for fructose (663 M^{-1}) in water should allow its recruitment to the bundle surface for selective catalysis, and future work will focus in this direction.



The selective recruitment of oligosaccharides, or even simple sugars, in water solvent is an unsolved molecular recognition problem.[1] Even monosaccharides exist as mixtures of conformational and constitutional isomers, and under aqueous conditions,[2] the hydroxyl groups that distinguish one sugar, or one isomer, are effectively camouflaged by bound water molecules.[3] Lectins, natural carbohydrate-binding proteins make use of several strategies to overcome this complexity in vivo, but it has been difficult to translate these strategies into synthetic lectins[4] that achieve high affinity and selectivity under aqueous conditions.[3d,5] Challenges exist even in noncompetitive solvents such as $CHCl_3$ and DMSO, but some recent and notable successes can be identified[5n] especially using boronic acids.[5b,f,6]

We recently reported the three-dimensional structure of the octameric, boronic acid-containing, β-peptide bundle EYBK (Figure 1A) and described its interactions with certain polyol and carbohydrate metabolites.[7] The EYBK bundle bound catechol, sorbitol, and dopamine in neutral aqueous solution with equilibrium dissociation constants in the low millimolar concentration range, but interactions with more complex saccharides, such as furanose and pyranose carbohydrates, were not detected. The interactions of the EYBK bundle with polyols are mediated by eight phenylboronic acid (PBA) side chains, one per EYBK monomer (Figure 1B). Here we show that structure-guided, electrostatic redesign of the phenylboronic acid environment provides new insight into factors governing effective sugar complexation in water solution and resulted in an improved β-peptide bundle, EOBK with enhanced affinity for fructose (663 M^{-1}).

The eight PBA side chains in the EYBK β-peptide bundle occupy one of two distinct environments.[7] Type 1 PBAs lie at the interface of two parallel 14-helices, whereas type 2 PBAs lie near the C-termini of adjacent helices that are not parallel (Figure 1A). The two sites differ in atomic detail but share one common feature, overwhelmingly negative electrostatic potential (Figure 1B), which would only intensify upon sugar binding to form a negatively charged boronate ester−diol complex. Previously, we observed that dopamine, whose pendant amine is protonated at neutral pH (pK_a = 8.9[8]), bound the EYBK bundle with higher affinity (814 M^{-1}) than did catechol (312 M^{-1}), a ligand that lacks a cationic appendage. This observation raised the possibility that the electrostatic environment surrounding each PBA side chain could be improved to

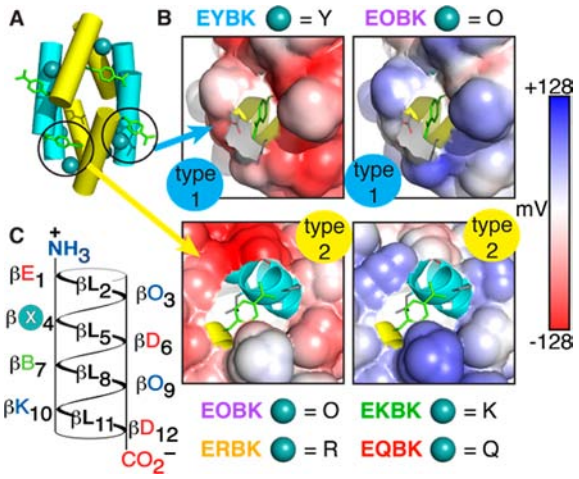


Figure 1. (A) Illustration of the EYBK octamer structure[7] showing type 1 and type 2 PBA side chains (green).[7] The Tyr residue at position 4 of each EYBK monomer is depicted as a blue sphere. Each bundle contains four type 1 sites and four type 2 sites. (B) Close-up of type 1 and type 2 sites in EYBK (left) and an EOBK model (right) illustrating the local electrostatic potential calculated using APBS.[9] (C) EYBK analogues evaluated in this work.

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generate β-peptide bundles with higher affinity for more complex saccharides.

Examination of the EYBK crystal structure[7] suggested that the electrostatic environment surrounding the PBA side chain at position 7 of each monomer would be influenced by the side chain at position 4. The position 4 side chain (β-Tyr in EYBK) is proximal and cofacial with the PBA side chain and thus is unlikely to interfere with formation of either the leucine-rich bundle core or stabilizing salt bridges, both of which employ residues on alternate 14-helix faces. Specifically, we hypothesized that β-peptides carrying a positively charged side chain in place of β-Tyr at position 4 would still assemble as octameric bundles but would possess a more positive electrostatic environment around each PBA side chain. Indeed, the calculated electrostatic surface potentials of EYBK analogs carrying β-Orn, β-Lys, or β-Arg at position 4 are significantly more positive in the region surrounding each PBA site (Figure 1B and Figures S2 and S3). By contrast, the electrostatic surface potential of an analog carrying β-Gln at position 4 resembles that of EYBK. These calculations suggested that EOBK, EKBK, and ERBK would interact with polyol ligands with higher affinity than EYBK, while EQBK would not.

To test this hypothesis, we prepared EOBK, EKBK, ERBK, and EQBK (Figure 1C) using previously reported methods[7] and evaluated their ability to assemble into β-peptide bundles and bind polyol ligands. As expected, the four EXBK peptides showed little 14-helix structure at low concentration (20 μM), with minimal ellipticity between 205 and 215 nm. In each case, the extent of 14-helix structure increased gradually between 50 and 400 μM and then plateaued (Figure 2A). The concentration dependence of the ellipticity changes at 212 nm suggested that all four EXBK peptides assemble into higher order quaternary structures. In no case, however, did the fits define the bundle stoichiometry with precision (Figure S2 and Table S2, Supporting Information). The inadequacy of CD for precise determination of peptide bundle stoichiometry has been observed previously.[10]

To define the stoichiometry more precisely, we turned to sedimentation equilibrium–analytical ultracentrifugation (SE–AU). The SE–AU data for EOBK, EKBK, and ERBK[11] fit well to monomer–octamer equilibria with values of ln K_a in line with previously analyzed β-peptide bundles, between 55.33 ± 0.2 (ERBK) and 74.4 ± 0.3 (EKBK) (Figure 2B–D and Figure S5). Fits to other bundle stoichiometries were significantly worse (Figure 2E).[12] The value of ln K_a for the octameric EOBK assembly determined by SE–AU (63.4 ± 0.3) agrees with the value obtained from the CD data when n = 8 (ln K_a = 64.85 ± 0.17). The ln K_a values for the octameric EKBK and ERBK assemblies determined by SE–AU and CD also agreed well (Figures S4 and S5 and Table S2, Supporting Information).

We next turned to isothermal titration calorimetry (ITC) to assess the relative affinities of the EXBK bundle panel for carbohydrate ligands. We began with sorbitol to directly assess how changes in the PBA electrostatic environment influence polyol affinity. Titrations were performed using 800 μM of each EXBK monomer to ensure >99.99% bundle formation. All four EXBK analogues bound sorbitol more favorably than did EYBK. For bundles formed from EQBK, EKBK, and ERBK, the affinity increases were moderate, ranging from 15-fold (for EQBK, K_a = 758 M^{-1}) to 35-fold (for ERBK, K_a = 1660 M^{-1}). By contrast, the affinity increase observed for the EOBK bundle was large, almost 2 orders of magnitude (K_a = 4620 M^{-1})

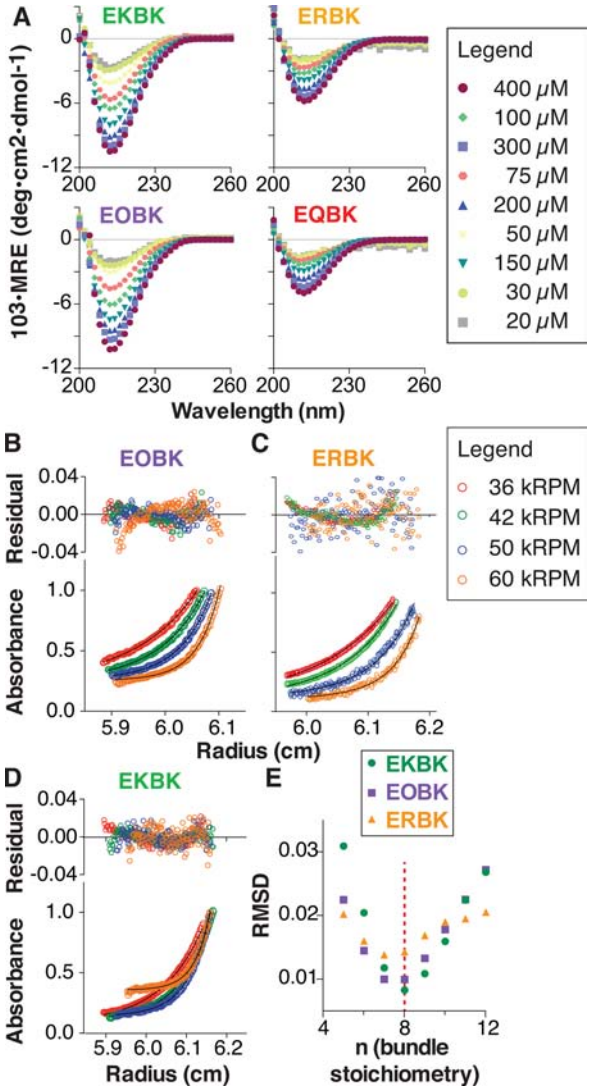

Figure 2. (A) Circular dichroism analysis of EXBK assembly at concentrations between 20 and 400 μM. (B–D) Sedimentation equilibrium analytical ultracentrifugation analysis of (B) EOBK, (C) ERBK, and (D) EKBK assembly showing the fit to an ideal monomer–octamer equilibrium. Traces shown are for 1.5 mM peptide samples. (E) Plot of the RMSD of each EXBK SE–AU fit as a function of n (n = 8 for an octamer). CD and SE–AU experiments were performed in 30 mM phosphate buffer containing 200 mM NaCl (pH 8).

(Figure 3). Of particular note is the significant difference between the sorbitol affinity of the EOBK and EKBK bundles. These two assemblies differ by a single methylene unit per β-peptide monomer, yet the EOBK octamer binds sorbitol 5-fold better.

The affinity of the sorbitol•EOBK complex was sufficient to unambiguously define the binding stoichiometry as 1:2 sorbitol/$EOBK_{monomer}$ (Table S3). This stoichiometry implies that each octameric EOBK bundle binds four sorbitol molecules. Each octamer contains four type 1 and four type 2 PBA sites, and sorbitol is too small to span the 12 Å separating even the closest PBA side chains on the bundle surface. This observation in turn would suggest that sorbitol binds preferentially to either the type 1 or type 2 PBA environment on the EOBK bundle surface and not simultaneously to two different PBA side chains (Figure 1A). To probe for differences

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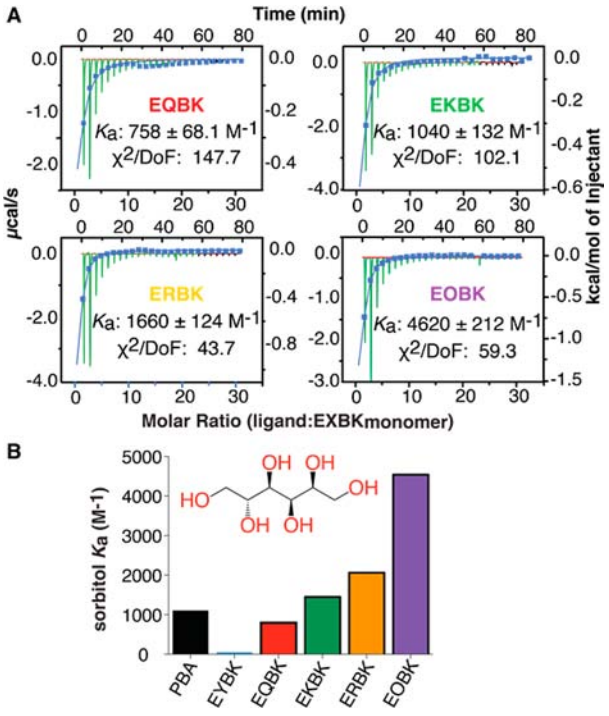

Figure 3. . ITC analysis of the interactions of sorbitol with the EOBK, EQBK, EKBK, and ERBK β-peptide bundles. (A) ITC experiments were performed by titrating sorbitol into a solution containing 800 μM of each β-peptide, conditions under which bundle assembly is >99% complete ([bundle] > 99 μM). The ITC output is shown in green; the integrated heat per injection is shown in blue. All experiments were performed at 25 °C in 30 mM phosphate buffer (pH 8). The concentration shown refers to the monomeric peptide. (B) Equilibrium association constants as determined by ITC. In each case, the value shown reflects a sorbitol/β-peptide stoichiometry of 1:2.

between the type 1 and type 2 sites that would explain this preference, we developed models based on the EYBK structure[7] to predict differences in both electrostatic potential and solvent accessibility. The total electrostatic surface potential within 4 Å of each type 1 and type 2 site on each EXBK octamer, calculated using Adaptive Poisson−Boltzmann Solver (APBS)[9] revealed significant positive potential in the cases of EOBK, EKBK, and ERBK, with little differences between the type 1 and type 2 sites (Figure 4A). In contrast, solvent-accessible surface area calculations[13] suggest that the boronic acids in a type 1 site are roughly twice as accessible as those at a type 2 site, irrespective of the identity of the substituent at position 4

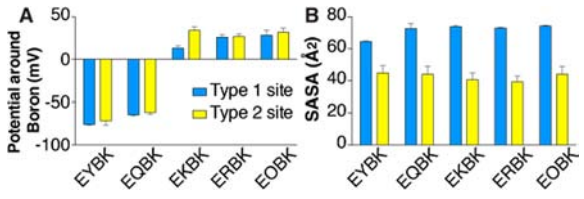

Figure 4. Electrostatic and accessibility differences between type 1 and type 2 PBA sites in EXBK β-peptide bundles. (A) Plot of the average potential within 4 Å of type 1 and type 2 PBA sites on the indicated EXBK β-peptide bundle. (B) Comparison of the solvent-accessible surface area (SASA) of type 1 (blue) and type 2 (yellow) $B(OH)_2$ groups in each EXBK bundle. Error bars reflect standard error of the mean.

(Figure 4B). Although structural work is necessary to confirm these predictions, these calculations suggest that type 1 sites present fewer steric barriers to ligand binding and may represent a unique environment for binding a polyol ligand in neutral aqueous solution.

We next investigated whether the improved diol affinity of the EOBK and EKBK bundles would extend to simple monosaccharides. Although ITC failed to detect an interaction between the EOBK or EKBK octamers and glucose, galactose, or sucrose, the ketose fructose bound well, with affinities for EOBK and EKBK of 663 and 364 M^{-1}, respectively (Figure 5).

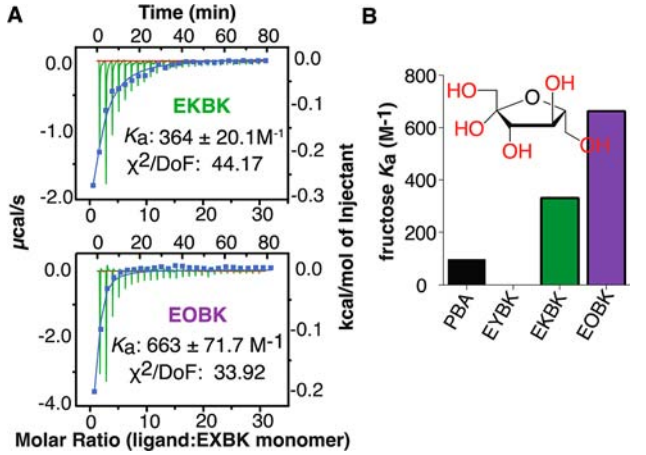

Figure 5. ITC analysis of EXBK interactions with fructose. (A) ITC experiments were performed by titrating fructose into a solution containing 800 μM of each EXBK peptide. Under these conditions, the β-peptide bundle concentration is >99 μM. The ITC output is shown in green; the integrated heat per injection is shown in blue. All experiments were performed at 25 °C in 30 mM phosphate buffer (pH 8). Concentration refers to the monomeric peptide. (B) Equilibrium association constants as determined by ITC (fructose/β-peptide stoichiometry = 1:2).

The bundle formed from the β-peptide EOYK, which carries a β-Orn residue at position 4 but lacks a phenylboronic acid side chain, also assembled into a bundle (concentration dependent CD data shown in Figure S6) but failed to detectably bind sorbitol or fructose, confirming that boronic acids are required for binding diols to EXBK bundles.

In summary, here we show that structure-guided electrostatic redesign led to a significant increase in the affinity of a β-peptide "borono-bundle" for simple sugars in neutral aqueous solution. The affinity of the EOBK borono-bundle for fructose (663 M^{-1}) in water is on par with that of both a previously reported benzoboroxle-based boronolectin (K_a = 339 M^{-1})[14] as well as a multivalent nanoparticle of undefined valency (K_a = 1150 M^{-1}).[15] Although further work is necessary to confirm interaction with the more accessible type 1 site, the affinities observed here could be sufficiently high to recruit sugars to the bundle surface for selective catalysis.[16]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02187.

Experimental procedures and supplementary data (PDF)

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Notes

The authors declare no competing financial interest.

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