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Highly Selective Oligosaccharide Sensing by a Curdlan−Polythiophene Hybrid

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Supporting Information

ABSTRACT: An in situ hybrid complex of Curdlan with water-soluble polythiophene functioned as a highly sensitive and selective saccharide chemosensor in aqueous media, enabling us to discriminate tetrasaccharide acarbose at 1 μM from 24 mono-, di-, tri-, tetra-, and pentasaccharides.

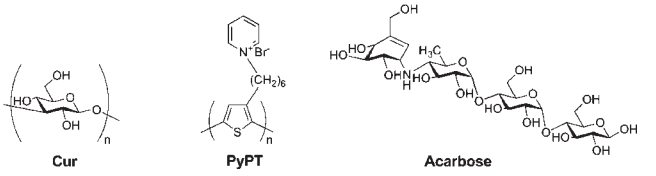
Saccharide sensing in aqueous media is one of the most intriguing but highly challenging targets in current chemistry. In particular, achieving sufficient sensitivity and selectivity for practical clinical uses is indeed a demanding task because of the structural diversity, heavy hydration, and low human physiological level (typically ≤5 mM).[1] Recent covalent approaches using the boronate formation with 1,2- and 1,3-diols have met with great success in mono- and disaccharide recognition.[2] However, this strategy may have some limitations when extended to higher oligosaccharides, and a noncovalent approach that adopts nature's strategy of building a highly ordered multiply hydrogen-bonded architecture with a specific saccharide[3] seems more promising.

Curdlan (Cur) is essentially a linear glucan composed of (1→3)-linked β-D-glucose units and forms a right-handed triple helix.[4] An interesting feature of the Cur helix is the reversible denaturing−renaturing driven by switching the solvent [from water to dimethyl sulfoxide (DMSO)] or the pH of an aqueous solution (from acidic to alkaline).[4,5] We have recently shown that chromophore-modified Cur can be used as a hydrogen-bonding saccharide sensor in aqueous solution by reading out the circular dichroism (CD) responses, which enabled us to selectively sense tetrasaccharide acarbose (with a concentration of ≥30 mM) from 24 mono-, di-, tri-, and tetrasaccharides.[6]

Shinkai and co-workers[7] found that the branched glucan schizophyllan forms stable complexes with water-soluble polythiophenes (PTs) to give helical triplexes that exhibit bisignate CD signals. This observation provoked us to test the combined use of Cur and PT in saccharide sensing. In this work, we have demonstrated highly selective and sensitive oligosaccharide sensing based on in situ formation of a hybrid complex of Cur with 2,5-poly(3-(1-pyridinium)hexylthiophene) (PyPT) (Chart 1). By using Cur as a hydrogen-bonding receptor for saccharide and water-soluble PyPT as a signal-amplifying reporter,[8] we were able to avoid the prior synthesis of chromophore-modified Cur yet greatly enhance the sensitivity to the micromolar level without losing the original high specificity for acarbose, a drug for treating type-2 diabetes mellitus and obesity.[9]

Regioregular head-to-tail 2,5-poly(3-(6-bromohexyl)thiophene) prepared by McCullough's method[10] was modified with pyridine in *N*,*N*-dimethylacetamide (DMA) to afford novel water-soluble PyPT (see the Supporting Information). The chiroptical properties of the

Chart 1. Structures for Cur, PyPT, and Acarbose



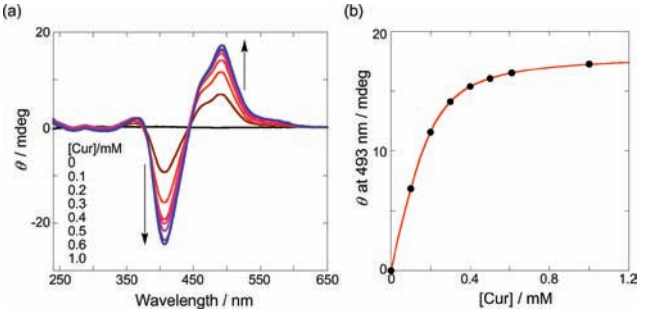


Figure 1. (a) CD spectra of a 1:9 (v/v) DMSO/H_2O solution of PyPT (0.20 mM in monomer units) in a 1 cm cell in the presence of Cur (0−1.0 mM in monomer units) at 25 °C. (b) Ellipticity induced at 493 nm and the nonlinear least-squares fit assuming the 1:1 stoichiometry, from which the association constant was found to be K_a = 24 300 ± 300 M^{-1}.

Cur−PyPT system were examined using UV−vis and CD spectroscopies.[11] Gradual addition of an aqueous PyPT solution to a DMSO solution of Cur induced an intense positive exciton couplet at the main band of PT (Figure 1a).[12] A nonlinear least-squares fit of the ellipticity change at 493 nm upon titration (Figure 1b), assuming the 1:1 stoichiometry, gave an association constant of 24 300 M^{-1}. This indicates the formation of a stable Cur−PyPT complex with a right-helical PT backbone (according to the exciton chirality theory),[13] as was the case with the schizophyllan−PT pair.[7]

Similar experiments were repeated in the presence of 24 mono- to pentasaccharides to reveal the saccharide sensitivity of the Cur−PyPT system at fixed PyPT and various Cur concentrations. As exemplified in Figure 2, addition of 1 μM acarbose caused a significant hypochromic effect with an appreciable hypsochromic shift, while the couplet amplitude was increased,[14] as a consequence of the shortened effective conjugation length and helix pitch of the PT backbone in the presence of saccharide. Crucially, the ellipticity change caused by the addition of saccharide was linearly dependent

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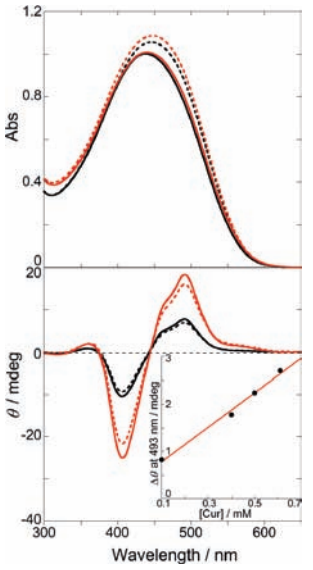

Figure 2. (top) UV−vis and (bottom) CD spectra of a 1:9 (v/v) DMSO/H_2O solution of PyPT (0.20 mM in monomer units) and 0.1 mM (black) or 0.5 mM (red) Cur (concentrations in monomer units) at 25 °C in a 1 cm cell in the presence (solid line) and absence (dashed line) of 1.0 μM acarbose. The inset shows the ellipticity change as a function of [Cur].

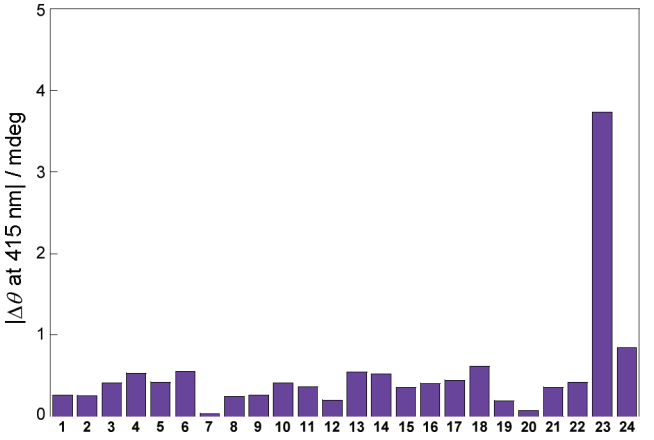

Figure 3. CD spectral changes induced in a 1:9 (v/v) DMSO/H_2O solution of PyPT (0.2 mM) and Cur (1.0 mM) (both in monomer units) at 25 °C upon addition of 1.0 μM mono- (**1**−**11**), di- (**12**−**17**), tri- (**18**−**20**), tetra- (**21**−**23**), and pentasaccharides (**24**): **1**, D-glucose; **2**, D-galactose; **3**, D-mannose; **4**, D-allose; **5**, D-fructose; **6**, D-sorbose; **7**, D-tagatose; **8**, D-fucose; **9**, D-ribose; **10**, 2-deoxy-D-ribose; **11**, D-arabinose; **12**, sucrose; **13**, lactose; **14**, D-maltose; **15**, D-trehalose; **16**, D-turanose; **17**, D-cellobiose; **18**, D-raffinose; **19**, D-melezitose; **20**, D-maltotriose; **21**, D-maltotetraose; **22**, stachyose; **23**, acarbose; **24**, D-maltopentaose (for the structures, see the Supporting Information). The error in $\Delta\theta$ was ≤4%.

on the Cur concentration (Figure 2 inset), indicating that the Cur moiety plays a key role in both saccharide recognition and signal transduction to PyPT. Under the optimized conditions of [Cur] = 1 mM, the detection limits were as low as 1.0 μM for most oligosaccharides.

Figure 3 illustrates the absolute ellipticity changes ($|\Delta\theta|$) at 415 nm upon addition of 1 μM saccharide to an aqueous solution of PyPT (0.2 mM) and Cur (1 mM).[15] All of the mono- to pentasaccharides examined more or less augmented the ellipticity, indicating that the PT helix is further twisted by incorporation of saccharide into the Cur−PT complex. Interestingly, among the oligosaccharides examined, acarbose was most effective in enhancing the ellipticity. Despite the rather limited variation of the tetrasaccharides examined in this study, the strikingly weak responses to the other tetrasaccharides and the higher homologue, maltopentaose, clearly indicate that the terminal valienamine moiety is one of the essential elements for the precise recognition of acarbose, while the maltotriose moiety plays a minor role.

In conclusion, we have demonstrated that the hybrid Cur−PT complex formed in situ in aqueous analyte solution can efficiently discriminate acarbose from 24 mono-, di-, tri-, tetra-, and pentasaccharides. The spectral range used (>400 nm), the high selectivity, and the low detection limit (1 μM) make this sensor attractive for use in diabetes research, diagnosis, and therapy, where the typical doses are 50−300 mg for oral administration.[9] Further studies to elucidate the roles of valienamine and the detailed sugar recognition mechanism are currently in progress.

■ ASSOCIATED CONTENT

Supporting Information. Experimental details, synthesis and characterization of PyPT, and CD spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Titration procedures: A 0.182 mL portion of aqueous PyPT solution (3.30 mM in monomer units) was added to a DMSO solution (0.3 mL) containing Cur at a particular concentration (up to the solubility limit of 1.0 mM). The resulting mixture was diluted with water (2.518 mL), sonicated for 10 min, and then subjected to spectral examinations (Figure 1). In saccharide sensing, a concentrated DMSO solution of saccharide was added to a DMSO solution containing Cur at a particular concentration, and the mixture was diluted with an aqueous solution of PyPT (3.30 mM) and water, sonicated for 10 min, and then subjected to spectral examinations (Figures 2 and 3 and Figures S3 and S4 in the Supporting Information).

(12) For the UV−vis spectra, see Figure S2.

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(14) For the UV−vis and CD spectral changes at 0.4 and 0.6 mM Cur, see Figure S3.

(15) For the CD spectra, see Figure S4.