Oligosaccharide sensing with chromophore-modified curdlan in aqueous media†

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A newly synthesized chromophore-modified curdlan functions as a saccharide chemosensor in aqueous solution, enabling us to discriminate tetrasaccharide acarbose from 24 mono-, di-, tri-, and tetrasaccharides.

Selective sensing of oligosaccharides in aqueous media is a challenge in current chemistry due to their heavy hydration and stereochemical diversities.[1,2] Hence, the use of aqueous or protic media is often avoided in saccharide recognition studies, except for some recently reported host–guest systems.[3] The precise saccharide recognition in aqueous media is a tricky task, demanding the host to form a highly structured multiple hydrogen-bonding network upon complexation with a specific saccharide that is heavily hydrated in the midst of bulk water. Thus, the development of selective saccharide sensor that functions in aqueous media is of particular significance and benefit not only from the scientific but also from the application point of view.[1–3]

Curdlan (Cur) is a linear glucan composed of (1 → 3)-linked β-D-glucose units and is known to form a triple helical structure.[4] The most intriguing feature of Cur is the ability to reversibly denature/renature by simply changing the solvent from water or aqueous acidic solution to DMSO or aqueous alkaline solution.[4,5] Recently, Sakurai, Shinkai and coworkers revealed that polynucleotide is merged with glucan, *i.e.* schizophyllan, to form a hetero triplex by replacing one of the three glucan components.[6] This inspired us to use the glucan triplex as a key tool for sensing only such saccharides that nicely splice into the triple-helical glucan's hydrogen-bonding network.

For this purpose, we synthesized chromophore-modified Cur, 6-*O*-(4-(dimethylamino)benzoyl)curdlan (**DABz-Cur**, Fig. 1), as a saccharide sensor, and investigated its ability for sensing a variety of oligosaccharides by using circular dichroism (CD) spectroscopy to find a specifically high sensitivity for one of the tetrasaccharides, *i.e.* acarbose (Fig. 1).

DABz-Cur was prepared in 92% yield by the reaction of 4-(dimethylamino)benzoyl chloride with commercially available native Cur, which was swollen overnight in *N*-methyl-2-pyrrolidinone at 100 °C prior to the reaction.[7] The degree of substitution of the obtained **DABz-Cur** was determined as 0.12 from the integrated areas of aromatic *versus* sugar proton signals in the ^{1}H NMR spectrum (see Fig. S1 in the ESI†).

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† Electronic supplementary information (ESI) available: Experimental details, synthesis and characterization of **DABz-Cur**, and the CD spectra. See DOI: 10.1039/c0cc02568b

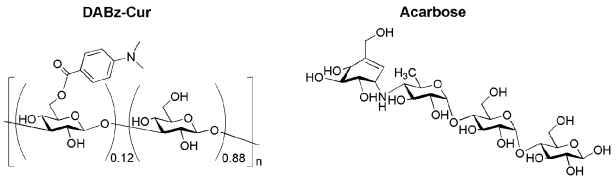

Fig. 1 Structures for **DABz-Cur** and acarbose.

The CD spectrum of **DABz-Cur** measured in DMSO exhibited a very weak negative Cotton effect at the charge-transfer (CT) band of DABz[8] centered at 310 nm (Fig. 2, red line),[9] indicating that the Cur triplex is disassembled to a single strand. In keen contrast, an intense bisignate CD signal was observed in the same region in an aqueous solution containing 10% DMSO (Fig. 2, black line). The negative exciton couplet observed suggests a left-handed helical arrangement of the DABz chromophores attached to the triple helical backbone of Cur, according to the exciton chirality theory.[10] Such contrasting chiroptical behavior in DMSO *versus* aqueous solution indicates that the original feature of Cur to reversibly denature/renature in the two solvents is preserved even after the DABz modification.

Acarbose is a drug to treat type-2 diabetes mellitus and obesity by inhibiting α-glucosidase that releases glucose from higher carbohydrates,[11] and therefore its detection is of particular significance from the diagnostic viewpoint. Acarbose added to an aqueous solution of **DABz-Cur** caused a significant reduction of the CD intensity without altering the couplet pattern (Fig. 2, blue line), indicating strong interactions of acarbose with **DABz-Cur**.[12] Although such a dramatic decrease in CD intensity is attainable either by disassembling the Cur triplex or by altering the DABz conformation on the triplex, the latter mechanism seems more plausible, since the CD intensity was enhanced upon

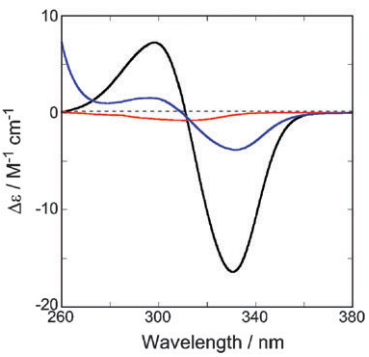

Fig. 2 CD spectra of **DABz-Cur** in DMSO (0.341 mM in monomer unit; red) and in 1 : 9 (v/v) DMSO–H_2O (0.462 mM in monomer unit) in the absence (black) and presence (blue) of acarbose (30 mM) at 25 °C.

addition of mono-, di- and trisaccharides at least at 30 mM concentration (Fig. 3 and 4 and the ESI†). This CD enhancement may indicate that the DABz chromophores are better aligned for exciton coupling.

For better understanding the saccharide recognition by **DABz-Cur**, we performed the CD spectral titrations[12] with tetrasaccharides, acarbose and stachyose, as well as disaccharide D-maltose.[13] As can be seen from Fig. 3 (black line), the negative CD intensity linearly decreased upon addition of acarbose of up to 40 mM to eventually reach a plateau of almost zero CD at 40–150 mM, indicating the strong binding to **DABz-Cur**. On the other hand, the CD spectral changes upon addition of stachyose gave a typical sigmoidal curve to reach the same plateau at 100–150 mM (Fig. 3, red), indicating weaker binding that requires multiple stachyose

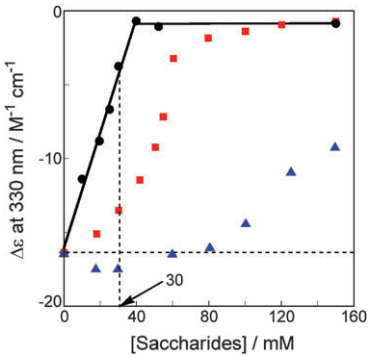

Fig. 3 CD spectral titration of **DABz-Cur** (0.462 mM in monomer unit) with acarbose (black circle), stachyose (red square), and D-maltose (blue triangle) in 1 : 9 DMSO–H_2O at 25 °C.

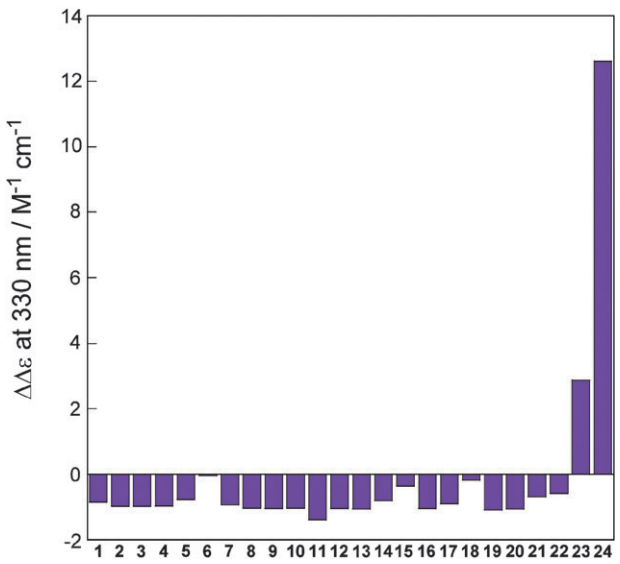

Fig. 4 CD spectral changes of **DABz-Cur** (0.462 mM in monomer unit) in 1 : 9 DMSO–H_2O upon addition of 30 mM mono- (**1–13**), di- (**14–19**), tri- (**20–22**), and tetrasaccharides (**23**, **24**): D-glucose (**1**), L-glucose (**2**), D-galactose (**3**), D-mannose (**4**), D-allose (**5**), D-fructose (**6**), D-sorbose (**7**), D-tagatose (**8**), D-fucose (**9**), D-ribose (**10**), 2-deoxy-D-ribose (**11**), D-arabinose (**12**), L-arabinose (**13**), sucrose (**14**), lactose (**15**), D-maltose (**16**), D-trehalose (**17**), D-turanose (**18**), D-cellobiose (**19**), D-raffinose (**20**), D-melezitose (**21**), D-maltotriose (**22**), stachyose (**23**), and acarbose (**24**); see the ESI† for structures.

molecules to induce appreciable CD changes. Thus, the addition of 150 mM acarbose or stachyose to a **DABz-Cur** solution made the CD spectra nearly silent. Crucially, the CD spectra of **DABz-Cur** in DMSO without and with 150 mM stachyose (Fig. 2 (red) and Fig. S3b (ESI†)) were very similar to each other in shape and intensity, suggesting that the DABz chromophore in the triplex randomly oriented on average even in the presence of a large amount of the tetrasaccharide. D-Maltose led to an initial enhancement of CD intensity, which was followed by a slow decrease in CD intensity (Fig. 3, blue). It was thus revealed that **DABz-Cur** strongly interacts with the tetrasaccharides with different affinities, but only weakly with the disaccharide. This result prompted us to further examine the CD spectral behavior of **DABz-Cur** with an expanded set of mono/di/tri/tetrasaccharides.

Fig. 4 illustrates the CD intensity change ($\Delta\Delta\varepsilon = \Delta\varepsilon - \Delta\varepsilon_0$, where $\Delta\varepsilon_0$ and $\Delta\varepsilon$ are the molar ellipticities at 330 nm in the absence and presence of saccharide, respectively) observed upon addition of a fixed concentration (30 mM) of saccharide to an aqueous solution of **DABz-Cur**. Interestingly, **DABz-Cur** precisely discriminated the tetrasaccharides from lower homologues, showing only minor changes in CD intensity for mono-, di-, and trisaccharides at least at this concentration, but much larger changes in the opposite direction for acarbose and stachyose.[13] Crucially, acarbose induced the largest variation in CD among the oligosaccharides examined, which was significantly larger than those caused by stachyose (and other lower homologues), suggesting the potential use of **DABz-Cur** as a chemosensor for tetrasaccharides, in particular for acarbose, in diabetes research, where typical dosage was 50–300 mg in one tablet for oral use.[11]

In this study, we have demonstrated for the first time that the use of chromophore-modified Cur enables us to detect oligosaccharides even in aqueous solution by reading out the conformational change of the attached chromophores by monitoring the CD response. It is to note that the DABz-appended Cur sensor responds preferentially to tetrasaccharides, in particular to acarbose (with almost linear dependence), which is pharmaceutically important as a drug to treat diabetes and obesity. This sensing strategy, which utilizes the glucan as a recognition device and the appended chromophore as a reporter, may be expanded to other analytes which are difficult to sense in aqueous media. Further studies to elucidate the detailed chiroptical behavior and sugar recognition mechanism and also to enhance the sensitivity are currently in progress.

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12 Sample preparation and titration experiments: a given amount of saccharide was added to a DMSO solution of **DABz-Cur**. The resulting mixture was sonicated to make a homogeneous solution, which was diluted with water and sonicated for 10 min for the use in UV and CD titration measurements (Fig. 3 and Fig. S3 (ESI†)). The titration experiments were run up to 150 mM, which is the solubility limit of acarbose.

13 All the examined CD spectra were shown in the ESI†.

