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Research paper

Analytical platform for sugar sensing in commercial beverages using a fluorescent BODIPY "light-up" probe

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ABSTRACT

Because of the globally increasing prevalence of diabetes, the need for accurate, efficient and at best miniaturized automated analytical systems for sugar detection in medical diagnostics and the food industry is still urgent. The development of molecular probes for sugars based on boronic acid receptors offers an excellent alternative to the kinetically slow enzyme-based sugar sensors. Moreover, by coupling such chelating units with dye scaffolds like BODIPYs (boron–dipyrromethenes), highly fluorescent sugar sensing schemes can be realized. In this work, a boronic acid-functionalized BODIPY probe was developed, which binds selectively to fructose's adjacent diols to form cyclic boronate esters. Placement of an amino group in direct neighborhood of the boronic acid moiety allowed us to obtain a broad working range at neutral pH, which distinguishes the probe from the majority of systems working only at pH > 8, while still meeting the desired sensitivity in the micro-molar range due to a pronounced analyte-induced fluorescence increase. To enhance the applicability of the test in the sense described above, integration with a microfluidic chip was achieved. Here, fructose was selectively detected by fluorescence with similar sensitivity in real time on chip, and an assay for the straightforward detection of sugar in (colored) sodas without sample clean-up was established.

1. Introduction

The prevalence of diabetes is globally increasing at an alarming pace [1,2]. As a metabolic disease, diabetes is not only directly associated with enormous costs and serious economic impact [3,4], but is also known to interact in many different ways with other global diseases such as cancer, tuberculosis, neurodegenerative or cardiovascular diseases [5–8]. Owing to changing lifestyles across the globe, sugar-sweetened beverages became one of the primary sources of added sugars in daily diets [2,9], shifting especially fructose and its metabolism into focus [10]. As a consequence, the need for the determination of fructose and other sugars is not only urgent in medical diagnostics [11], but also in the food industry [12]. The determination of the sugar content of beverages is thus not only of paramount importance to guarantee the specific taste of a soft drink, but also because consumers are very concerned about a drink's quality especially when products are labeled "light" or "zero". Besides laboratory-based methods using for instance HPLC or capillary electrophoresis [13,14], accurate, efficient, miniaturized and potentially automatable analytical systems are thus very attractive to guarantee optimal process control. Here, Raman spectroscopic, enzymatic and non-enzymatic chemiluminescence-based approaches have been developed, which however still require rather elaborate microscopy setups and/or specific timing [15–17]. Particularly interesting in this respect would be the use of molecular probes based on boronic acid receptors that are well-known to bind a sugar molecule through its diol motif [18–21]. Moreover, by coupling such chelating units with rhodamine, fluorescein or BODIPY (boron-dipyrromethene) moieties, colorimetric and/or highly fluorescent sugar sensing schemes can be obtained. In particular, fluorescence being a very sensitive, versatile and easily miniaturizable technique, it is well suited for on-line detection [22,23]. However, most of these fluorescent probes suffer from weak signaling responses due to the lack of sufficient electronic changes upon complexation between saccharide and boronic acid moiety. Moreover, facile complexation of saccharide occurs only at pH >8, where the favorable boronate anion is formed [21,24]. To circumvent these problems, additional photoinduced electron transfer- (PET) active amine groups are placed adjacent to the boronic acid moiety [25–27]. In line with these efforts, our present aim was to advance the probe component of such assays by shifting

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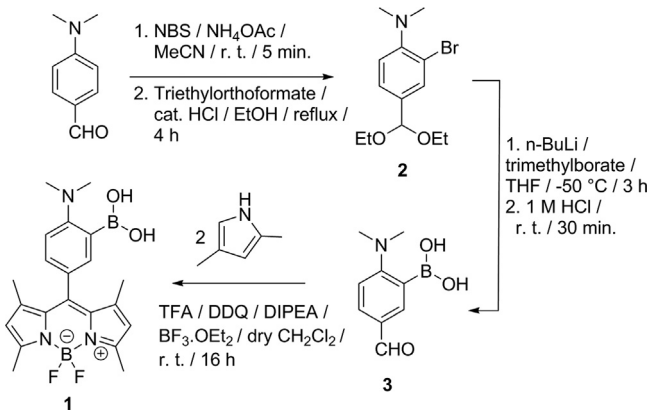

Scheme1. Synthesis of molecular probe **1**.

the working range toward neutral pH while generating favorable "light-up" responses. The probe architecture was designed in such a way that a tertiary amino group was placed adjacent to a boronic acid receptor, creating a more basic environment [28] and thus showing enhanced fluorescence responses through modulation of an intramolecular charge transfer (ICT) process within a considerably broad pH range, pH 6–10.

To improve the applicability of the assay toward process integration, the molecular probe was implemented with a microfluidic chip, which shows good selectivity and sensitivity for fructose detection in real time. This straightforward approach was used to measure the sugar content of selected commercial beverages, which showed good agreement with the reported values, and was confirmed by an independent IFU 55/56 method, rendering this assay suitable for sensitive and quantitative online measurements.

2. Materials and methods

2.1. Synthesis

BODIPY-boronic acid probe **1** was synthesized by condensation of 2,4-dimethyl-1*H*-pyrrole and 2-(dimethylamino)-5-formylphenylboronic acid (**3**), which was obtained from 4-(dimethylamino)benzaldehyde as shown in Scheme 1. Synthesis and characterization of intermediates **2** and **3** are described in detail in Section I of the Supplementary Data.

BODIPY probe (***1***) To a solution of 2,4-dimethylpyrrole (132 mg, 1.4 mmol) in 25 mL of dry dichloromethane, 2-(dimethylamino)-5-formylphenylboronic acid **3** (102 mg, 0.5 mmol) was added followed by a catalytic amount of trifluoroacetic acid (TFA). After stirring the resulting reddish solution overnight, 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ, 144 mg, 0.6 mmol) was added and the reaction was continued for 2 h. To this mixture, *N*,*N*-diisopropylethylamine (DIPEA, 0.83 mL, 4.8 mmol) and $BF_3.OEt_2$ (0.60 mL, 4.8 mmol) were added. After stirring for 3 h, the mixture was concentrated in vacuo, re-dissolved in EtOAc and washed with water. The water layer was extracted with EtOAc and the combined organic layers were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified by column chromatography using neutral alumina (95:5 EtOAc:MeOH) to obtain pure **1** (40 mg, 18%) as a red colored solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.76 (s, 2H), 7.42 (t, 1H, J = 8.8 Hz), 7.32 (d, 2H, J = 8.8 Hz), 6.17 (s, 2H), 2.78 (s, 6H), 2.50-2.45 m, 6H), 1.39 (s, 6H). MS-ES+ m/z (MH +) 412.201 (calculated 412.210).

2.2. Materials and instrumentation

All reagents, including sugars: D-fructose (Fru), D-glucose (Glu), D-sucrose (Suc), D-galactose (Gal), D-mannose (Man), and solvents were obtained from commercial suppliers and used without further purification unless otherwise indicated. All air- and moisture-sensitive reactions were carried out under argon atmosphere in oven-dried glassware. TLC was performed on Merck silica gel 60 F254 TLC plates with a fluorescent indicator for 254 nm excitation. Compounds were visualized under UV light at 254 nm. NMR measurements were carried out on a Bruker AV 400 using residual protonated solvent signals as internal standard. Chemical shifts are reported in ppm and coupling constants ($J_{X\text{-}X'}$) are reported in Hz. Mass spectra were measured on a Waters LCT Premier XE. UPLC was performed with a Waters UPLC Acquity equipped with a Waters LCT Premier XE mass detector for UPLC–MS, with Waters Alliance systems (consisting of a Waters Separations Module 2695, a Waters Diode Array detector 996 and a Waters Mass Detector ZQ 2000) equipped with an Acquity BEH C18 (2.1 × 50 mm) column, and with Shimadzu LC-10A systems equipped with a photodiode array detector (PAD). UV–vis absorption spectra were recorded on an Analytik Jena Specord 210 Plus spectrophotometer. Steady state fluorescence measurements were carried out on a Horiba Jobin–Yvon FluoroMax-4P spectrofluorometer using standard 10 mm path length quartz cuvettes. Fluorescence lifetimes were determined with a unique customized laser impulse fluorometer with picosecond time resolution described elsewhere [29,30]. The fluorescence lifetime profiles were analyzed with a PC using the software package FLA900 (Edinburgh Instruments). All solvents employed for the spectroscopic measurements were of UV spectroscopic grade (Aldrich). Milli-Q water was used throughout the study. Stock solutions of saccharides were prepared in phosphate buffer and the probe solution was prepared in ethanol. The pH was monitored with a digital pH meter (pH lab 827, Metrohm GmbH) equipped with a glass electrode (Biotrode).

3. Results and discussion

3.1. Synthesis

Traditionally, boronic acid derivatives are synthesized as their boronic ester due to the ease of its purification through silica gel chromatography compared to the boronic acid. However, our attempt for the conversion of the bromine in **2** into a boronic ester with bis(pinacolato)diboron was not successful. Recently, such failure has been reported for the same compound due to the steric hindrance of the *N*,*N*-dimethylamino group present in **2**, avoiding the bulky substitution [31]. Hence, we proceeded our synthesis with the boronic acid group itself and the purification was achieved through alumina chromatography. Finally, molecular probe **1** was obtained in pure form with an overall yield of 2.5 %.

3.2. Spectroscopic features of probe *1*

Molecular probe **1** showed weakly Stokes-shifted, largely mirror image-shaped absorption and emission bands centered at ca. 500 and 510 nm that are typical for 1,3,5,7-tetraalkyl-substituted BODIPY dyes [32]. These features are not significantly altered as a function of solvent polarity or proticity, covering the entire polarity range between hexane and water (Fig. 1, Table 1). Interestingly, however, for aprotic solvents an increase of the polarity from hexane to acetonitrile induced a dramatic decrease of the fluorescence quantum yield (Φ_f), while for protic solvents the reverse phenomenon was observed, with a maximum Φ_f = 0.84 in water (Table 1).