FLUORESCENT SACCHARIDE SENSORS

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3.1. INTRODUCTION

"In the field of observation, chance favours only the prepared mind"
Louis Pasteur 1822-1895

In order to set the context for this chapter, we will outline why saccharide sensors are important. Saccharides and related molecular species are involved in the metabolic pathways of living organisms, therefore, the detection of biologically important sugars (D-glucose, D-fructose, D-galactose, etc.), is vital in a variety of medicinal and industrial contexts. The recognition of D-glucose is of particular interest, since the breakdown of glucose transport in humans has been correlated with a number of diseases: renal glycosuria,¹,² cystic fibrosis,³ diabetes⁴,⁵ and also human cancer.⁶ Industrial applications range from the monitoring of fermenting processes to establishing the enantiomeric purity of synthetic drugs.

This chapter will discuss fluorescent saccharide sensors constructed using ‘boronic acid’ receptor units. Current enzymatic detection methods of sugars offer specificity for only a few saccharides; additionally, enzyme based sensors are unstable under harsh conditions. Stable boronic acid based saccharide receptors offer the possibility of creating saccharide sensors which through design can be selective and sensitive for any chosen saccharide.

A growing number of excellent reviews exist in the literature covering the use of boronic acids in the development of saccharide receptors.⁷-¹⁵

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Our aim with this review is to provide a specific and more personal perspective on current and future directions in the development of fluorescent saccharide sensors. We have structured the chapter to follow the same conceptual journey we ourselves take when developing new sensor systems. The two components required for a sensor are a selective interface and a read-out mechanism. The two main sections of this review are for that reason interface and read out. The read out or fluorescence will be discussed first since selectivity is only important when an output is possible.

Recognition of saccharides by boronic acids has a unique place in supramolecular chemistry. The pair-wise interaction energy is large enough to allow single-point molecular recognition, and the primary interaction involves the reversible formation of a pair of covalent bonds (rather than non-covalent attractive forces). Despite a long history—the first structural and quantitative binding constant data were reported in the 1950’s—there is general agreement that boronic acids covalently react with 1,2 or 1,3 diols to form five or six membered cyclic esters. The adjacent rigid cis-diols of saccharides form stronger cyclic esters than simple acyclic diols such as ethylene glycol. With saccharides the choice of diol used in the formation of a cyclic ester is complicated by the possibility of pyranose to furanose isomerization of the saccharide moiety. Lorand and Edwards first determined the selectivity of phenylboronic acid towards saccharides and this selectivity order seems to be retained by all monoboronic acids (D-fructose > D-galactose > D-glucose).

The equilibria involved in the phenylboronate binding of a diol are conventionally summarized as a set of coupled equilibria (equation 1). In aqueous solution phenylboronic acid reacts with water to form the boronate anion plus a hydrated proton thereby defining an acidity constant $K_a$. The formation of a diol boronate complex, defined by $K_{\text{tet}}$, formally liberates two equivalents of water, but this stoichiometric factor is usually ignored as a constant in dilute aqueous solution. In a formal sense, phenylboronic acid could also bind diols to form a trigonal complex ($K_{\text{trig}}$), and this species would itself act as an acid according to $K_a'$. The “acidification” of solutions containing phenylboronic acid and diols is always discussed in terms of the trigonal complex being a stronger acid than the parent phenylboronic acid, i.e. $K_a' > K_a$. As a result, $K_{\text{tet}} > K_{\text{trig}}$.

We have recently examined associations of the boronic acids with buffer conjugate bases (phosphate, citrate and imidazole). What we discovered was that binary boronate-X complexes are formed with Lewis bases (X), together with ternary species (boronate-X-saccharide). The most important discovery as far as sensor design was the discovery of ternary complexes. In some cases, these previously unrecognized species persist into acidic solution and under some stoichiometric conditions they can be the dominant components of the solution. These complexes suppress the boronate and boronic acid concentrations leading to a decrease in the measured apparent formation constants.

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$\text{Equation 1 shows an explicit water molecule “coordinated” to the trigonal boronic acids. There is undoubtedly water in rapid exchange on the Lewis acidic boron in the same way that hydrated Lewis acidic metal ions exchange bound water. A good analogy is } Zn^{2+} (aq), \text{ which ionizes in water to give a pK}_a = 8.8, \text{ i.e. } Zn-OH_2 \rightarrow Zn-OH + H^+. $