

Fluorescent Chemosensors of Carbohydrates. A Means of Chemically Communicating the Binding of Polyols in Water Based on Chelation-Enhanced Quenching¹

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Fluorescent sensors that bind small molecules with signal transduction are of current interest.² Various mechanisms for the construction of intensity and ratiometric probes have been elucidated and utilized in the synthesis of ion sensors functioning in aqueous solution. However, fewer chemical sensing mechanisms (other than bulk quenching) have been described for neutral molecules; nevertheless, many small analytes of interest are uncharged. For example, blood glucose levels are closely regulated to about 4.5 mM. Of course, most carbohydrates are neither fluorescent nor are they fluorescence quenchers; novel signal transduction mechanisms are required. We now report the first fluorescent chemosensor that signals polyol binding.

Anthrylboronic acid **1**³ in water displays a fluorescence emission centered at 416 nm of similar structure to that displayed by anthracene itself. Upon addition of base, fluorescence decreases; because the emission can be modulated reversibly, the change is due not to decomposition but to ionization leading to borate **2** (Figure 1). While there are several reasons why the fluorescence of borate **2** might be quenched compared with that of **1**, the oxidizability of borates suggests electron-transfer quenching; photoinduced electron transfer from alkyltriphenylborate salts with resulting fluorescence quenching has been described by Schuster.⁴ The pH-fluorescence profile of **1** obtained without buffer is shown in Figure 2, from which a pK_a of 8.8 is calculated; this compares favorably to the known phenylboronic acid pK_a of 8.83.⁵

Upon addition of fructose, the apparent pK_a value decreases, leading to the remaining four curves shown in Figure 2. The explanation for this observation lies in the fact that the fructose complex of **1** is a stronger acid than is **1** itself. This result was predicated on the work of Edwards, who reported the same trend in polyol complexes of phenylboronic acid in 1959.⁶ As determined in the presence of a near-saturating amount of fructose (100 mM), the apparent pK_a of the fructose-**1** complex is 5.9. The greatest signal range available is therefore at a pH that is the average of $pK_a(\mathbf{1})$ and $pK_a(\mathbf{1}\text{-fructose})$ or 7.35. Titrations of selected polyols, determined at pH 7.4, are shown in Figure 3; the apparent fructose dissociation constant at that pH is 3.7 mM.⁷ The stability trends we observe are in good agreement with the reported trends using phenylboronic acid in water.⁶ Chelation-enhanced quenching (CHEQ) obtained on polyol binding at constant pH results, in essence, from a shifting of the Figure 1 equilibrium from **1** (higher fluorescence) toward **4** (lower fluorescence).

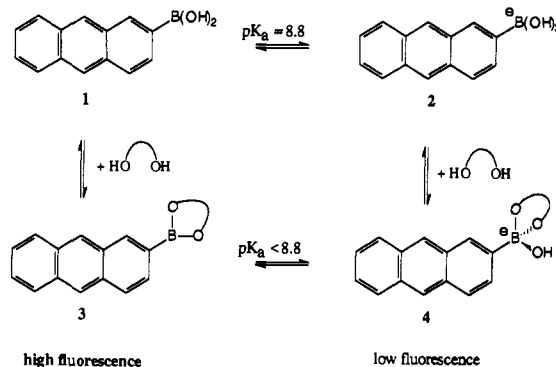


Figure 1. Equilibria available to an aqueous solution of anthrylboronic acid **1** in the presence of a generic polyol. Species **1** and **3** display higher fluorescence than do species **2** and **4**.

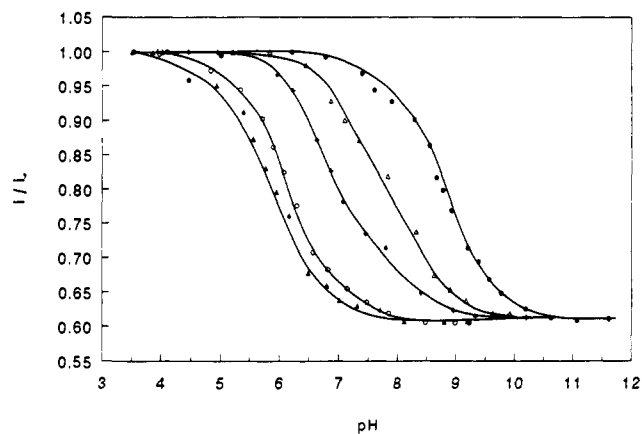


Figure 2. pH-fluorescence titrations of anthrylboronic acid **1** (0.75 μM) as a function of fructose concentration (●, 0 mM; Δ, 2 mM; +, 5 mM; ○, 50 mM; ▲, 100 mM). All solutions contain 1% (v/v) DMSO. The apparent pK_a 's at these concentrations are, respectively, 8.8, 7.8, 7.0, 6.2, and 5.9.

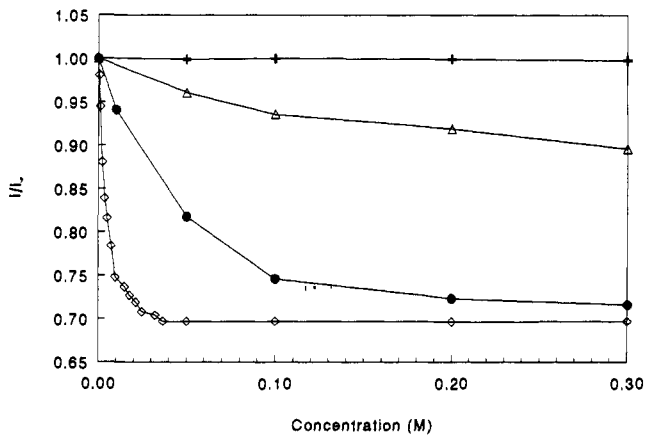


Figure 3. Fluorescence titrations of anthrylboronic acid **1** (0.75 μM) at pH 7.4 (20 mM phosphate buffer) as a function of polyol concentration (◇, fructose; ●, 1,1,1-tris(hydroxymethyl)ethane; Δ, glucose; +, ethylene glycol). All solutions contain 1% (v/v) DMSO.

We have shown that pK_a modulation in an anthrylboronic acid serves as a viable mechanism for signaling carbohydrate binding. Useful fluorescent chemosensors require the following: (1) tight, reversible association mechanisms, and (2) signal transduction mechanisms that communicate the association fluorometrically. Work by various groups on the synthesis of polyol-specific boronic acids provides direction to the former goal.⁸ The present work

(1) This paper is dedicated to Professor Nelson J. Leonard, a pioneer in fluorescent probe chemistry, on the occasion of his 75th birthday.

(2) For leading references to work by Bouas-Laurent, de Silva Masilamani, Sousa, Tsien, Ueno, Valeur, and this group, see: Czarnik, A. W. In *Frontiers in Supramolecular Organic Chemistry and Photochemistry*; VCH: Weinheim, 1991; pp 109-122.

(3) We synthesized 2-anthrylboronic acid (**1**, mp 320 °C dec) from 2-bromoanthracene via the Grignard reagent, followed by reaction with trimethyl borate and hydrolysis. Full synthetic and characterization details are available as supplementary material.

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(7) Binding constants were determined by using the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1LA, United Kingdom.

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provides a working mechanism for the latter.

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Supplementary Material Available: Experimental details, a listing of spectral data, and the ^{13}C NMR spectrum for 2-anthrylboronic acid (2 pages). Ordering information is given on any current masthead page.

Entropy of Association of Methane in Water: A New Molecular Dynamics Computer Simulation

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Hydrophobic interactions are important in clathrate-hydrate formation, protein folding, micelle formation, and other fields.¹⁻⁹ Entropy should play a major role in the interaction of hydrophobic solutes, just as it dominates the thermodynamics of hydrophobic solvation. Theoretical and computer studies have calculated the free energy of association of hydrophobic solutes,¹⁰⁻¹⁷ yet none have evaluated directly the role of entropy. We present here a computer simulation of the entropy of association of two methane-like molecules in aqueous solution. The entropic contribution to the free energy of association is attractive at short separations, when the solutes are in contact.

The free energy of association of two methane molecules in water at 25 °C is shown in Figure 1a, together with the potential energy used to model the methane-methane interaction. This free energy, or potential of mean force (PMF), is calculated using a traditional thermodynamic integration technique.¹⁸ Two distinct minima are evident in the PMF, and the contact minimum is significantly deeper than the solvent-separated minimum. The

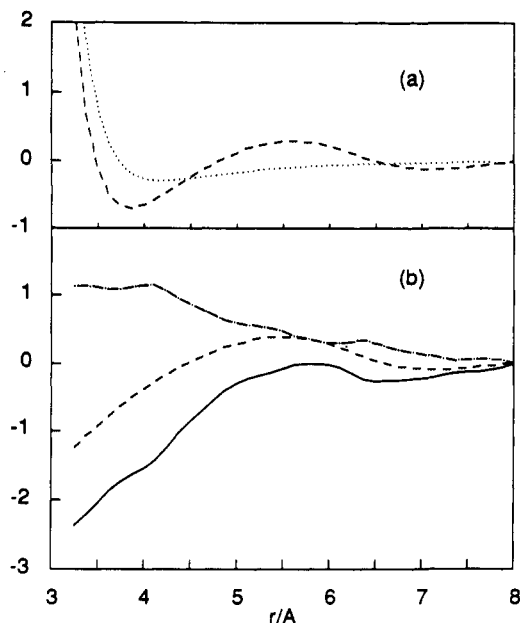


Figure 1. (a) Methane-methane potential of mean force (dashed line) and potential energy (dotted line), in kcal mol⁻¹. (b) Solvent contribution to the potential of mean force (dashed line); entropy of association, $-TS(r)$ (solid line); and configurational energy of association (dot-dashed line).

position of the solvent-separated minimum is in conflict with a recent discussion of solvent-induced interactions by Ben-Naim¹⁹ and will be examined later.²⁰ The SPC model²¹ is used for water, and a united atom approximation for the two methane molecules. The methane-methane (MM) and methane-oxygen (MO) interactions are pairwise additive, spherically symmetric Lennard-Jones potentials, with parameters $\sigma_{\text{MM}} = 3.73 \text{ \AA}$, $\epsilon_{\text{MM}} = 0.2931 \text{ kcal mol}^{-1}$, $\sigma_{\text{MO}} = 3.4475 \text{ \AA}$, and $\epsilon_{\text{MO}} = 0.2134 \text{ kcal mol}^{-1}$.

Subtracting the methane-methane potential energy from the PMF yields the so-called "indirect"⁶ (solvent) contribution to the PMF, displayed as the dashed line in Figure 1b, along with its energetic and entropic components. Unlike the full PMF, the "indirect" component is purely attractive at short separations.

Our new result is the entropy of association, $-TS(r)$, the solid line displayed in Figure 1b, calculated from an equation introduced below. Our central result is that *the entropy causes an attraction between the solutes* at separations less than approximately 5.5 Å. Experiments by Pollack and others²² document that the entropy of hydration for hydrophobic solutes is unfavorable, presumably due to increased solvent ordering around the solutes. Hence solvating two solutes in contact (where their solvation shells are shared) should be favored entropically over solvating each solute individually.¹⁷ The data of Figure 1b are, to our knowledge, the first direct confirmation of this hypothesis.

Our data predict that as the temperature increases, (i) the magnitude of the attraction at short separations increases and (ii) the contact well in the PMF becomes deeper. The latter conclusion disagrees with the temperature dependence of the PMF for hard spheres in water calculated by Pratt and Chandler,¹⁰ probably due to a different choice of the interaction potentials.²⁰ Our result is in qualitative agreement with the single-point (1.533 Å) experimental estimate of Yaacobi and Ben-Naim²³ of the entropy of association for methane.

The energy of association is determined by subtracting the entropy of association from the free energy (PMF). The energy (dot-dashed line in Figure 1b) is slightly repulsive at short range.

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