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On the Rate of Boronate Ester Formation in ortho-Aminomethyl Functionalized Phenyl Boronic Acids†

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Abstract

The role of the ortho-aminomethyl functional group in phenyl boronic acids for sugar complexation is a topic of debate. To decipher its effect on the kinetics of boronate ester formation, we first performed pseudo-first order kinetics analyses at five pH values up to 4 mM in fructose, revealing a first-order kinetic dependence upon fructose. Under these conditions, the reaction is in equilibrium and does not reach completion, but at 50 mM fructose saturation is achieved revealing zero-order dependence upon fructose. This indicates rate-determining creation of an intermediate prior to reaction with fructose, which we propose involves leaving group departure of inserted solvent. Further, the region of kinetics displaying zero-order dependence has a kinetic isotope effect (KIE) of 1.42, showing involvement of a proton transfer in the leaving group departure. The ratio of forward and reverse rate constants branching from the intermediate shows that fructose is several thousand times more nucleophilic than the solvent. Overall, the data supports a mechanism where the o-aminomethyl group lowers the pK_a of the proximal boronic acid and acts as a general-acid (as an ammonium) to facilitate leaving group departure. Consequently, by microscopic reversibility the resulting amine must perform general-base catalysis to deliver fructose.

Introduction

Boronic acids have been extensively utilized for the detection of carbohydrates, as well as various other diol-containing compounds.¹ Selective detection of carbohydrates is important not only in monitoring diseases such as cancer and diabetes,² but also because of the challenge they present through their vast structural diversity. In the field of supramolecular chemistry, boronate ester formation is recognized as a unique interaction because it is one of a few rapidly reversible interactions that involves the making and breaking of covalent bonds.³

Phenyl boronic acid is limited in scope as a diol receptor due to the fact that it only operates efficiently above physiological pH.⁴ In a landmark study by Shinkai and James, anthracene based sensor **1** was used to signal the presence of various carbohydrates in neutral aqueous solution.⁵ The sensor was proposed to operate via a photo-induced electron transfer (PET) interaction between the anthracene fluorophore and the appended amine. The key hypothesis was that the B–N bond in the boronic acid was not strong enough to interfere with PET quenching of the anthracene by the amine. Upon boronate ester formation, the Lewis acidity of the boron is increased, which enhances B–N bonding and disrupts PET quenching

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†Dedicated to Prof. A. P. de Silva on the occasion of his 60th birthday.

(Scheme 1, 1a to 2a). Wang *et al.* published an alternative explanation.⁶ He proposed that formation of a boronate ester could significantly reduce the pK_a of the boronic acid, so that the first pK_a of the boronate ester corresponded to hydroxylation at boron to form a solvent inserted species (2d), suggesting that amine protonation could more effectively 'tie up' the nitrogen lone pair (Scheme 1, 1a to 2d) than enhancement of the B–N bond.

Our group conducted an investigation of B–N bonding vs. solvent insertion using a combination of X-ray crystal structures, ^{11}B NMR, and computational analysis, with all results showing that the solvent inserted species is preferred in protic media for both an acid and ester of an *o*-aminomethyl phenyl boronic acid.⁷ Further, it was found that the first pK_a is hydroxylation of the boron in both the boronate ester and the free boronic acid (Scheme 2). We also found that in the solid state the inserted solvent molecule is nearly fully dissociated, giving a zwitterionic species (ammonium cation and borate anion, such as the central structure of Scheme 2), as opposed to a neutral, solvent 'coordinated' structure.⁸

Most recently, Benkovic has thoroughly analyzed the kinetics of boronate ester formation between unsubstituted phenyl boronic acid and alizarin Red S (ARS).⁹ He found that the reaction mechanism of this simple boronic acid proceeds via two competing pathways. The dominant pathway involved nucleophilic addition of ARS to the neutral trigonal boronic acid. However, an anionic tetrahedral boronate can also undergo direct reaction with ARS, analogous to an S_N2 reaction. **Further, Ishihara found that even plain phenyl boronic acid under basic conditions reacts through a trigonal planar boron.**¹⁰ As described herein, with an *o*-aminomethyl group appended to the boronic acid, we propose that the species which undergoes reaction with the added diol is a trigonal neutral boronic acid, even at neutral pH.

Irrespective of the role of the ortho-aminomethyl group in the optical response of receptor **1** to sugars, this motif has been adopted as a standard structural design in the creation of boronic acid-based receptors. This is evidenced by the plethora of receptors reported since Shinkai's initial report,¹¹ most all of which incorporate a ortho-aminomethyl functionality yielding good thermodynamics of binding sugars at neutral pH. **Traditionally, those boronic acid receptors missing the ortho-aminomethyl group have other electronwithdrawing groups on the phenyl ring that lower the pK_a of the boronic acid.** The exact role of the amino group of Shinkai-type receptors in sugar binding is still not completely clear. Because our structural studies⁷ showed that a solvent is inserted between the amine and the boron in both the boronic acid and boronate esters, the amine most likely plays a role in the formation of the boronate ester, either via: hydrogen bonding, salt-pair formation, or as a general-acid/general base catalyst. Any of these phenomena should have an **effect** on the kinetics of sugar binding. Moreover, it has not been demonstrated whether or not there is a direct structural interaction in the form of an intimate ion-pair between the boronate anion and the protonated ammonium in solution, as is seen in X-ray crystal structures,⁸ nor if such an interaction affects the kinetics and mechanism of boronate ester formation. Herein, we explore these issues via kinetics and isotope effect studies.

Results and Discussion

Kinetics with **1** and D-fructose

Much of the work regarding the question of whether or not a B–N bond is present in *o*-aminomethyl phenyl boronic acids and esters has been done with sensor **1** directly, so it was decided that this would be the most relevant starting point for our kinetic investigation. We choose 2 to 1 water/methanol solvent system for solubility purposes, and because our group has used this solvent system in many previous studies.

The first step was to generate the sensor and verify that it behaved as previously predicted. Figure 1 shows the pH titration of sensor **1** both with and without D-fructose. As previously reported for pure water,¹² a large fluorescence decrease is observed around pH 7 and another smaller decrease at pH 11. In the presence of fructose, there is a small decrease at neutral pH followed by a large decrease at high pH. We attribute these two pK_a 's to the same physical processes with and without the presence of fructose. The first pK_a is hydroxylation of the boronic acid, while the second pK_a corresponds to deprotonation of the ammonium.

Because we now know that the solvent insertion occurs for both the boronic acid and the boronate ester, there must be a different mechanism than both Shinkai/James or Wang propose for the fluorescent enhance, as shown in Figure 1. Although it will be the topic of a future paper, we herein reveal our postulate. We propose a subtle combination of the previous two theories; the increased Lewis acidity of the boronate ester over the boronic acid leads to a stronger B-OH (solvent) interaction, which concomitantly leads to greater protonation of the amine. This in turn would cause the observed change in fluorescence upon hydroxylation of the boron by reducing PET quenching from the amine lone pair. Note that the boronate ester formed between **1** and D-fructose is shown in Figure 1 with fructose acting as a tridentate ligand, as has been demonstrated by Norrild and co-workers.¹³

With the recognition that solvent inserted forms of the boronic acid and boronate dominate in protic media, we envision three distinct mechanistic pathways that could lead to boronate ester formation depending on the solution pH (Scheme 3). At a pH below pK_{a1} , the rate-determining step is likely nucleophilic addition, because its reverse to create sp^2 boron gives the experimentally determined boron hybridization at this pH, while all the other steps are rapid proton transfers (Scheme 3A). After the initial addition step, ring closure steps will be more rapid because they are intramolecular. At a high pH, those above pK_{a2} , the amine is deprotonated and therefore should not participate in the reaction mechanism to any appreciable degree (Scheme 3C).

At a pH between the first and second pK_a (the regime we have chosen to analyze, see below) we postulate there is an equilibrium requiring loss of the inserted solvent (water or methanol) from the boron prior to nucleophilic addition, creating what we label as intermediate **B** (Scheme 3B). The proximal amino group could directly facilitate this step by donating a proton to the hydroxyl group as a general-acid catalyst, creating a molecule of solvent as the leaving group. This would lead to intermediate **B** in which neither the amine nor the boronic acid are in their preferred protonation states at this pH. Because of the creation of this intermediate, it seemed probable that this would be the rate-determining step. By microscopic reversibility, the following nucleophilic addition step would be enhanced by the nearby deprotonated amine via general-base catalysis. Of course, all three mechanistic possibilities of Scheme 3 can occur at any pH, but the dominant pathway is predicted to be a function of pH as presented above.

We measured the rate of boronate ester formation at five pH values in a range above, near, and below the two pK_a values previously measured from Figure 1. All reactions were monitored at 4 to 6 different fructose concentrations that were at least ten-fold excess over the concentration of **1**, where [F] can be assumed to be $[F]_0$ (*pseudo*-first order). After data collection, the individual rate profiles were fit to a single exponential rate equation. After fitting, a plot of k_{obs} vs. [fructose] was generated for each pH (Figure 2). If the reaction is taken to be a reversible bi-molecular association, the rate of product formation can be described with Eq. 1 (F=Fructose, H=**1**, P=**1**:H). Expressing the concentration of the product in terms of the concentration of **1** and substituting into Eq. 1 gives Eq. 3. After simplifying, one derives Eq. 5. Therefore, a plot of k_{obs} vs. fructose concentration will give a linear plot

where the slope and y-intercept correspond to k_1 and k_{-1} respectively (Figure 2 and Table 1). This is an oversimplification because it does not take into account the distribution of protonation states of the boronic acid and boronate ester at the five pH values. However, it does give an estimate of the overall forward and reverse rates, k_1 and k_{-1} .

$$d[P]/dt = k_1[H][F]_0 - k_{-1}[P] \quad \text{Eq. 1}$$

$$[P] = [H]_0 - [H] \quad \text{Eq. 2}$$

$$d[P]/dt = k_1[H][F]_0 - k_{-1}[H]_0 + k_{-1}[H] \quad \text{Eq. 3}$$

$$d[P]/dt = [H](k_1[F]_0 + k_{-1}) \quad \text{Eq. 4}$$

$$k_{\text{obs}} = k_1[F]_0 + k_{-1} \quad \text{Eq. 5}$$

The forward (k_1) and reverse (k_{-1}) rate constants decrease from pH 5.3 to 9.5 as the hydroxide ion concentration increases and the reverse rate constant nearly plateaus. However, at pH 11.3 k_1 increases. This is because of increasing formation of fructose anion ($pK_a = 12.2$), which is a more nucleophilic ligand capable of faster reaction. The most important insight that this data gives is that in all cases the reaction shows a first-order rate dependence on fructose up to 4 mM in concentration. The fact that we find first order dependence upon fructose up to a concentration of 4 mM reveals that the loss of water from **1** at pH 8.7 in the 2:1 water:methanol must not be rate-determining. Instead, the association of fructose is still part of the rate-determining step(s) of the reaction. Yet, it also was evident that the reactions at these concentrations were not nearing completion at equilibrium, meaning we had not yet achieved a high enough concentration to saturate receptor **1** with fructose. We will return to this point below.

Kinetic Isotope Effects with **1** and D-fructose

One of the most important questions we sought to answer was whether or not the amino group acted as a general-acid catalyst to facilitate departure of the inserted solvent prior to nucleophilic addition of fructose. This would mean a significant degree of proton transfer in the transition state of the rate-determining step. The most direct way of detecting such an interaction is through kinetic isotope effects.

We decided to measure the reactions at a pH (**no comma here**) where both the boronic acid and fructose were in predominantly only one protonation state. For this reason, we chose pH 8.7. At this pH, the boronic acid is predominantly in its anionic form, and the amine is predominantly protonated. Likewise, fructose ($pK_a = 12.2$) is predominantly protonated.

The first step toward measuring an isotope effect was to perform pH titrations in hydrogen and deuterium solvents to see if there were any dramatic differences in the pK_a 's of either the boronic acid or boronate ester between these two solvents (Figure 3). The most important observation was that the pK_a 's of the boronic acid and boronate ester remained nearly constant on going from protio to deuterio solvent. The pK_a 's were approximately 0.5 units higher in the deuterated solvent, which is close to the 0.4 difference found for pH values read by a pH-meter.¹⁴

Also, the fluorescence in deuterio solvent was slightly higher than in protio solvent for both the free acid and boronate ester. It is known that deuterium has a lower zero-point energy than hydrogen. Therefore, in a scenario such as Figure 4, where the hydrogen or deuterium can reside either on oxygen or nitrogen, deuterium should prefer nitrogen slightly more than hydrogen does. If our explanation that the position of the proton controls the fluorescence regulation in **1** is correct, then replacing the ammonium hydrogen with deuterium should make **1** behave as if it were 'more' protonated, and the fluorescence should increase, as observed.

Next, the binding constants were determined under the conditions to be studied during the kinetic analysis (Figure 5). The binding constants were measured in both hydrogen and deuterium solvents. Both curves were fit to a 1:1 binding isotherm, to give association constants of $3.6 \times 10^3 \text{ M}^{-1}$ and $4.0 \times 10^3 \text{ M}^{-1}$ in hydrogen and deuterium solvents, respectively.

Once we were comfortable with the experimental conditions, we were ready to continue with our kinetic analysis. The reaction between **1** and D-fructose was monitored at pH 8.7, in protio and deuterio solvents (Supporting information, Figure S1). The rates of the reactions in both solvents increase, as well as the overall fluorescence, as the concentration of fructose increases. At high concentrations of fructose, the fluorescent signal starts to saturate, suggesting that the reaction is approaching 100% completion. This is in agreement with the binding isotherms above, where the signals saturate at 5 to 10 mM of fructose. The kinetic traces were fit individually to a single exponential rate profile, and a plot of k_{obs} as a function of fructose concentration shows distinct curvature at higher fructose concentrations (Figure 6). The plot is analogous to that given in Figure 2, except that the maximum fructose concentration in Figure 2 is 4 mM, while in Figure 6 it is approximately 18 mM. The curvature in the plots in Figure 6 tells us that as the concentration of fructose increases, the reaction changes from first-order to zero-order with respect to fructose concentration.

Saturation kinetics arises in reactions displaying a rate law in the form shown in Equation 6. Such reactions can be described as having a prior equilibrium, followed by a rate-determining step (Eq. 7–8, A=boronic acid, B=intermediate, and P=boronate ester). We postulated that the prior equilibrium is the loss of the inserted solvent, while the subsequent step is nucleophilic addition of fructose to the boronic acid (Scheme 3B). This reaction scheme gives rise to a rate law shown in Equation 9, where $[F]=[F]_0$ because we are using pseudo-first order conditions. Deriving the rate equation under the aforementioned considerations gives the rate law in Equation 11. When the fructose concentration becomes very large so that $k_2[F] \gg k_{-1}$, the equation simplifies to Eq. 13 which has no rate dependence on fructose concentration.

$$-d[A]/dt = \frac{a[A][B]}{1+b[B]} \quad \text{Eq. 6}$$

$$d[P]/dt = k_2[B][F]_0 \quad \text{Eq. 9}$$

$$[B] = \frac{k_1[A]}{k_{-1} + k_2[F]_0} \quad \text{Eq. 10}$$

$$d[P]/dt = \frac{k_1 k_2 [A][F]_0}{k_{-1} + k_2 [F]_0} \quad \text{Eq. 11}$$

$$k_{\text{obs}} = \frac{k_1 k_2 [F]_0}{k_{-1} + k_2 [F]_0} \quad \text{Eq. 12}$$

$$d[P]/dt = k_1[A] \quad \text{when } k_2[F]_0 \gg k_{-1} \quad \text{Eq. 13}$$

Saturation kinetics is nicely explained by the **loss** of an inserted solvent, giving rise to intermediate B in Scheme 3. Because we have previously shown that the inserted solvent is greatly dissociated in the complex, the **loss** of the solvent must involve proton transfer from the ammonium to the boronate anion leaving hydroxide or alkoxide, creating water or methanol respectively. Thus, the ammonium would be serving as a general-acid catalyst. Evidence for such a mechanism would be the observation of a primary kinetic isotope effect (KIE) on the k_1 step.

Equation 12 was used as a model to fit the two curves of Figure 6. Using the program Origin to optimize rate constants to best fit the curves (best fit lines are shown), the k_1 values were found to be 0.34 and 0.24 s⁻¹ for H₂O/CH₃OH and D₂O/DOCH₃) solvents, respectively. Hence, the KIE on k_1 is 1.42. While small, this KIE value demonstrates that the k_1 step does involve the breakage of a bond to deuterium. This supports our postulate that the *o*-aminomethyl group is acting as an intramolecular general-acid catalyst (as an ammonium), supplying a proton to the leaving group.

Yet, the fact that the value is small is also informative. In the solvent inserted species, the hydrogen bridges between the nitrogen of the amine and oxygen of the boronic acid, albeit is primarily on the nitrogen in the solid state.⁸ This small normal KIE points to the fact that the inserted solvent molecule is likely not fully dissociated, such that a full proton transfer is not required to facilitate leaving group departure. In the extreme, if the solvent were simply inserted between N and B and the O-H bond of the solvent not dissociated at all, no KIE would be expected. The KIE is therefore smaller than that associated with the breaking of a full covalent N-H bond, but is still positive. In summary, the small KIE value is suggestive of a state of sharing the hydrogen between the N and O in the solvent inserted boronic acid.

The denominator of Eq. 12 can be used to determine the relative rate constants for trapping the neutral and trigonal boronic acids with solvent (k_{-1}) or with fructose (k_2). This ratio defines the concentration that must be achieved by fructose to out compete the solvent for reaction with the intermediate. In H₂O/CH₃OH, the ratio of k_2 to k_{-1} is 200, while in D₂O/CH₃OD it is 160. However, we must remember that the k_{-1} value inherently includes the concentration of the solvent nucleophiles ($k_{-1} = k_{-1}'([H_2O] + [CH_3OH])$). We estimate that a 2:1 water:methanol mixture is about 45 M in total nucleophilic oxygen atoms (see supporting information). Hence, the k_2 to k_{-1} ratios should be multiplied by 45 to estimate the k_2 to k_{-1}' ratio, which in H₂O/CH₃OH results in a value of 9000 and in D₂O/DOCH₃ is 7,500. Therefore, in the water-methanol mixtures, fructose is approximately 7 to 9 thousand times more nucleophilic than the solvent mixture. This is likely due to the lower pK_a of

fructose relative to the solvent, which creates a significantly better nucleophile when involved in a general-base catalyzed addition reaction.

Conclusion

The kinetic studies described herein reveal for the first time saturation behavior in the reaction of *o*-aminomethyl phenyl boronic acids with sugars. The saturation is indicative of a prior equilibrium loss of a leaving group, followed by nucleophilic addition of the sugar. The mechanism is perfectly analogous to an S_N1 reaction, where the leaving group in the boronic acid is an inserted solvent. But, because the leaving group is the solvent, one needs a very large excess of sugar to reveal saturation kinetics. The large excess is required, even though we find that the nucleophilicity of fructose toward the intermediate is several thousand times greater than the solvent. Curve fitting of the kinetic data shows a KIE of 1.42, which albeit small, supports the role of the neighboring ammonium acting as a general-acid catalyst to facilitate solvent departure. By microscopic reversibility, the resulting amine would be a general base to facilitate delivery of a sugar nucleophile.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
pH Titration of **1** (1 μ M) alone (♦) and in the presence of 50 mM D-fructose (■), 33% aq. MeOH with 50 mM NaCl.

Figure 2.
Plot of observed rate versus fructose concentration for kinetic data collected at pH 5.3 (♦), 6.2 (●), 7.4 (■), 9.5 (▲), and 11.3 (+), with $[I]=(20\ \mu M)$.

Figure 3. pH profiles of **1** (10 μM) in 2:1 $\text{H}_2\text{O}:\text{CH}_3\text{OH}$ alone (◆), with 100 mM D -fructose (▲), and in 2:1 $\text{D}_2\text{O}:\text{CH}_3\text{OD}$ alone (■), and with 100 mM D -fructose (●).

Figure 4.
Potential energy diagram showing the differences in ZPE for the O–H(D) and N–H(D) bonds in **1**.

Figure 5. Binding isotherm for **1** (10 μM) and D-fructose in (◆) 2:1 $\text{H}_2\text{O}:\text{CH}_3\text{OH}$ and (■) 2:1 $\text{D}_2\text{O}:\text{CH}_3\text{OD}$ with 50 mM NaCl at pH(D) 8.7.

Figure 6. Plot of k_{obs} as a function of fructose concentration in 2:1 $\text{H}_2\text{O}:\text{CH}_3\text{OH}$ (♦) and in 2:1 $\text{D}_2\text{O}:\text{CH}_3\text{OD}$ (■) at $\text{pH}(\text{D}) = 8.7$, $10 \mu\text{M}$ 1, 50 mM NaCl .

Scheme 1.

Overview of the species postulated by the B–N bond (top) and solvent insertion (bottom) mechanisms.

Scheme. 2.

Scheme 3.

Predicted reaction mechanisms for boronate ester formation at A) low, B) intermediate, and C) high pH. 'An' represents the anthracene functionality in **1**.

Table 1

Overall forward and reverse rate constants measured from the rate data shown in Figure 2.

pH	k_1	k_{-1}
5.3	36.0	0.44
6.2	33.0	0.09
7.4	16.4	0.04
9.5	10.3	0.03
11.3	23.6	0.05