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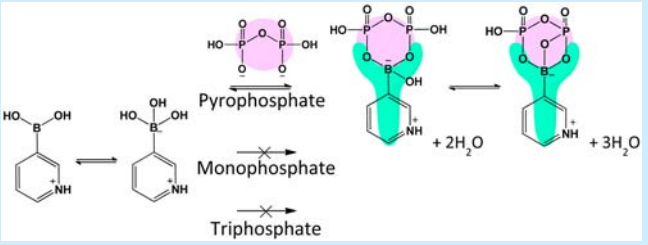
Boronate Based Metal-Free Platform for Diphosphate-Specific Molecular Recognitions

Mai Sanjoh,[†] Daisuke Iizuka,[†] Akira Matsumoto,*[,†] and Yuji Miyahara*[,†]

[†]Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Kanda-surugadai 2-3-10, Chiyoda-ku, Tokyo 101-0062, Japan

Supporting Information

ABSTRACT: A reversible boronate−diol interaction provides a versatile synthetic platform for molecular recognitions whose binding specificity can be molecularly tailored. We found that boronate derivatives with relatively strong acidity generally undergo a diphosphate-specific recognition among other phosphates under weakly acidic pH conditions, a feature relevant to DNA sequencing. ^{11}B and ^{31}P NMR studies identified "tetrahedral boronate and divalent diphosphate" as a pair responsible for forming a 1:1 stoichiometric complex, which manifests as a unique pH-dependent stability.



Phosphates and organophosphorus compounds are commonly found structural components of life and play many determinant roles in cellular processes including signal transduction and metabolic control. Phosphorylation, or the addition of a phosphate group to a protein, is a dynamic process of post-translational modification that provides a regulatory mechanism for function and activity of the protein.[1,2] In drug therapy, many nucleoside analogues, which are currently in use as antiviral and antitumor agents, are dosed in the form of inactive prodrugs, whose intracellular activations also rely on kinase-driven phosphorylation.[3,4] Therefore, abilities to control and monitor (de)phosphorylation and the related metabolites would aid in both diagnostic and therapeutic purposes. Pyrophosphate (PPi) is of particular interest due to its involvement in several important cellular enzymatic reactions.[5−7] For example, during DNA replication reaction catalyzed by DNA polymerase, it is stoichiometrically produced on each occasion of the single base synthesis. Therefore, the detection of PPi is relevant to DNA sequencing, a technique known as pyrosequencing.[8,9] Further, a change in fluid PPi concentration has been implicated in several pathological conditions including tumors.[10,11] Aside from the gold-standard enzymatic determination methods, there are some reports on synthetic PPi sensors capitalizing on binuclear metal coordination chemistry in which each metallic center is designed to chelate with two PPi oxygen atoms thereby inducing changes in optical and electrical properties of the complex.[12−14] These compounds show remarkably high selectivity and binding stability toward PPi, whose binding constants (K) are typically found on the order of 10^8 M^{-1}.[14] However, these interactions are in practice irreversible and thus may not be suitable for continuous monitoring and other potential biomedical applications, where more dynamic, temporal, and reversible interactions prevail with biological significance as illustrated above.

Herein, we describe a selective and yet reversible type of complexation between PPi and boronic acid derivatives that may offer a synthetic molecular basis for spatiotemporal interaction and monitoring of PPi and its derivatives in an environmentally sensitive fashion. Boronic acid derivatives readily complex with 1,2- and 1,3-*cis*-diols including those found on carbohydrates, ribose, and catechol through reversible boronate ester formation in an aqueous solution.[15] These interactions have been applied to a number of chemo-sensing, separation, diagnostic, and therapeutic applications.[16−21] Boronic acid compounds are compatible with human physiology, as exemplified by the fact that some of them have been used as chemotherapeutic agents and in other therapies.[22−24] Advantageously, the binding strength and the target specificity can be, to some extent, tailored on the basis of stereochemistry and the control of stereoelectronic effects.[25,26] Nonetheless, to our knowledge, any diphosphate-specific molecular recognition by boronic acids has yet to be reported. We found that derivatives exhibiting relatively strong acidity (or low pK_a) generally do show such a binding specificity.

Evidence for the specific interaction between boronic acid and PPi was first disclosed in ^{11}B and ^{31}P NMR studies on 3-pyridylboronic acid (3-PyBA) (Figure 1). ^{11}B NMR is commonly used to elucidate boronic acid−diol interactions where the observed chemical shift is sensitive to hybridization state of the boron nucleus, i.e., trigonal or tetrahedral.[27,28] Typically, the tetrahedral state or boronate displays the chemical shifts at around 0 ppm, whereas the trigonal state (boronic acid) results in a significant downfield resonance. In the absence of binding targets, due to a rapid interconversion between the two hybridization states, only one signal appears at

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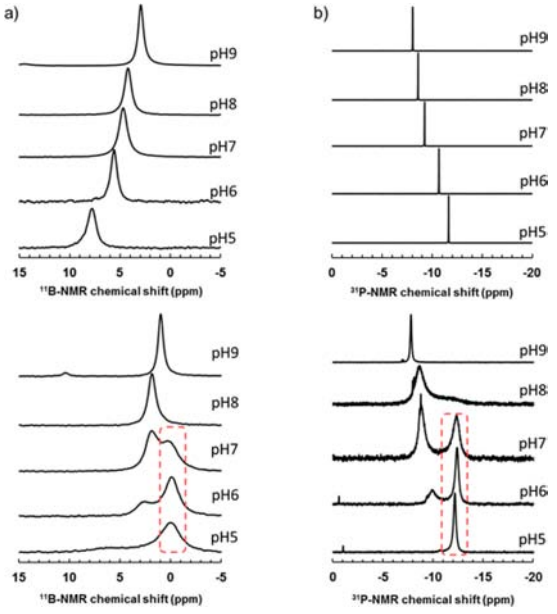

Figure 1. (a) ^{11}B NMR spectra of a 20 mM 3-PyBA aqueous solution for various pH's without (top) and with (bottom) 20 mM PPi. An area highlighted with red dots indicates the resonance attributable to the PPi-bound boronate. (b) ^{31}P NMR spectra of a 20 mM PPi aqueous solution without (top) and with (bottom) 20 mM 3-PyBA. The red-dot area highlights the resonance attributable to the boronate-bound PPi.

a field corresponding to the ratio of the two states at a given pH; as a result, the chemical shift undergoes a downfield shift with decrease of the pH over the range of the pK_a value of 3-PyBA (ca. 4.4) (Figure 1a: top). In contrast, when allowed to complex with PPi (Figure 1a: bottom), since the rate of exchange between the free and the PPi-complexed forms of boronic acid/boronate is now slow relative to the ^{11}B NMR time scale, the spectrum consists of two peaks: a free boronic acid/boronate peak, whose chemical shift shows a downfield shift with decrease of the pH similar to the above, and a peak attributable to those complexed with PPi at a higher field (around 0 ppm). Figure 1a (bottom) also reveals that the 3-PyBA/PPi complex peak becomes dominant with decrease of the pH and, correspondingly, that for the free state is dramatically weakened. This characteristic feature of the 3-PyBA/PPi complex was also confirmed from ^{31}P NMR analysis (Figure 1b). That is, PPi alone shows only a single peak for all range of the pH (Figure 1b: top), but in the presence of 3-PyBA an additional resonance appears at around −12 ppm when the pH is decreased, which is attributable to the complex (Figure 1b: bottom). Many other derivatives of boronic acid were also examined for the interaction with PPi under neutral to acidic pH conditions. So far, the binding has been unique to those exhibiting relatively strong acidity with pK_a values no greater than 7.1, most prominently with 3-PyBA, which had the lowest pK_a value (ca. 4.4) of all the investigated series. These observations, along with the fact that the complex peak appears at around 0 ppm of ^{11}B NMR attributable to the tetrahedral state (Figure 1a: bottom), suggest that only the boronate form is favored for the PPi-binding; in this regard, more supporting arguments will be provided in later paragraph.

From ^{11}B NMR peak intensity ratios, equilibrium binding constants (K) between 3-PyBA and a series of (organo)-phosphates were determined, and some representatives are shown in Figure 2 (see also Table S1, Supporting Information). Note that the maximum K value obtained for the 3-PyBA/PPi

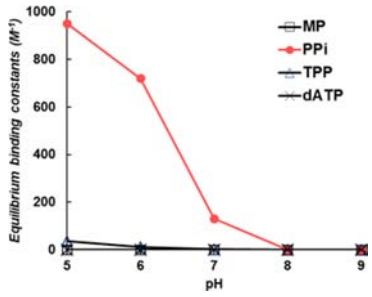

Figure 2. Binding constants (K) between 3-PyBA and various (organo)phosphates as a function of pH, as determined by ^{11}B NMR analysis; monophosphate or MP (open squares), diphosphate or PPi (red solid circles), triphosphate or TPP (open triangles), deoxyadenosine triphosphates or dATP (crosses).

complex in Figure 2 (950 M^{-1} at pH 5) is categorized as the highest class of all the boronic acid involved interactions hitherto reported.[29−31] Also worthy of mention is that 3-PyBA has no interaction with deoxyadenosine triphosphate (dATP) within the detection limit of ^{11}B NMR (Figure 2, see also Figure S6 and Table S1, Supporting Information). As mentioned above, this PPi-specific molecular recognition out of a mixture of deoxynucleotide triphosphates (dNTP) such as dATP is directly applicable to the pyrosequencing and the monitoring of polymerase chain reactions (PCR). Interestingly enough, 3-PyBA hardly binds with mono- (MP) or triphosphate (TPP), resulting in a strikingly diphosphate-specific interaction (Figure 2). It may be reasonable to observe that MP, which lacks 1,2-*cis*-diol configuration, is unable to bind with 3-PyBA. Somewhat surprising is an inferior outcome for TPP compared with PPi, despite its superiority in the number of hydroxyl groups (five) to that for PPi (four), which appears to be an advantage for the boronate binding. The contribution of electrostatic repulsion, which should matter between (both anionic) boronate and phosphates and to a further extent with TPP (due to its greater anionic charges compared to PPi), thus hampering its approach to the boronate more significantly, is unlikely to be the reason; the PPi-selective binding behavior remained unchanged even with excessively increased salt concentration of up to 500 mM NaCl in order to exclude such an effect (Table S1, Supporting Information). Although more detailed investigation is required to account for the origin of the selectivity, suggested possibilities include an unfavorable steric hindrance and a higher intramolecular mobility of TPP as well as a different level of Lewis acidity between these binding targets, which has also been proposed as a rate-determining factor in the 3-PyBA-involved binding.[32] Other relevant organophosphates, including deoxyadenosine diphosphate (dADP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP), were also assessed for the interaction with 3-PyBA (Figures S4, S5, and S7, Supporting Information, respectively). In this series, only the latter two, ADP and ATP, were able to bind with 3-PyBA. Comparative ^{11}B and ^{31}P NMR analyses uncovered that these bindings had occurred only at their ribose functionality. Deoxyadenosine diphosphate (dADP) was not able to bind with 3-PyBA, despite its available diphosphate functionality. On the other hand, a nucleoside analogue (chemotherapeutic agent) gemcitabine diphosphate (GDP), which is less bulky in the structure compared to the

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above-mentioned molecules, did show the diphosphate-boronate binding (Figure S9, Supporting Information). Taken together, the steric factor seems to be an important determinant for the plausibility and the choice of the binding sites i.e., ribose or diphosphate, in these molecular recognitions. Also worthy of mention is that an N-substituted derivative, 3-(*N*-carboxymethyl)pyridinium boronic acid, could also bind with PPi (Figures S10 and S11, Supporting Information). These observations may raise the possibility of using the diphosphate−boronate interaction as a route for facile and reversible chemical conjugation (along with further room for N-substitution) and a bioreversible (e.g., kinase-dependent) way of protection of the nucleoside analogues, which may help improve the pharmacodynamics and thus eventually the efficacy of these drugs.[33]

The NMR titration experiment shown in Figure 3 validates the prior hypothesis that the existence of boronate (as opposed

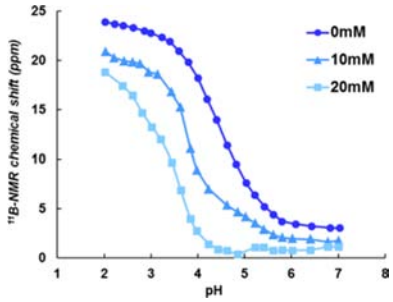

Figure 3. Plots of the centroid values of the chemical shifts obtained in ^{11}B NMR spectra of a 20 mM 3-PyBA aqueous solution for various pH's and PPi concentrations; 0 mM (navy solid circles), 10 mM (sky blue solid triangles), and 20 mM (aqua solid squares).

to the boronic acid form) is a prerequisite for the stable PPi-binding. In the absence of PPi, an inflection point of the curve is noted at around pH 4.4, in agreement with its reported value of the pK_a.[34] With addition of PPi, the apparent pK_a of 3-PyBA (inflection point of each titration curve) undergoes a marked downward shift, indicating an apparently increased acidity of the boronate. This trend, characteristic of a majority of reported boronic acid-diol interactions, confirms that the tetrahedral boronate indeed predominates as a form responsible for the stable binding.[23] This situation is schematically presented in parts a and b of Figure 4 together with a proposed 1:1 manner complex structure in accordance with the result of mass-spectrometric analysis (Figure S12, Supporting Information). Both diester and triester formations are possible in the complex,

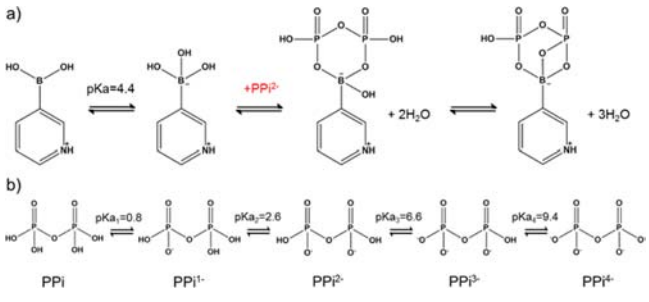

Figure 4. (a) pH- and PPi-dependent equilibria of 3-PyBA along with the proposed complex structures. (b) pH-dependent equilibria of PPi.

which are yet to be discriminated.[35] Therefore, these two possible structures are presented as equilibria in Figure 4a.

A question remains as for the observed pH-dependent stability of the complex in Figure 2. As judged from the titration curve under the condition of 20 mM PPi (Figure 3), it is evident that at pH 5 and above the boronate solely exists (with no fraction of the boronic acid). Nonetheless, Figure 2 reveals that a marked pH-dependent decay of the complex stability occurs with increase of the pH over an apparently irrelevant range to that for the boronic acid/boronate equilibrium. A change in the protonation state of PPi is most likely a cause, for which four distinct pK_a's have been identified at 0.8, 2.6, 6.6, and 9.4, respectively (Figure 4b). Among these, the equilibrium between PPi^{2-} and PPi^{3-} (pK_a = 6.6) appears to coincide well with the profile of the pH-dependent complexation (Figure 2), signifying that the divalent PPi^{2-} is critical for the boronate interaction. This interpretation has been further supported from analysis of other boronic acid derivatives with systematically differentiated acidity or pK_a. That is to say, for those derivatives shown in Figure 5a possessing pK_a values of 6.3−7.1

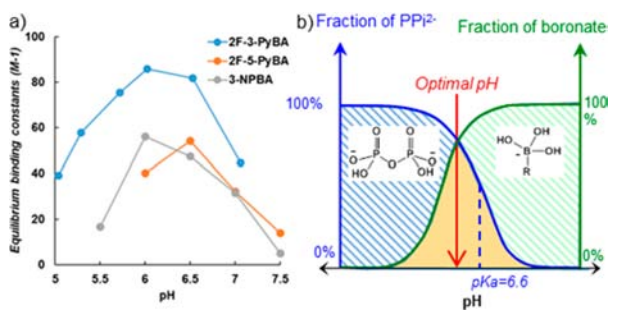

Figure 5. (a) Binding constants (K) between PPi and three boronic acid derivatives with distinct pK_a's, as determined by ^{11}B NMR analysis, as a function of the pH; 2-fluoro-3-pyridylboronic acid or 2F-3-PyBA (blue, pK_a = 7.1), 2-fluoro-5-pyridylboronic acid or 2F-5-PyBA (orange, pK_a = 7.0), 3-nitrophenylboronic acid or 3-NPBA (gray, pK_a = 6.3). (b) Schematic model to explain both pH- and pK_a-dependent complex stability.

(close to that for the PPi^{2-}/PPi^{3-} equilibrium, i.e., 6.6), somewhat optimal pH's for the complex stability appear at around 6−6.5 (Figure 5a; see also Figure S8, Supporting Information). As illustrated in Figure 5b, the appearance of these optimal pH's may be ascribed to the occurrence of maximal boronate/PPi^{2-} pairings (available for the complexation), which are sensitive to each derivative's pK_a's relative to that for PPi. Such a relationship also accounts for the observation that the boronate/PPi interaction was unique to those derivatives with the pK_a values no greater than 7.1; above this threshold pK_a, a chance is lost for the boronate and the divalent PPi (PPi^{2-}) to coexist. Thus, these observations safely establish that the diphosphate-boronate interaction herein demonstrated stems from an exclusive affinity between the boronate and the divalent PPi (PPi^{2-}), probably due to its suitable strength in the Lewis acidic interaction.

Our finding demonstrates that a controlled boronate−diol interaction, which is probably the simplest chemistry of all the relevant reports, can manifest itself as a diphosphate-selective molecular basis in a reversible and pH-dependent manner. This relatively weak and thus dynamic mode of the interaction may address the otherwise difficult challenges such as continuous monitoring of organophosphates and other biointeractive

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applications, to which temporal and reversible enzymatic events (involving organophosphates) are inherent. Such a reversible nature of the interaction may also be translated into solid-state electronics and optics, e.g., in the form of washable and reusable electrodes. The generality of the binding and extensibility toward more complex molecular designs, as validated for the N-substituted system, may suggest other possibilities including the controlled pharmacodynamics and the delivery of nucleoside analogues.

■ ASSOCIATED CONTENT

Supporting Information

More detailed procedures for ^{11}B and ^{31}P NMR studies along with additional spectral data, preparation of boronic acid derivatives, mass-spectral analysis, and a comprehensive table of equilibrium binding constants of the boronates for various targets. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: matsumoto.bsr@tmd.ac.jp.
*E-mail: miyahara.bsr@tmd.ac.jp.

Notes

The authors declare no competing financial interest.

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