CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200701911

Complexation of L-Lactate with Boronic Acids: A Solution and Holographic Analysis

Felicity K. Sartain,[a, b] Xiaoping Yang,[a] and Christopher R. Lowe*[a]

Abstract: Boronic acids have been used as receptors for the detection of diols and α-hydroxy acids. The incorporation of 3-acrylamide phenyl boronic acid (3-APB) into a hydrogel generates a suitably responsive and fully reversible holographic sensor for L-lactate. However, it was also found that the use of 3-APB resulted in the sensor being responsive towards a number of other compounds containing two hydroxy groups. This report details the further investigation into the reaction between L-lactate and three boronic acid-based receptors, both in the holograms and in solution, in order to establish the mechanism of binding. A novel boronate receptor is proposed based on this understanding.

Keywords: boronic acid · lactate · ligands · receptors · sensors

Introduction

Boronic acids are known to form complexes with bidentate compounds in aqueous solution.[1,2] The reaction tends to be rapid and reversible, and has resulted in boronates being utilised as affinity ligands for the separation of carbohydrates, as well as recognition ligands in saccharide sensors.[3] Boronates have also been shown to bind *o*-diphenols, *o*-hydroxy acids, dicarboxylic acids and α-hydroxy acids,[4–9] but of late less research has focussed on these reactions.

L-Lactate is a key metabolite, the detection of which is important across a number of disciplines, including clinical diagnostics[10,11] and sports medicine.[12,13] The integration of boronic acids into polymers has been reported previously[14] and more recently a hydrogel was generated that enabled the holographic determination of L-lactate.[15] Although it was demonstrated that the response towards L-lactate was fully reversible under physiological conditions, the sensor was found to respond to other small metabolites that could potentially interfere with eventual in vivo determination of L-lactate. The concentration of pyruvate, galactose and fructose in blood is <0.1 mM,[16,17] thus it is unlikely that these compounds will interfere significantly with the L-lactate reading. Glucose, however, is present in significantly higher concentrations (≈ 5 mM[18]), hence it is probable that this would be the primary interferent if this holographic sensor were to be used on a physiological sample. It has been demonstrated that this selectivity problem can be overcome simply by adjusting the composition of the hydrogel, which in turn can alter the sensitivity of the 3-acrylamide phenyl boronic acid (3-APB) holographic sensor for L-lactate over glucose[15] or vice versa.[19] However, this approach merely masks the interference problem. Through gaining a more thorough understanding of the reactions involved it is anticipated that this issue can be resolved, thus enabling the development of a selective receptor for L-lactate.

The main difference between these two bidentate chelators is that one is a diol and the other is an acid anion at physiological pH. Furthermore, glucose is a more rigid molecule and considerably less acidic, and such characteristics are believed to be of mechanistic significance.[7] Thus, glucose and L-lactate cannot be treated as comparable ligands.

Previous holographic studies[15] showed that a stable signal was gained much quicker following addition of any concentration of L-lactate than after addition of the same concentration of glucose, for example, the response of the holographic sensors to 2 mM lactate ($t_{90\%}=9$ min) compared to 2 mM glucose ($t_{90\%}=31.5$ min). In general, the chemically induced kinetics of a hydrogel-based sensor are dependent on the systemic free energy of a hydrogel matrix and the affinity of a receptor. In this instance, because the sensor responds to both glucose and L-lactate, it is believed that the

[a] Dr. F. K. Sartain, Dr. X. Yang, Prof. C. R. Lowe
Institute of Biotechnology, University of Cambridge
Tennis Court Road, Cambridge, CB2 1QT (UK)
Fax: (+44) 1223-334162
E-mail: crl1@biotech.cam.ac.uk

[b] Dr. F. K. Sartain
Current address: Smart Holograms Ltd., 291 Science Park
Milton Road, Cambridge, CB4 0WF (UK)

Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author.

difference in the rate of response of these two molecules to the sensor is most likely due to differences in their binding-site structures. Because L-lactate and glucose have fundamentally different chemistries, it is likely that they bind to boronic acid through different mechanisms, and the mechanism of a reaction can significantly affect the rate of reaction.[20]

A considerable amount of research has been conducted to elucidate the mechanism by which diols (such as glucose) bind to boronic acids.[1–3,21] It has been proposed that the mechanism of binding of a diol to a boronic acid proceeds via an associative transition state, by which proton transfer from the incoming ligand to the leaving hydroxyl from the boron occurs, with two water molecules being produced.[21] In each case it is the B–O bonds that are broken, not the C–O bonds.[22] Glucose is thought to behave in this manner, with most researchers believing that the reaction occurs more readily with the tetrahedral form of the boronate.[1,7,23]

Conversely, the reaction between α-hydroxy acids, such as L-lactate, and boronic acids has not been so closely investigated. Pizer and co-workers[5,6] have specifically analysed the reaction between L-lactate and phenylboronic acid and boric acid, respectively. They suggest that the mechanism by which L-lactate binds to the boronate centre occurs through attack of the hydroxyl oxygen on the boron, and this association orients the carboxylate properly for chelation, with the loss of a hydroxyl.[6] This mechanism is similar to that determined for glucose, yet it is apparent[5–8] that L-lactate preferentially binds to the trigonal boronic acid conformer.

In later work by Pizer et al.,[21] it was intimated that the mechanism by which these two analytes bind is indeed different; however, this has not been proven conclusively. Thus, by conducting further analysis into the binding reaction between L-lactate and three boronate ligands, utilising multinuclear NMR techniques and the holographic data gained, it was anticipated that the mechanism of binding between L-lactate and boronic acids could be confirmed.

This paper reports the investigation into the reaction between the L-lactate and three boronic acid receptors, in particular with respect to the mechanism of binding of L-lactate compared to that observed with glucose.

Results

Solution analysis of the reaction of L-lactate with 2-, 3- and 4-APB utilising $^{11}B/^{1}H$ NMR spectroscopy and the pH-depression method: It is suggested that the reaction between a boronic acid and an α-hydroxy acid occurs preferentially with the trigonal form of the boronate,[5,6] but it is also implied that the reaction can occur with the tetrahedral form, albeit at a slower rate.[4] At pH 7.4 the three boronate receptors have been shown to adopt different conformations at the boron centre,[14,24] 3- and 4-APB are predominantly trigonal whereas 2-APB adopts a zwitterionic tetrahedral form. Previous studies have shown that ^{11}B NMR spectroscopy is a suitable analytical tool for the determination of boronate species,[20,23] and more information concerning the identity of boronate compounds can be obtained by employing this technique, rather than the traditional solution titration methods. Thus, by utilising multinuclear NMR spectroscopy to monitor the reaction between each of these receptors and L-lactate in aqueous solution it is possible to demonstrate whether a binding reaction occurs and a complex between the receptor and analyte is formed.

Solutions of each boronate were made up in D_2O and pD adjusted to about 7.4, and L-lactate solutions were prepared in each of the boronate solutions. The L-lactate solution was added to each boronate solution in turn and the reactions monitored by using $^{11}B/^{1}H$ NMR spectroscopy and the pH-depression method. The pH-depression method measures the increase in acidity as the diol is added to the boronic acid and is used to determine complex formation upon the assumption that the boronate ester is far more acidic than the boronic acid.[1]

As L-lactate was added to the solutions of 3- and 4-APB, respectively, it was apparent that a new species was formed, as indicated by the resonance at $\delta = -10.00$ ppm, which is indicative of the generation of the tetrahedral boronate–L-lactate complex. Two peaks are present for the majority of the experiment, this shows that the boronate ester is stable and that species exchange is slow on the ^{11}B NMR timescale. The positive chemical shift of the other signals ($\delta = \approx 10$ ppm) indicates that trigonal species are present (Figure 1). In each case there is a marginal upfield shift of

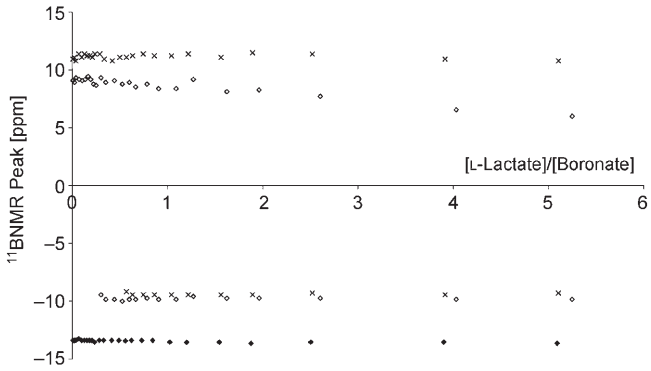


Figure 1. Comparison of ^{11}B NMR peak shifts upon addition of L-lactate to solutions of 10 mM 2-APB (◆), 3-APB (◇) and 4-APB (×) prepared in D_2O and pD adjusted to 7.0, at room temperature.

these resonances, which implies that the boronic acid equilibrium is moving very slowly towards the tetrahedral species. This slight shift in the equilibrium may be due to the change in pD, which overall increased by only about 0.3 of a pD unit as the experiments progressed.

The addition of L-lactate did not affect the ^{11}B NMR spectrum of 2-APB at all, as only one peak at $\delta = -13.46$ ppm was observed throughout (Figure 1). Again a change in pD was recorded that was comparable to that seen with 3- and 4-APB, with an overall increase to pD ≈ 7.54. ^{1}H and ^{11}B NMR spectroscopy have been shown to be suitable ana-

lytical tools for the determination of various borate species,[20,23,29,30] and thus it is thought that any conclusions drawn from these experiments should rely mostly on the results gained from these two techniques. This is not to say that the fluctuations in pD should be ignored: Instead, the changes can be viewed as providing a pD monitor during the addition rather than as definite proof of complex formation.

Ultimately, these solution assays have demonstrated that the response observed previously with the holographic sensors[15] reflects what is observed in solution, with L-lactate binding to 3- and 4-APB but apparently not to 2-APB.

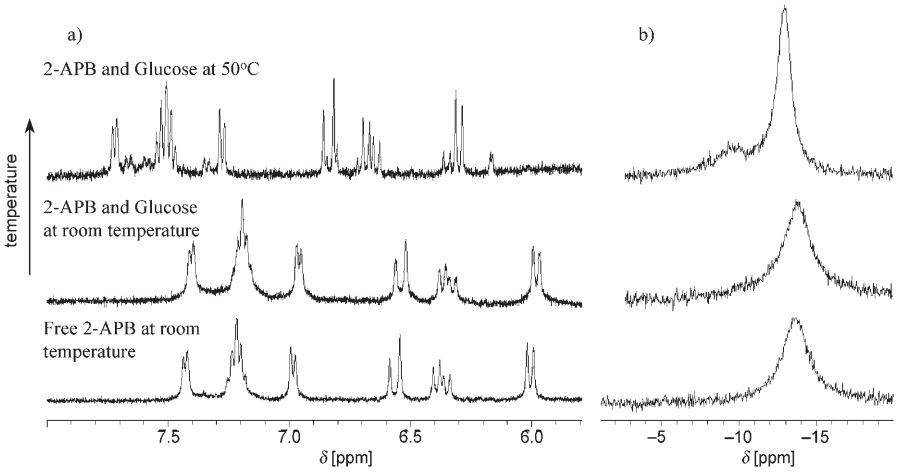


Figure 2. a) Comparison of ^{1}H NMR spectra recorded for ≈20 mM 2-APB in D_2O (pD adjusted to ≈7.7) in the absence of glucose, with 2–3 mg glucose at room temperature and with glucose at 50 °C. b) Comparison of ^{11}B NMR spectra recorded for ≈20 mM 2-APB in D_2O (pD adjusted to ≈7.7) in the absence of glucose, with glucose at room temperature and with glucose at 50 °C.

Solution assays with 2-APB and glucose and L-lactate, respectively: A comparison of the reaction between 2-APB and L-lactate and glucose, respectively, in solution was performed and monitored by ^{1}H/^{11}B NMR spectroscopy. This would determine whether or not it was possible for glucose to bind to 2-APB, as it had become apparent that L-lactate did not. Because the reaction between glucose and a boronic acid is thought to occur more readily with the tetrahedral form,[1,7,22] it was assumed that this analyte would be able to bind to the zwitterionic tetrahedral 2-APB receptor.

A 2-APB solution (≈20 mM) in D_2O was made up and pD adjusted to about 7.7. Subsequently ^{11}B and ^{1}H NMR spectra were recorded to compare the difference between 2-APB alone (control) and in the presence of about one equivalent of glucose. At room temperature no change in either spectrum was observed; however, when the temperature was raised to 50 °C, the peak at $\delta = -13.46$ ppm in the ^{11}B NMR spectrum became sharper, and shifted slightly downfield, but also an additional smaller peak was observed at $\delta = -9.95$ ppm. Comparable changes were also noted in the ^{1}H NMR spectrum (Figure 2). The additional resonances observed at 50 °C in the ^{1}H NMR spectrum and the peak at $\delta = -9.95$ ppm in the ^{11}B NMR spectrum further indicate that glucose forms a complex with 2-APB at 50 °C.

The experiment was then repeated with L-lactate (≈1 equivalent). Interestingly, the increase in temperature caused the resonances to shift downfield slightly, but no additional peaks were observed in the ^{1}H NMR spectrum, indicating that no complexation had occurred. Furthermore, there was no change in the peak observed in the ^{11}B NMR spectrum in response to increasing temperature. Again this suggests that L-lactate does not bind with 2-APB (Figure 3).

These results suggested that heating the 2-APB complex to 50 °C affected the ability of the monomer to bind glucose, but still prevented L-lactate from chelating. A plausible explanation is that in aqueous conditions 2-APB may self-associate to form a trimer that is dissociated on heating to 50 °C, releasing the hydroxyl groups that are then free to react with glucose (Scheme 1). It has also been observed[24] that there are very strong hydrogen bonds between the OH

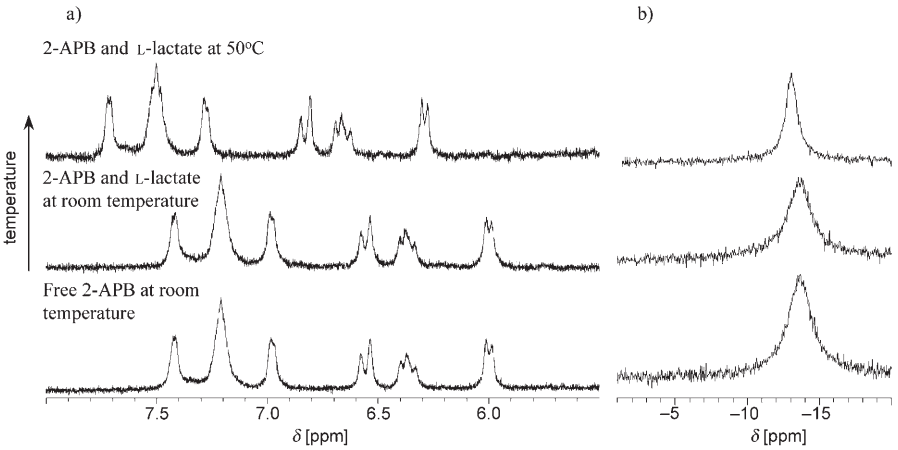
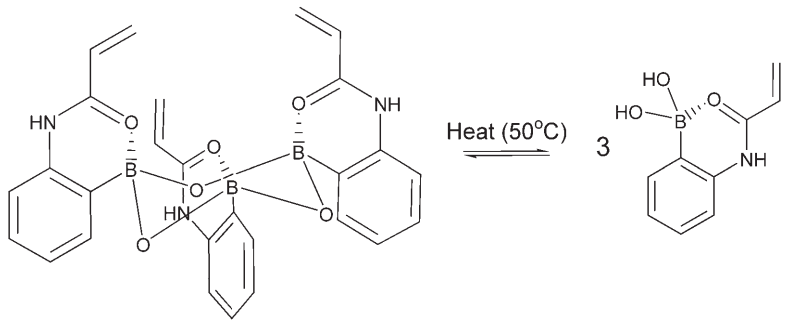


Figure 3. a) Comparison of ^{1}H NMR spectra recorded for ≈20 mM 2-APB in D_2O (pD adjusted to ≈7.7) in the absence of L-lactate, with 2–3 mg L-lactate at room temperature and with L-lactate at 50 °C. b) Comparison of ^{11}B NMR spectra recorded for 2-APB (≈20 mM) in D_2O (pD adjusted to ≈7.7) in the absence of L-lactate, with L-lactate at room temperature and with L-lactate at 50 °C.



Scheme 1. Proposed trimer of 2-APB at room temperature which dissociates on heating to 50°C.

groups of 2-APB, which may partly explain why 2-APB binds only weakly to glucose relative to the "trimer." However, further experimental studies are necessary to confirm which of these hypotheses is most probable.

Response of the 2-APB holographic sensor to L-lactate and glucose, respectively: A 20 mol% 2-APB holographic sensor was made and subsequently its response to L-lactate and glucose, respectively, was monitored. It was found that L-lactate did not induce a response, however, the addition of 12 mM glucose resulted in a contraction of about 30 nm.[24] These holographic experiments further demonstrate that glucose and L-lactate behave differently towards the 2-APB monomer. It is apparent that L-lactate is unable to chelate with the zwitterionic tetrahedral form of 2-APB, whereas glucose can. This lack of binding between L-lactate and 2-APB indicates that this analyte does not bind to a boronate centre through the same mechanism as glucose.

Two-dimensional NMR analysis of the species generated from reaction of L-lactate with 2-, 3- and 4-APB: This analysis aimed to confirm that L-lactate bound to the trigonal form of a boronic acid, thereby generating the tetrahedral ester complex. In an attempt to elucidate the complex structures for the boronate ester generated upon reaction of each of the boronic acid receptors with L-lactate, a series of ^{1}H and ^{11}B NMR spectra were recorded for each of the boronates investigated in the presence and absence of L-lactate in dimethyl sulfoxide ([D_6]DMSO). [D_6]DMSO was used to eliminate any pH effects in an attempt to simplify the system and also enables OH peaks to be identified. For each receptor, three sets of ^{1}H and ^{11}B NMR spectra were analysed; initially the receptor alone was in solution, then L-lactate was added in a 1:1 molar ratio, and finally the solution mixture was analysed after a D_2O shake. Figure 4 summarises the results obtained. (Spectra are shown in Supporting Information).

It was assumed that the quasi-tetrahedral form of 2-APB was observed in the ^{11}B NMR spectra, as a broad peak was recorded at $\delta = -4.97$ ppm, whereas with 3- and 4-APB a broad signal was recorded at $\delta \approx 11$ ppm, which is indicative of a trigonal conformation. In the ^{1}H NMR spectra, the first noticeable difference between the three compounds was that the OH peaks from 2-APB were not observed, however, it is not always possible to detect the OH resonances even if they are run on a broad scale. Upon the addition of L-lactate to the 2-APB solution a clear upfield shift was observed in the ^{11}B NMR spectrum (from $\delta = -4.97$ ppm to $\delta = -10.17$ ppm) and changes in the resonances were also observed in the ^{1}H NMR spectrum, indicating that in neutral non-aqueous solvent L-lactate can bind to 2-APB. It is suggested that under these conditions the complex generated is similar to that observed with methanol,[31] in which the hydroxyl group binds to the boron centre[23] (Scheme 2).

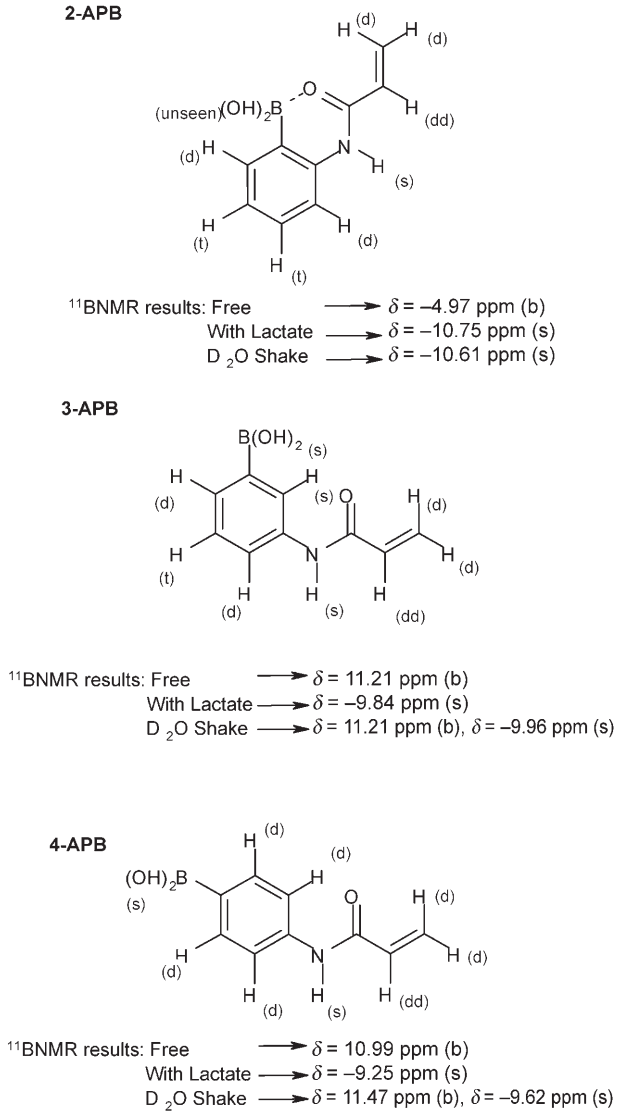
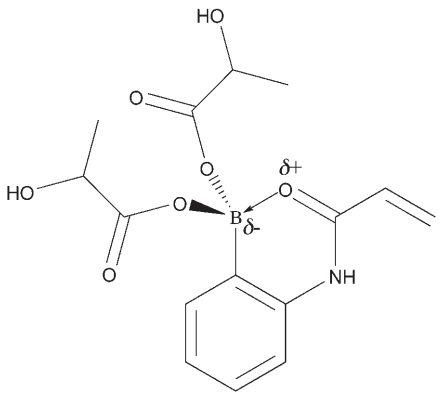


Figure 4. Summary of NMR results obtained in [D_6]DMSO. The proton splitting for the "free" receptors are given in parentheses and the resonances recorded from the ^{11}B NMR analysis are tabulated below each boronate molecule.



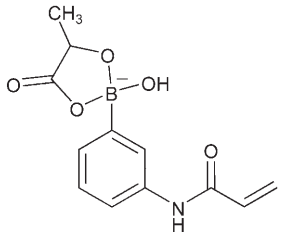
Scheme 2. Structure of the complex formed between 2-APB and L-lactate in [D_6]DMSO.

A comparison of the resonances gained from each ^{1}H and ^{11}B NMR spectrum for the complex formation of L-lactate with 2-APB to those obtained with L-lactate and 3- and 4-APB, respectively, was made. It was apparent that the resonances that dominated in the 2-APB–L-lactate reaction were different to those noted with L-lactate and 3- and 4-APB, respectively. The behaviour of 3-APB and 4-APB was similar: both were initially in the trigonal form and upon the addition of L-lactate there was a switch to a tetrahedral form. Furthermore, new peaks appeared in the ^{1}H NMR spectrum after L-lactate addition, and thus it was apparent that a complex had formed. After the D_2O shake, a mixture of boronate species was observed, and similar resonances ($\approx$10.5–6 ppm) in the ^{1}H NMR spectra were affected.

It was difficult to distinguish from this one-dimensional data which peaks belonged to which species, and thus a series of two-dimensional NMR spectral analyses were conducted with only 3-APB and L-lactate, as it was assumed that 4-APB would behave in the same fashion.

The COSY spectrum revealed that there were four different species of L-lactate present in the solution. One was confirmed to be "free" L-lactate ($\delta = 1.13$ ppm, $\delta = 3.62$ ppm). The most prominent peaks ($\delta = 1.19$ ppm, $\delta = 4.03$ ppm) were indicative of the "complexed" L-lactate. The triplet peak at $\delta = 1.32$ ppm was found to couple to the two small quartet peaks at $\delta = 4.26$ ppm and $\delta = 4.20$ ppm; because the triplet was coupled to both quartet peaks it is assumed that the triplet is in fact a result of the overlap of two doublet peaks, and hence it is believed that there are two separate species of L-lactate present. What exactly these latter two species are is unclear, but from the HMBC and HMQC results it is apparent that they are independent of the "free" and "complexed" L-lactate species that were considered to be the most significant in these analyses. Furthermore, after the D_2O shake, these peaks could barely be distinguished, and thus it may be that these species are an artefact of working in [D_6]DMSO. Interestingly, these peaks have similar shifts and splittings to those observed in the 2-APB–L-lactate ^{1}H NMR spectrum. This may suggest that the complex formed between 2-APB and L-lactate can also be formed with 3- and 4-APB; however, for the latter cases this type of complex does not predominate and is also destabilised upon the addition of D_2O.

Considering all of the information collected it became apparent that L-lactate binds well to the trigonal form of 3- and 4-APB to produce the 1:1 complex shown in Scheme 3. The formation of this complex with other similar boronic acids is also suggested in the literature.[6,8]



Scheme 3. Structure of the complex formed between 3- or 4-APB and L-lactate.

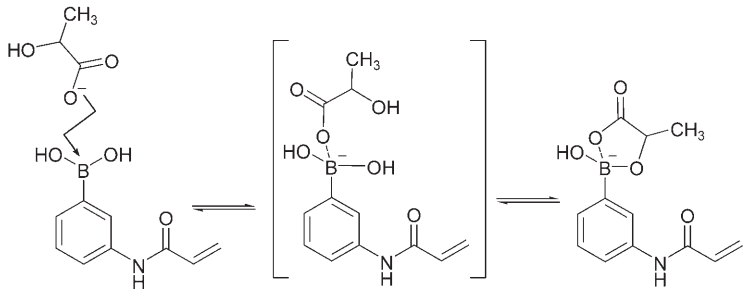
Discussion

It is apparent from this work that L-lactate binds to 3- and 4-APB, but not to 2-APB in aqueous solution. Considering just 2- and 3-APB, the difference in the structure at pH 7.4 is that 3-APB predominantly has a trigonal conformation, whereas 2-APB is thought to adopt a zwitterionic tetrahedral form. The reason for the lack of binding to 2-APB is unclear, but it may be due to an electrostatic repulsion between the ligand and the analyte. After all, at pH 7.4, L-lactate is primarily in its acid anion form, and the boron atom in 2-APB carries a negative charge due to the interaction between itself and the carbonyl oxygen. Thus, when L-lactate and 2-APB are in solution, it is feasible that there will be repulsion between the two, potentially preventing L-lactate from binding. However, it has been demonstrated[4] that complex formation can occur between acid anions and a tetrahedral boronate conformer, and only the rate of reaction is slower than that recorded with the equivalent fully protonated form.

Previously, Pizer et al.,[5,6] postulated that the mechanism by which α-hydroxy acids reacted with either the trigonal or tetrahedral conformer of a boronate was through initial attack on the boron atom by the hydroxyl oxygen, followed by ring closure via the carboxyl. Hence, the slower rate observed with the acid anions was attributed to electrostatic repulsion between the leaving, negatively charged hydroxyl and the incoming anionic donor. If this mechanism is correct, then it seems likely that L-lactate would react with 2-APB to form a cyclic boronate ester, only more slowly than that observed with 3-APB. Because no binding was observed in either the aqueous solution or holographic assays, it implies that this mechanism is not correct, and thus L-lactate must be binding by an alternative mechanism.

Boronic acids are both Broønsted acids and weak Lewis acids.[31] This means that they are able to donate a proton from an incoming ligand and also accept a lone pair of electrons. Although only weak Lewis acids, it is evident from pK_a titration curves that, for the boronic acids investigated, this is a significant part of their chemistry. The strong interaction seen in 2-APB, in which the lone pair of electrons from the carbonyl oxygen is donated to the boron, is also justified if the boron is considered as a Lewis acid.

In this instance, in which the acid anion is dominant, it seems feasible that the mechanism of binding will begin by nucleophilic attack on the boron atom by the more acidic carboxyl group, followed by ring closure via the less acidic hydroxyl group (Scheme 4). This hypothesis has also been suggested in more recent work by Pizer and Tihal,[19] and the proposed mechanism provides an explanation as to why no binding is observed with 2-APB in water.



Scheme 4. Proposed mechanism of binding for L-lactate with a boronic acid (3-APB).

The zwitterionic structure adopted by 2-APB gives the boron atom a negative charge, which will not only experience charge repulsion on encountering another anion, but also renders it a weaker Lewis acid, making attack by the carboxylate unfavourable. Additionally, the boron centre is bound to three atoms, with a fourth binding orbital involved in a strong interaction with the carbonyl oxygen, which leaves little room for nucleophilic attack to occur.

If the proposed mechanism is correct, then this represents a potential way forward to develop a boronic acid ligand that is able to distinguish between bidentate chelators that have two equivalent hydroxyls or which "lack protons of appreciable acidity" (e.g., glucose), and those that are bifunctional (e.g., L-lactate). The mechanism by which glucose is thought to bind is via an associative transition state with the loss of two water molecules[18]. In this case, two hydroxyls are required on the boron atom to enable the boronate–ester formation. For a bifunctional or acid anion it is thought that only one hydroxy group is required on the boron atom to stabilise the boronate–ester complex, as the initial nucleophilic attack occurs directly on the boron centre. Therefore, by using a boronic acid that has only one hydroxyl bound to the boron centre it is thought that some selectivity can be introduced.

To test this hypothesis a simple assay was performed using a solution of (5-amino-2-hydroxymethylphenyl)boronic acid HCl dehydrate (5A2HMPBA), and the response of the addition of glucose and L-lactate, respectively, was monitored by ^{11}B NMR spectroscopy. The solution of 5A2HMPBA (in D_2O) resulted in a resonance at $\delta =$ 13.33 ppm, which implied that the boron was predominantly in a trigonal, and therefore, neutral state. Addition of about 2–3 mg of glucose to the solution resulted in very little change being observed—an upfield shift of 0.14 ppm—which indicated, as expected, that glucose did not bind to 5A2HMPBA. However, when 2–3 mg sodium L-lactate was added to a fresh 5A2HMPBA solution the spectrum recorded showed a strong, sharp peak at $\delta = -5.44$ ppm and a weak, broad peak at $\delta = 13.11$ ppm (Figure 5). This result indicated that L-lactate was not only able to bind to 5A2HMPBA, but that a stable, negatively charged tetrahedral complex was also generated. This result does appear to demonstrate that the proposed L-lactate binding mechanism is indeed correct, and an overall charge change occurs when 5A2HMPBA binds to L-lactate in solution. Thus, if this response can be induced when a similar boronic acid receptor is incorporated into a hydrogel upon the addition of L-lactate then it is feasible that a selective holographic response can be observed.

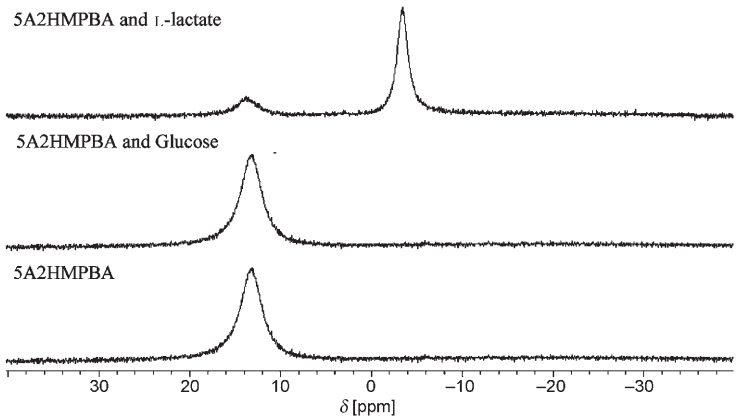


Figure 5. Comparison of ^{11}B NMR spectra recorded for 5A2HMPBA ($\approx$30 mM in D_2O) alone, and subsequently with glucose ($\approx$2–3 mg) and L-lactate ($\approx$2–3 mg), respectively. The addition of L-lactate induced a clear shift in the ^{11}B signal from $\delta = 13.33$ ppm to $\delta = -5.44$ ppm, which indicates a conformational change occurred at the boron centre. This shift was not observed upon the addition of glucose.

Conclusion

The combined analysis of solution and holographic data obtained from the experiments shows that the zwitterionic tetrahedral 2-APB receptor was able to bind glucose but not L-lactate in both aqueous solution and within the hologram. By considering this difference, and analysing the reaction of L-lactate with 2- and 3-APB, respectively, it became apparent that the binding mechanism of L-lactate was different to that of glucose.

Consequently, the hypothesis that L-lactate binds to the trigonal form of 3-APB by means of nucleophilic attack on the boron atom by the more acidic carboxyl group followed by ring closure with the less acidic hydroxyl was proposed, and a simple solution assay has demonstrated that this mechanism is indeed correct and an overall charge change occurs upon addition of L-lactate. If this can be realised in a hologram, then it may be possible to generate a selective holographic sensor for L-lactate. Currently work is focused on designing a novel boronic acid-based receptor that can be incorporated into a suitable hydrogel to achieve this aim.

Experimental Section

Materials: All chemicals were of analytical grade unless otherwise stated. Acrylamide, acryloyl chloride, 1,1′-diethyl-2,2′-cyanine iodide (QBS photosensitising dye), 2,2′-dimethoxy-2-phenyl acetophenone (DMPA), dimethyl sulfoxide (DMSO), *N,N′*-methylene-bis-acrylamide (electrophoresis grade), phosphorous pentoxide, potassium bromide, silver nitrate (1 M, volumetric standard), sodium hydroxide and sodium L-lactate were purchased from Sigma–Aldrich Chemical Company. 3-Aminophenylboronic acid monohydrate was purchased from Avocado Research Chemicals and glucose was purchased from ICN Biomedicals. 2-Aminophenylboronic acid hydrochloride, 4-aminophenylboronic acid and (5-amino-2-hydroxymethylphenyl)boronic acid HCl dehydrate were purchased from Combi-Blocks and phosphate buffered saline (PBS) tablets were purchased from Oxoid. Deuterium oxide (D_2O), sodium deuteroxide (NaOD), [D_6]dimethyl sulfoxide ([D_6]DMSO) and deuterium chloride (DCl) were purchased from Goss Scientific.

Equipment: Microscope slides (Super Premium, 1–1.2 mm thick, Low Iron) were purchased from BDH (Merck). Aluminised 100-μm polyester film (grade MET401) was purchased from HiFi Industrial Film. UV exposure unit (≈350 nm, model no. 555–279), was purchased from RS components. A micro PerpHect pH electrode and bench-top meter were purchased from Orion.

Instrumentation: A frequency-doubled Nd:YAG laser (350 mJ, 532 nm, Brilliant B) was used in the hologram recording and subsequently analysed by using a LOT-ORIEL MS127i Model 77480 imaging spectrograph in single-channel mode with a 256×1024 pixel InstaSpec IV CCD detector, processing software and a tungsten halogen light source. Spectrometer calibration was achieved by using a spectral Calibration Lamp (37–4405) purchased from Ealing Electro Optics. The NMR analysis was carried out by using either a JNM-LA 400 MHz from Jeol, or a Bruker DRX-500 (500 MHz).

Syntheses of 2-, 3- and 4-acrylamidophenyl boronic acids (2-, 3-, 4-APB): The syntheses of 2-APB,[24] 3-APB[25] and 4-APB[15] have been reported previously. For each compound the ^{1}H NMR ^{13}C NMR, ^{11}B NMR and MS data obtained was consistent with that published.

Synthesis of 2-APB polymer films: The 2-APB hydrogels were produced by UV-initiated co-polymerisation. Appropriate quantities of the monomers were dissolved in 2% (w/v) DMPA in DMSO at a ratio 1:2.21 (w/v) of the monomers to solvent. Glucose was also added (4:1 (w/w) ratio glucose:2-APB) to the solution, which was then heated for 30 min at 50 °C to encourage the dissolution of 2-APB. A 100 μL aliquot of the polymer mixture was pipetted onto the aluminium side of an aluminium/plastic reflective sheet and a silanised glass slide was placed on top of the solution. The slides were then left exposed to UV light for 1 h. The polymerised films were subsequently submerged in deionised water, peeled off from the aluminium sheet and washed in some fresh deionised water. Any excess polymer material on the edges of the slide was removed using a scalpel blade.

Hologram construction: The polymer films initially had to be photosensitised by introducing a silver halide emulsion into the polymer matrix. The technique used was based on the principle of the diffusion method described by Blyth et al.[26] For these 2-APB acrylamide-based copolymers the procedure described previously[15,24] was used. All of this work was carried out under safe red lighting.

Monitoring the holographic response: The holograms were interrogated by using an in-house-built reflection spectrophotometer as described by Mayes et al.[27] A piece of hologram was cut from the whole slide (≈8 mm wide) and placed in a 4 mL plastic cuvette with the polymer side facing inward. PBS buffer (1 mL, pH 7.4, buffer concentration 10 mM, ionic strength 160 mM) was added, the cuvette was covered and left to stir at a constant rate with a magnetic microflea/stirrer arrangement. The set-up was left at 30 °C to allow the system to reach equilibrium, subsequently a 0.1 M solution of sodium lactate was made up in PBS buffer, 20 μL of this solution was added to the cuvette and the system left to equilibrate. This process was repeated with a 0.1 M solution of glucose.

NMR analysis: Each sample (2–5 mg) was taken and dissolved in about 0.5 mL of a suitable NMR solvent. A 5 mm quartz tube (Wilmad −528-pp) was used for all runs of ^{11}B NMR and referenced externally to $B(OH)_3$ in D_2O, $\delta = 1.40$ ppm. The 1D analysis was carried out using the JNM-LA 400 MHz, and the COSY, NOESY, HMBC and HMQC spectra were run using the Bruker DRX-500 (500 MHz).

pH-Depression method for following binding of lactate to the boronate ligands: Solutions of 10-mM 3-APB prepared in D_2O (pD adjusted with NaOD), 20 mM and 200 mM sodium lactate solutions prepared in the 10 mM 3-APB solution were made up. Initially, 300 μL of 3-APB solution was analysed by ^{11}B and ^{1}H NMR spectroscopy using the JNM-LA 400 MHz, and on removal, the pD of the sample was determined and recorded. The pD was calculated using pD = pH* + 0.4,[28] in which pH* was the direct value measured by the pH meter that had been calibrated with standard aqueous buffer solutions. This process was repeated for subsequent samples whereby a specific amount of either of the lactate solutions was added to this first sample in order to follow the binding of lactate to 3-APB in solution. This experiment was carried out at pD ≈ 7.4 and repeated in triplicate for 2-, 3- and 4-APB.

Acknowledgement

The authors thank the BBSRC, GlaxoSmithKline and Smart Holograms Ltd. for their financial support.

[1] G. Springsteen, B. Wang, *Tetrahedron* **2002**, *58*, 5291–5300.
[2] J. P. Lorand, J. O. Edwards, *J. Org. Chem.* **1959**, *24*, 769–774.
[3] T. D. James, S. Shinkai, *Topics Curr. Chem.* **2002**, *218*, 159–200.
[4] L. Babcock, R. Pizer, *Inorg. Chem.* **1980**, *19*, 56–61.
[5] R. Pizer, R. Selzer, *Inorg. Chem.* **1984**, *23*, 3023–3026.
[6] S. Friedman, B. Pace, R. Pizer, *J. Am. Chem. Soc.* **1974**, *96*, 5381–5384.
[7] R. D. Pizer, L. Babcock, *Inorg. Chem.* **1977**, *16*, 1677–1681.
[8] K. Ishihara, Y. Mouri, S. Funahashi, M. Tanaka, *Inorg. Chem.* **1991**, *30*, 2356–2360.
[9] R. Pizer, C. Tihal, *Inorg. Chem.* **1992**, *31*, 3243–3247.
[10] S. D'Auria, Z. Gryczynski, I. Gryczynski, M. Rossi, J. R. Lakowicz, *Anal. Biochem.* **2000**, *283*, 83–88.

[11] H. Nakamura, Y. Murakami, K. Yokoyama, E. Tamiya, I. Karube, *Anal. Chem.* **2001**, *73*, 373–378.
[12] D. B. Pyne, T. Boston, D. T. Martin, A. Logan, A. *Eur. J. App. Phys.* **2000**, *82*, 112–116.
[13] A. Poscia, D. Messeri, D. Moscone, F. Ricci, F. Valgimigli, *Biosens. Bioelectron.* **2005**, *20*, 2244–2250.
[14] E. Pringsheim, E. Terpetschnig, S. A. Piletsky, O. S. Wolfbeis, *Adv. Mater.* **1999**, *11*, 865–868.
[15] F. K. Sartain, X. Yang, C. R. Lowe, *Anal. Chem.* **2006**, *78*, 5664–5670.
[16] J. Landon, J. K. Fawcett, V. Wynn, *J. Clin. Pathol.* **1962**, *15*, 579–584.
[17] T. D. James, K. Sandanayake, S. Shinkai, *Angew. Chem.* **1994**, *106*, 2287–2289; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2207–2209.
[18] L. Stryer, *Biochemistry*, 4th ed., Freeman, **1995**.
[19] S. Kabilan, A. J. Marshall, F. K. Sartain, M.-C. Lee, A. Hussain, X. Yang, J. Blyth, N. Karangu, K. James, J. Zeng, D. Smith, A. Domschke, C. R. Lowe, *Biosens. Bioelectron.* **2005**, *20*, 1602–1610.
[20] M. Van Duin, J. A. Peters, A. P. G. Kieboom, H. Van Bekkum, *Tetrahedron* **1985**, *41*, 3411–3421.
[21] R. D. Pizer, C. A. Tihal, *Polyhedron* **1996**, *15*, 3411–3416.
[22] H. K. Garner, H. J. Lucas, *J. Am. Chem. Soc.* **1950**, *72*, 5497–5501.
[23] M. Van Duin, J. A. Peters, A. P. G. Kieboom, H. Van Bekkum, *Tetrahedron* **1984**, *40*, 2901–2911.
[24] X. Yang, M.-C. Lee, F. K. Sartain, X. Pan, C. R. Lowe, *Chem. Eur. J.* **2006**, *12*, 8491–8497.
[25] S. Chapelle, J.-F. Verchere, *Tetrahedron* **1988**, *44*, 4469–4482.
[26] K. Ishihara, A. Nagasawa, K. Umemoto, H. Ito, K. Saito, *Inorg. Chem.* **1994**, *33*, 3811–3816.
[27] M. P. Hughes, B. D. Smith, *J. Org. Chem.* **1997**, *62*, 4492–4499.
[28] A. K. Covington, M. Paabo, R. A. Robinson, R. G. Bates, *Anal. Chem.* **1968**, *40*, 700–706.
[29] J. Blyth, R. B. Millington, A. G. Mayes, C. R. Lowe, *Imaging Sci. J.* **1999**, *47*, 87–91.
[30] A. G. Mayes, J. Blyth, R. B. Millington, C. R. Lowe, *Anal. Chem.* **2002**, *74*, 3649–3657.
[31] P. K. Glasoe, F. A. Long, *J. Phys. Chem.* **1960**, *64*, 188–190.

Received: December 4, 2007
Published online: March 11, 2008

