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Nitrogen-15 NMR Spectroscopy of Sugar Sensor with B–N Interaction as a Key Regulator of Colorimetric Signals

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Nitrogen-15 NMR spectroscopy showed strongly upfield values of the chemical shift for one of the azo nitrogen atoms of a boronic-acid-appended azo dye: this indicated the formation of a boron–nitrogen (B–N) dative bond. The B–N dative bond was cleaved by sugar addition.

Boronic acids are widely used for sugar sensors because they react with diol moieties of sugars to form boronate esters.[1] Sugar sensors based on boronic acids require the occurrence of signal changes upon sugar binding. James et al. have developed a novel fluorescent sensor that contains a nitrogen atom at a position adjacent to the boronic acid group.[2] The sensor compound shows a significant increase in fluorescent intensity upon sugar binding. They proposed that the fluorescent intensity of the sugar sensor was controlled by a boron–nitrogen (B–N) interaction. Since then, the B–N motif has been widely used not only in fluorescence sensors but also in electrochemical and colorimetric sensors.[3] In previous studies, our group has synthesized a colorimetric sugar sensor based on the B–N interaction between boronic acid and azo groups.[4]

Although B–N interactions have been recognized as the key regulators of many sensor signals, there are few ways to investigate B–N interactions in solution state, except for ^{11}B NMR spectroscopy.[5] Unfortunately, ^{11}B NMR is not able to provide conclusive evidence for the B–N interaction because ^{11}B chemical shifts only suggest a difference in the hybridization states of boron atoms. When a boron atom is tetrahedral, its chemical shift is upfield from that of the trigonal-planar geometry, where pure sp^3 and sp^2 are approximately 0 and 30 ppm, respectively.[5] In many cases, adding sugar induces a change in ^{11}B NMR spectra of sugar sensors, and this change is interpreted as a change in the B–N interaction. However, it is very difficult to identify the state of the B–N interaction from the limited information about boron hybridization because not only the adjacent nitrogen but also hydroxides, solvent molecules, and sugars interact with the boronic acid group, and all of these interactions are reflected in ^{11}B chemical shifts. Consequently, B–N interactions of sugar sensors have been investigated and debated for a long time.[5,6]

In order to gain insight into B–N interactions of sugar sensors, we utilize ^{15}N NMR spectroscopy because the formations of coordination bonds are sensitively reflected in the ^{15}N chemical shifts.[7] We synthesized a ^{15}N-labeled boronic-acid-appended azo dye (^{15}N-BA, Figure 1a) which shows the same character as the colorimetric sugar sensors reported previously.[4] We measured its ^{15}N NMR spectra and conducted density functional theory (DFT) calculations to confirm the validity of the ^{15}N chemical shifts values.

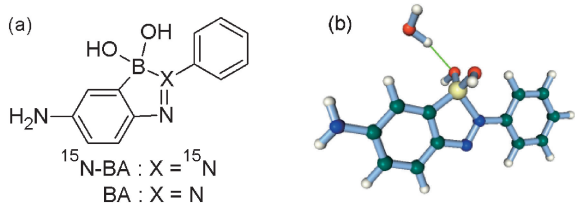


Figure 1. (a) Structures of ^{15}N-BA and BA, (b) an energy-minimized structure of BA containing a hydrogen bond with a water molecule. Hydrogen bonds are shown in light green.

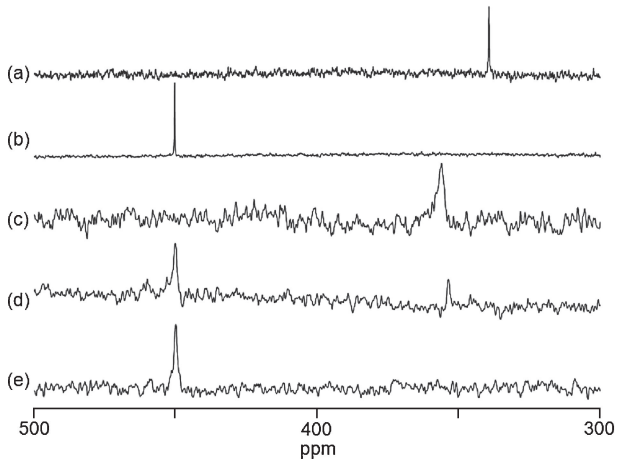


Figure 2. ^{15}N NMR spectra of ^{15}N-BA (20 mM) under various conditions: (a) in D_2O, (b) in a 1.0 M NaOD–D_2O solution, (c–e) in a mixed solvent (100 mM CHES buffer pH 10.0/DMSO-d_6 = 3/1, v/v), (c) without D-fructose, (d) in the presence of 0.10 M D-fructose, and (e) in the presence of 1.0 M D-fructose. The ^{15}N-frequency (0 ppm) is 81.07646745 MHz.

Figure 2a shows the ^{15}N NMR spectra of ^{15}N-BA in D_2O. The ^{15}N chemical shift was observed at 339 ppm; this value is strongly upfield shifted because ^{15}N chemical shifts of azo groups are generally observed at around 500 ppm.[8] On the other hand, the ^{15}N chemical shift of ^{15}N-BA in a 1.0 M NaOD–D_2O solution was observed at 450 ppm (Figure 2b).

Since nonlabeled boronic-acid-appended azo dye (BA) acted as a colorimetric sensor at pH 10.0 (Figure 3b), we investigated the effect of sugar on ^{15}N-BA in a mixed solvent (100 mM CHES buffer pH 10.0/DMSO-d_6 = 3/1, v/v). In the absence of sugar, the ^{15}N chemical shift was strongly upfield shifted (Figure 2c). In the presence of 0.10 M D-fructose, there were two distinct peaks at 353 and 450 ppm (Figure 2d). Further addition of D-fructose extinguished the peak at 353 ppm (Figure 2e).

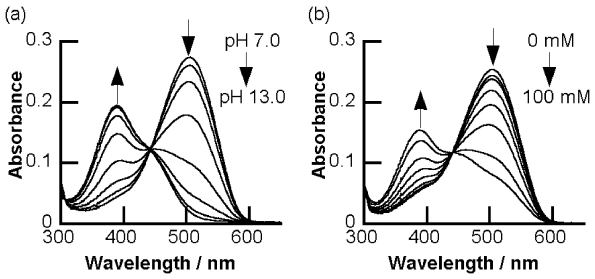


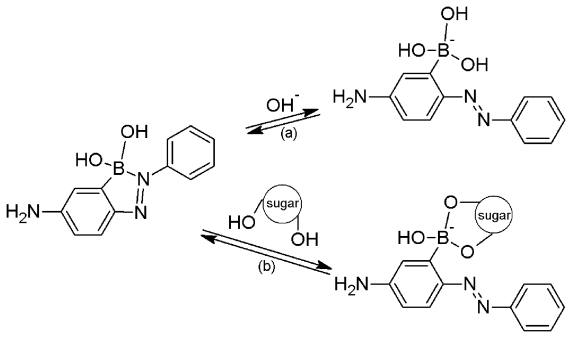
Figure 3. (a) UV–visible absorption spectra of BA (10 μM) in different pH solutions (pH 7.0, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, and 13.0), measured in a methanol/water mixture (1/1, v/v) containing HEPES (5.0 mM). (b) UV–visible absorption spectra of BA (10 μM) in the presence and absence of D-fructose (0, 1, 2, 5, 10, 20, 50, and 100 mM), measured in a methanol/water mixture (1/1, v/v) containing CHES (5.0 mM), pH 10.0.

Then, we investigated the structure responsible for the strongly upfield shift for one of the azo nitrogen atoms. We used the Gaussian suite of programs using DFT calculations at the B3LYP/6-311++G(2d,p) level of theory.[9] We conducted calculations of various structures and predicted their ^{15}N chemical shifts. We found that the structures which did not contain a B–N dative bond showed a predicted value of around 450 ppm (Figure S1, Table S1).[10] In contrast, the calculated structures that contained the B–N dative bond (Figure 1b obtained with Facio software[11]) showed a predicted value of 349 ppm, which is in the best agreement with the actual measured value (339 ppm). Such an upfield value was reported to be found in protonated azobenzene (360 ppm), in which the azo group coordinates with a proton.[8] Hence, it is reasonable to conclude that the strongly upfield value of the ^{15}N chemical shift is due to the existence of a B–N dative bond.

We assumed that ^{15}N-BA in alkaline solutions has a boron atom coordinating with three hydroxy groups because abundant hydroxide can coordinate with the boronic acid group. The energy-minimized structure shows a predicted value of 444 ppm (Table S1a),[10] which agrees well with the actual measured value of ^{15}N-BA in 1.0 M NaOD solution (450 ppm). On the basis of these ^{15}N NMR and DFT calculation results, an acid–base equilibrium of ^{15}N-BA can be presented as shown in Scheme 1a.

On the basis of the structural information obtained from ^{15}N NMR and DFT calculation results, we can explain the reason for the change in the ^{15}N chemical shift of ^{15}N-BA upon sugar binding in the following manner. In the conditions of Figure 2c, the major species of ^{15}N-BA has a B–N dative bond, which is responsible for the upfield value of the ^{15}N chemical shift (356 ppm). Adding sugar induces a B–N dative bond cleavage, which results in a recovery of the ^{15}N chemical shift in the normal range (450 ppm). This structural change (Scheme 1b) corresponds to a solvolysis mechanism, which was originally proposed by Wang's group.[6a,6b]

We also measured ^{11}B NMR spectra under the same conditions as that for the ^{15}N NMR spectra. In the absence of sugar, the ^{11}B chemical shift was observed at 13.0 ppm (Figure S2c).[10] Addition of D-fructose increased the ^{11}B chemical shift to around 8 ppm and decreased that around 13 ppm (Figures S2d and S2e).[10] Since both the ^{11}B chemical shifts at around 13 and 8 ppm correspond to quasi-tetrahedral boron, it is very difficult to describe the effect of sugar on the structures of ^{15}N-BA.



Scheme 1. (a) Acid–base equilibrium of BA and (b) equilibrium of BA and sugar in pH 10.0.

Figure 3 shows the effect of pH and sugar on UV–visible absorption spectra of BA. In the pH range from 7 to 10, the absorption maximum was observed at 505 nm, which is significantly red-shifted compared to that of 4-aminoazobenzene (365 nm).[12] A pH increase induced a decrease in the absorption maximum at 505 nm and an increase in a new band at 386 nm. Sugar addition induced a similar spectral change.

From the ^{15}N NMR and DFT calculation results, we can conclude that the red shift of the absorption maximum is due to a B–N dative bond and that the B–N dative bond is cleaved by pH increase or sugar addition (Schemes 1a and 1b). The upfield value of the ^{15}N chemical shift of BA is clear evidence for the formation of the B–N dative bond. The ^{15}N NMR investigation would also be applicable to other sugar sensors showing B–N interactions, in order to determine their structural details, which would contribute to further development of sugar sensors based on boronic acids.

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