2-Aminopurine Fluorescence Spectroscopy for Probing a Glucose Binding Aptamer

Chang Lu[1,2], Po-Jung Jimmy Huang[2], Jingkai Zheng[1] and Juewen Liu[2]*

1. Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China

2. Department of Chemistry, Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, ON, N2L 3G1, Canada

Email: liujw@uwaterloo.ca

Abstract

Glucose is the most important analyte for biosensors. Recently a DNA aptamer was reported allowing binding-based detection. However, due to a relatively weak binding affinity, it is difficult to perform binding assays to understand the property of this aptamer. In this work, we replaced the only adenine base in the aptamer binding pocket with a 2-aminopurine (2AP) and used fluorescence spectroscopy to study glucose binding. In the selection buffer, glucose increased the 2AP fluorescence with a K_d of 15.0 mM glucose, which was comparable with the 10 mM K_d previously reported using the strand displacement assay. The binding required two Na^+ ions or one Mg^{2+} that cannot be replaced by Li^+ or K^+. The binding was weaker at higher temperature and its van't Hoff plot indicated enthalpy-driven binding. While monosaccharides failed to achieve saturated binding even at high concentrations, two glucose-containing disaccharides, namely trehalose and sucrose, reached a similar fluorescence level as glucose although with over 10-fold higher K_d's. Detection limits in both the selection buffer (0.9 mM) and in artificial interstitial fluids (6.0 mM) were measured.

Keywords: aptamers, biosensors, glucose, diabetes, fluorescence

Introduction

Current commercial biosensors for the detection of glucose rely on enzymes such as glucose oxidase,[1-2] where glucose is converted to gluconic acid and hydrogen peroxide. For glucose-binding based detection, concanavalin A and boronic acid have been the main ligands, though their binding chemistry is not specific to glucose.[3-5] Aptamers are oligonucleotides that can selectively bind target molecules.[6-7] DNA aptamers are particularly attractive for the detection of small molecular targets, showing excellent affinity, specificity and versatility in signal transduction.[8-11]

Glucose is considered to be a challenging target for aptamers due to a lack of strong epitopes for binding. One strategy is to complex glucose with a biboronic acid ligand, and an aptamer with a K_d of around 3.8 mM glucose was obtained using this method.[12] Recently, the Stojanović group managed to select a DNA aptamer for binding free glucose with a K_d of 10 mM. This K_d is physiologically relevant since the blood glucose concentration can typically vary from 4 mM to 11 mM depending on the time of the day and meals.[13]

Most previously reported small molecular binding aptamers have much higher affinities (high nM to high μM being most common),[9, 14-15] and this glucose aptamer posed challenges for studying its binding.[16] So far, all the fluorescence-based assays using this aptamer relied on the strand displacement reaction, in which a quencher-labeled complementary oligonucleotide was required.[17-18] Such a system makes it difficult to study the effect of ionic strength and temperature, since DNA hybridization and melting are also affected by these factors. Due to its high K_d, the use of isothermal titration calorimetry (ITC) is also quite challenging. To acquire high quality data, more than 1 mM aptamer would be required.[19-20]

2-Aminopurine (2AP) is an adenine analog and its fluorescence is strongly affected by the local environment of nucleic acids.[21-22] When 2AP is more stacked with its neighboring bases, the fluorescence would be lower and vice versa.[23] Because of this unique property, 2AP has been widely used as a probe to study DNA aptamers, such as the aptamers for theophylline,[24] Hg^{2+} and Ag^+,[25] and Na^+.[26-27] Among these, the Na^+-binding aptamer has some similarity to the glucose aptamer (e.g., a high K_d of around 10 mM).[28]

Herein, we used 2AP fluorescence spectroscopy to study this glucose aptamer. In particular, we aimed to understand its metal and temperature-dependent binding properties. Since the current glucose detection method is gradually shifting from finger stick blood-based to using interstitial fluids for continuous monitoring,[29] we also tested our 2AP-based aptamer as a sensor in simulated interstitial fluids.

Results and Discussion

The 2AP-labeled aptamer. The secondary structure of the glucose binding aptamer is shown in Figure 1A. It has a small stem loop followed by two single-stranded regions, with the two ends being closed by five base pairs.[17] We suspected that glucose binding is from the two single-stranded regions marked in blue, which contained only one adenine (in red). Thus, we replaced this adenine with a 2AP (see Figure 1A for their structures). We hoped that this perturbation is small enough to still allow similar glucose binding.

In the aptamer selection buffer, we titrated the 2AP-modified aptamer with glucose, and the emission peak at 370 nm gradually increased (Figure 1B), suggesting binding of glucose by the aptamer. The saturated fluorescence reached around 180% of the initial value. The increased emission suggests that the 2AP base stacking was relaxed upon binding glucose.[21] We plotted the fluorescence enhancement as a function of glucose concentration (Figure 1C, black dots). Fitting the data in the rising region using the Hill equation, where the logarithm of $\Theta/(1-\Theta)$ is plotted against the logarithm of glucose concentration,[30] yielded a Hill coefficient of 1.03 (Figure 1D, Θ is the fraction of bound aptamer), suggesting the binding of one glucose. Thus, the data were fitted to the binding of one glucose with a K_d of 15.0 mM glucose, which is similar to that obtained from the strand displacement assay (10 mM),[17] confirming that the replacement of the adenine by a 2AP did not change the binding affinity much.

We also tested two 2AP-labeled scrambled control sequences that are not supposed to bind glucose. For both sequences, adding up to 50 mM glucose had minimal effects on the fluorescence intensity. Although a small increase was observed with higher concentrations of glucose for scrambled DNA1, a similar increase was also seen by adding galactose (Figure 1C, red and blue symbols). For scrambled DNA2, which had a more rigid structure (also folds in an overall hairpin), no fluorescence increase was observed in the presence glucose or galactose

(Figure 1C, green and purple symbols). We reason that the fluorescence increase in scrambled DNA1 was not due to specific binding but could be related to the increase of viscosity to fold the DNA.[31] The non-structured scrambled DNA1 can be folded by adding concentrated sugars. Scrambled DNA2 has a more rigid structure and thus was less affected by it. Overall, these two control sequences showed no specific binding to glucose, supporting the specific glucose binding of the aptamer.

We then studied the kinetics of the fluorescence change. After adding glucose to the aptamer and mixing, we placed the cuvette into the fluorometer, and the fluorescence was stable within 40 sec (Figure 1E). Therefore, for subsequent studies, we used a waiting time of 1 min.[32] The only previous binding assay was performed using the strand displacement reaction, where a fluorophore-labeled aptamer was hybridized to a quencher-labeled partially complementary DNA. Binding of glucose to the aptamer would displace the quencher-labeled strand to result in fluorescence enhancement.[17] The strand displacement reaction is typically slower since it involved three reactants.[33] The 2AP label allowed a faster signal change, which is important for biosensors.

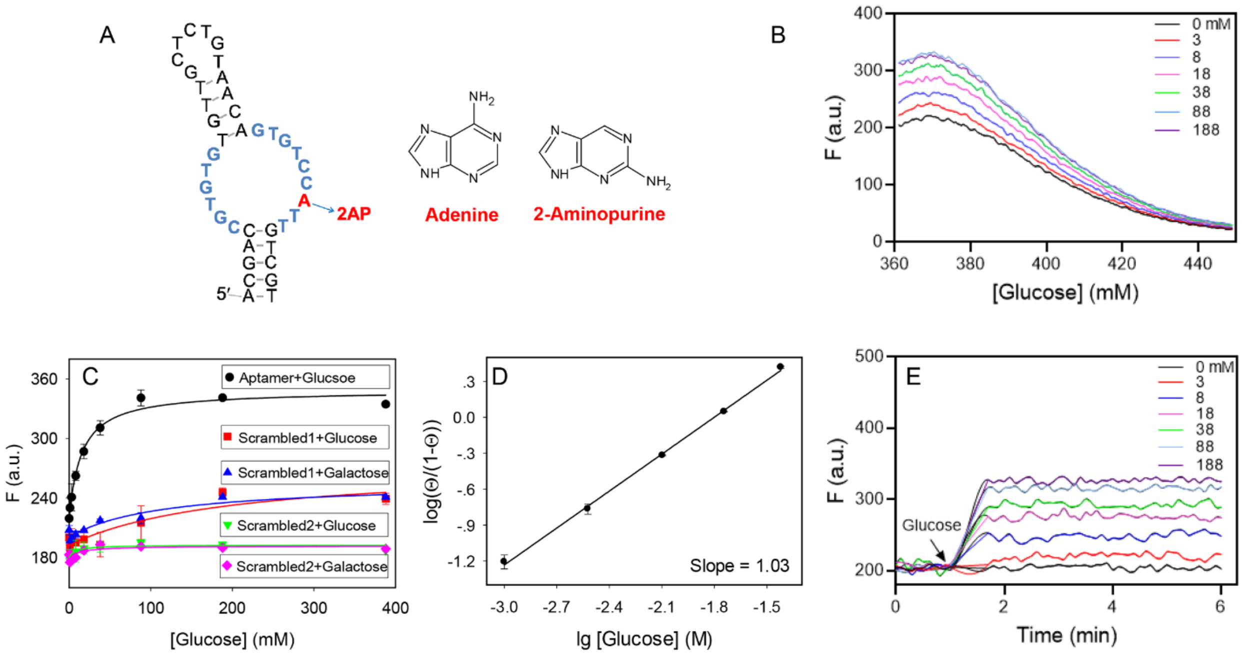

Figure 1. (A) The secondary structure of the glucose binding aptamer. The structures of adenine and 2AP are also shown. (B) Fluorescence spectra of 1 μM of the 2AP-labeled aptamer with 310 nm excitation in the presence of various concentrations of glucose in the selection buffer named

buffer A (1 M NaCl, 10 mM $MgCl_2$, 5 mM KCl, 20 mM HEPES, pH 7.6). (C) The fluorescence enhancement of the 2AP-labeled aptamer (black line) and the 2AP containing scrambled sequences. (D) Hill plot of the aptamer data in (C) in the rising region, and a slope of 1.03 indicates the binding of one glucose. (E) Kinetics of fluorescence increase of the 2AP-labeled aptamer, where different concentrations of glucose were added at 1 min.

Two sodium ions are required for glucose binding.

The aptamer selection was performed in a high-salt buffer (1 M NaCl, 10 mM $MgCl_2$, 5 mM KCl and 20 mM HEPES, pH 7.6),[17] while the ionic strength of blood is lower (142 mM Na^+, 5 mM K^+, 1.5 mM Mg^{2+} and 2.5 mM Ca^{2+}). Since aptamer binding might be affected by the concentration and type of salt,[34-35] we first aimed to understand the effect of salt. As illustrated above, using the selection buffer condition, we obtained a K_d of 15.0 mM glucose. We fixed the other components and decreased the concentration of NaCl gradually to 0 (Figure 2A). Interestingly, we observed binding in all cases. As long as more than 10 mM NaCl was added, the final fluorescence reached a similar level. These results suggest that saturated binding could be eventually reached. While binding was observed in all these cases, the K_d for glucose was significantly increased at lower concentrations of NaCl (Figure 2B). To measure the number of Na^+ ions involved in the binding, we mixed the aptamer with 100 mM glucose in a buffer free of Na^+ or Mg^{2+}. We then gradually titrated Na^+ and recorded the fluorescence (Figure 2C), which showed a sigmoidal response suggesting the involvement of multiple cooperative Na^+ ions for glucose binding. We then made the Hill plot (inset of Figure 2C), and a Hill coefficient of 2.3 was obtained from its slope, suggesting that two Na^+ ions were involved to promote glucose binding. The fitted K_d was 290 mM Na^+. Compared to the Na^+ binding aptamer with a K_d of around 10-20 mM,[26, 28, 36] the apparent K_d here for Na^+ was much lower. This can be explained by that Na^+ was used to mediate the binding of glucose, and two Na^+ ions were required.

When we replaced 1 M NaCl by 1 M of KCl or LiCl, the binding dropped significantly (Figure 2D, E, blue curves). Therefore, the effect of Na^+ was more than charge screening, since 1 M of these monovalent salts would have a similar and saturated charge screening effect. So, it is likely that Na^+ participated in binding, which was supported by the Na^+ binding curve in Figure 2C.

A careful comparison of the 0 or 10 mM NaCl data in Figure 2A with the 1 M Li^+ or K^+ data indicated that adding Li^+ or K^+ actually inhibited glucose binding. With 10 mM NaCl alone, the final fluorescence still reached around 350 fluorescence unit, while with 1 M LiCl or KCl, the fluorescence saturated at below 230 fluorescence units, suggesting that only a fraction of the aptamers bound glucose. The rest could not bind, which is attributable to the Na^+ binding site being occupied by Li^+ or K^+.

Note that the 20 mM HEPES buffer we used contained ~10 mM Na^+ from the buffer. To rule out this background Na^+, we prepared a buffer with the acid form of HEPES and adjusted it to pH 7.6 using LiOH. In this case, with 1 M LiCl, we observed that binding further dropped (Figure 2D, red curves), indicating the ~10 mM Na^+ in the buffer contributed to the binding of glucose. While glucose is non-charged, salt can bridge the highly negatively charged aptamer to promote aptamer binding. A DNA aptamer motif for specific binding of Na^+ is already known,[36] and DNAzymes specific for Na^+ and Li^+ were also reported.[37-38] Thus, it is understandable that this aptamer can achieve specific Na^+-mediated glucose binding.

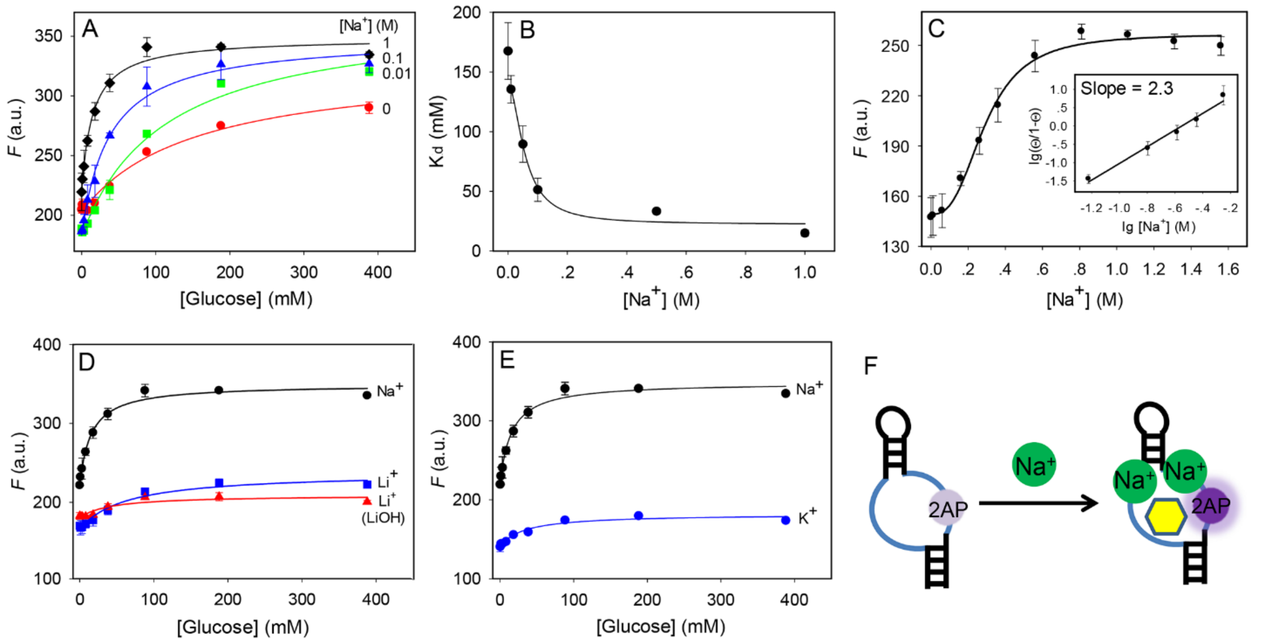

Figure 2. (A) Glucose-dependent 2AP fluorescence in buffers containing different concentrations of NaCl. The buffer contained 10 mM Mg^{2+}. (B) The fitted K_d from (A) as a function of Na^+ concentration. (C) Fluorescence of 1 μM aptamer as a function of Na^+ concentration in 20 mM HEPES, pH 7.6 adjust with LiOH. No Mg^{2+} was present for this

experiment. Inset: the Hill plot based on the rising region of the data in the main figure. Θ is the fraction of bound aptamer. (D) Fluorescence enhancement of the 2AP-labeled glucose aptamer in buffers with 1 M NaCl or LiCl as a function of glucose concentration. (E) The fluorescence enhancement of 2AP-labeled glucose aptamer in buffers with 1 M NaCl or KCl as a function of glucose concentration. In (D and E), the buffers were: Na^+ buffer (1 M NaCl, 10 mM $MgCl_2$, 5 mM KCl, 20 mM HEPES, pH 7.6), the Li^+ buffer (1 M LiCl, 10 mM $MgCl_2$, 5 mM KCl, 20 mM HEPES, pH 7.6), the Li^+ (LiOH) buffer (1 M LiCl, 10 mM $MgCl_2$, 5 mM KCl, 20 mM HEPES, pH 7.6 adjusted by LiOH), the K^+ buffer (1.005 M KCl, 10 mM $MgCl_2$, 20 mM HEPES, pH 7.6, adjusted by LiOH). (F) A cartoon showing that this aptamer requires two Na^+ ions to bind glucose.

Magnesium-dependent glucose binding.

In the above experiments, 10 mM Mg^{2+} was present (except for Figure 2B). We then studied the effect of Mg^{2+}. To understand binding close to physiological conditions, we fixed the NaCl concentration at 100 mM, and varied the Mg^{2+} concentration (20 mM, 10 mM, 5 mM, 1 mM, and 0). In this case, we also observed decreased glucose binding with decreasing Mg^{2+} concentration (Figure 3A). The apparent K_d increased to over 200 mM glucose when Mg^{2+} was absent (with 100 mM Na^+ alone, Figure 3B).

We noticed that the initial fluorescence gradually increased with increasing Mg^{2+} concentration (Figure 3A). We then plotted the initial fluorescence as a function of Mg^{2+} and Na^+ concentration for both the aptamer and the scrambled DNA2. For both DNA, the initial fluorescence (no glucose added) increased with increasing of Mg^{2+} concentration (Figure S1A, B). Nevertheless, the final fluorescence change after adding glucose remained similar (Figure S1C, D). Thus, these metal ions can relax the initial stacking of the 2AP bases, and after binding glucose, the relative change of stacking was similar regardless of the initial fluorescence.

To measure the number of Mg^{2+} ions involved in the binding, we dissolved the aptamer in the Na^+-free buffer and added 100 mM glucose. We then gradually titrated Mg^{2+} and recorded the fluorescence change (Figure 3C). The shape of the binding curve is typical for a one-site binding, which was confirmed by a slope of 1.2 in the fitted Hill plot (inset of Figure 3C). The fitted apparent *K*d was only 0.28 mM Mg^{2+}. Thus, this aptamer can use either two Na^+ ions (Figure 2F) or one Mg^{2+} ion (Figure 3F) to promote glucose binding, which may be explained by their charge difference. By comparing the apparent *K*d of these two metal ions, which differed by about 1000-fold, Mg^{2+} was much more efficient than Na^+ in promoting binding.

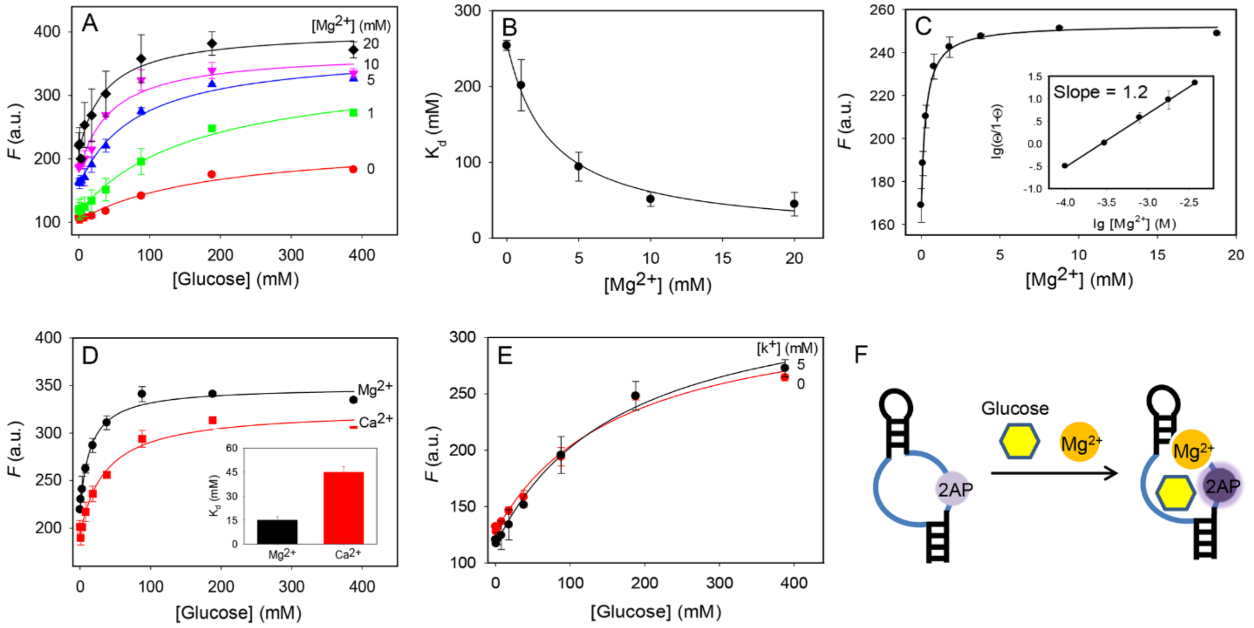

Figure 3. (A) Glucose-dependent 2AP fluorescence change in the presence of different $MgCl_2$ concentrations. The buffer contained 100 mM Na^+. (B) *K*d values as a function of $MgCl_2$ concentrations based on the data in (A). (C) Fluorescence of 1 μM aptamer as a function of Mg^+ concentration in 20 mM HEPES, pH 7.6 adjust with LiOH. No Na^+ was present for this experiment. Inset: the Hill plot based on the rising region of the data in the main figure. Θ is the fraction of bound aptamer. (D) The fluorescence enhancement in buffers with Mg^{2+} or Ca^{2+} as a function of glucose concentration. The buffers contained 1 M Na^+. Inset: *K*d values for glucose binding in 10 mM Mg^{2+} or Ca^{2+}. (E) The fluorescence enhancement of 2AP-labeled aptamer in buffers without or with 5 mM K^+ as a function of glucose concentration. The K^+ buffer contained 100 mM NaCl, 1 mM $MgCl_2$, 5 mM KCl, 20 mM HEPES, pH 7.6. (F) A cartoon showing that this aptamer requires one Mg^{2+} ion to bind glucose.

We then studied whether Mg^{2+} could be replaced by Ca^{2+}. With 1 M NaCl, we compared 10 mM $MgCl_2$ and 10 mM $CaCl_2$, where the binding in Ca^{2+} dropped a little with an increase of

K_d value (Figure 3D), indicating that Ca^{2+} can also participate in the binding although Mg^{2+} was more effective. One of the best metal-mediated target binding is probably the fluoride riboswitch, where three Mg^{2+} ions bridged the target F^- and the backbone phosphates of the aptamer.[39] A recently reported uric acid binding aptamer required three Na^+ ions but was independent of Mg^{2+}.[40] In 2002, Lorsch and Szostak reported a cyanocobalamine binding RNA aptamer, which required a high concentration of Li^+ but not Mg^{2+}.[41] These can be explained by their selection buffer composition. Finally, since the selection buffer contained 5 mM KCl, we also tested its effect. When KCl was fully removed, the binding and the K_d value barely changed (Figure 3E), indicating that the 5 mM K^+ did not influence glucose binding.

From Mg^{2+}-dependent binding to Na^+-dependent binding

Since this aptamer was able to use both Na^+ and Mg^{2+}, we then studied their relationship. To do this, we used the Na^+- and Mg^{2+}-free buffer and started with a kinetic study (Figure 4A). In this buffer, adding 50 mM glucose to the aptamer had almost no fluorescence change (the first arrow). When adding 10 mM Mg^{2+} (the red trace), the fluorescence increased around 100 fluorescence unit, and the fluorescence further increased when 1 M Na^+ was added. Based on the apparent K_d's for the metal ions, 10 mM Mg^{2+} or 1 M Na^+ can individually saturate the metal ion requirement already. Since the final fluorescence indicates that final state of the 2AP fluorophore, the binding state was different in the presence of Mg^{2+} and Na^+. This is not surprising since replacing one Mg^{2+} by two Na^+ ions is likely to result in a perturbation of the binding site. Thus, the further increase of the fluorescence suggested the transition of the aptamer from the Mg^{2+}-dependent binding to the Na^+-dependent binding, and the latter had a tighter binding for glucose. To confirm this hypothesis, we switched the order of addition and added Na^+ first, in which case, further addition of Mg^{2+} did not cause much fluorescence change (the blue trace). Their final fluorescence was the same as the sample of adding Mg^{2+} and Na^+ together (the black trace). Therefore, when sufficient Na^+ and Mg^{2+} were saturated, the binding state was dominated by Na^+.

We then quantitatively titrated glucose in the presence of buffers containing only Na^+ or only Mg^{2+} (Figure 4B). In this case, the glucose binding in the presence of Mg^{2+} alone was much weaker with a K_d of 194 mM glucose. On the other hand, with 1 M NaCl alone (no Mg^{2+}), the K_d (20 mM glucose) was comparable to that with 1 M Na^+ and 10 mM Mg^{2+} (15 mM glucose). Therefore, with 1 M Na^+, Mg^{2+} did not promote the effect of Na^+. The aptamer is more efficient in the presence of Na^+. This can explain that when we added Na^+ to the Mg^{2+} containing sample, the fluorescence can further increase, whereas when Mg^{2+} was added to the Na^+-containing sample, the fluorescence remained the same (Figure 4A). The transition from the Mg^{2+}-mediated glucose binding to the Na^+-mediated binding is shown in Figure 4C.

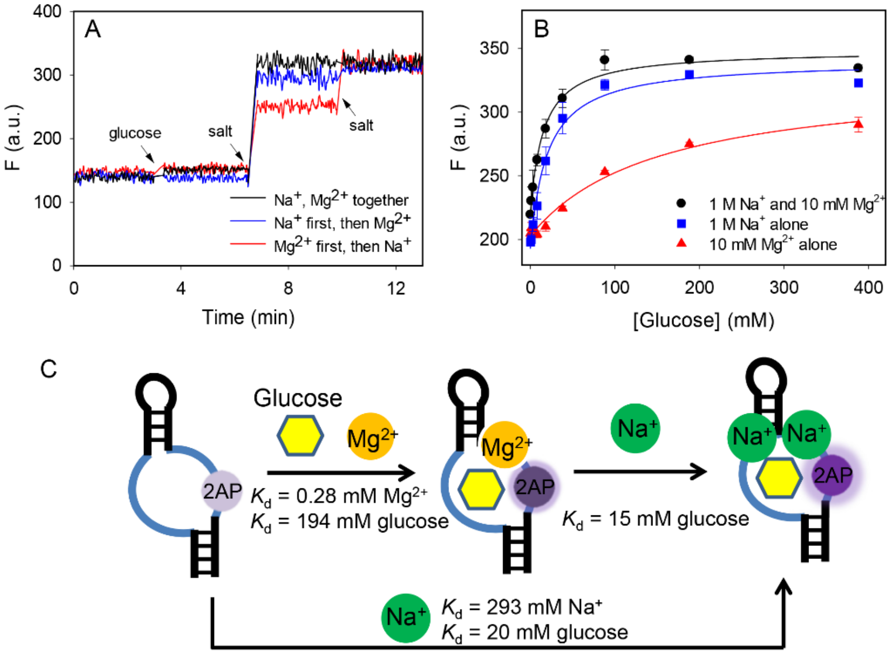

Figure 4. (A) Kinetics of the aptamer fluorescence response to the addition of 50 mM glucose, 10 mM Mg^{2+} followed by 1 M Na^+, or 1 M Na^+ followed by 10 mM Mg^{2+}, or a mixture of 1 M Na^+ and 10 mM Mg^{2+}. The initial buffer was 20 mM HEPES, pH 7.6 adjusted with LiOH. (B) Aptamer response to glucose in the presence of 1 M Na^+ alone, 10 mM Mg^{2+} alone and with 1 M Na^+ and 10 mM Mg^{2+} in the LiOH adjusted buffer. (C) A cartoon showing the Na^+ and Mg^{2+} mediated binding of glucose, and the transition of Mg^{2+}-mediated to Na^+-mediated binding.

Temperature dependent binding.

To obtain further insights, we then measured the thermodynamics of glucose binding. We titrated glucose to the 2AP-labed aptamer at various temperatures (Figure 5A). At each temperature, the binding curve was fitted to obtain the K_d. Glucose showed binding from 7°C to 37°C, with the fluorescence increase being higher at lower temperatures. Saturated binding was reached in all temperatures. The temperature data were then fitted to the van't Hoff equation (Figure 5B), from which the ΔH (-7.6±1.5 kcal mol^{-1}) and ΔS (-18.9±5.2 cal mol^{-1} K^{-1}) were obtained. ΔG of the reaction was calculated to be -1.7±0.1 kcal mol^{-1} at 310 K (the body temperature). Since the ΔS term is negative, the system has decreased entropy upon binding, which is thermodynamically unfavorable. Thus, glucose binding to this aptamer is an enthalpy-driven reaction. This is consistent with the fact that glucose is a quite hydrophilic and strongly hydrated molecule.

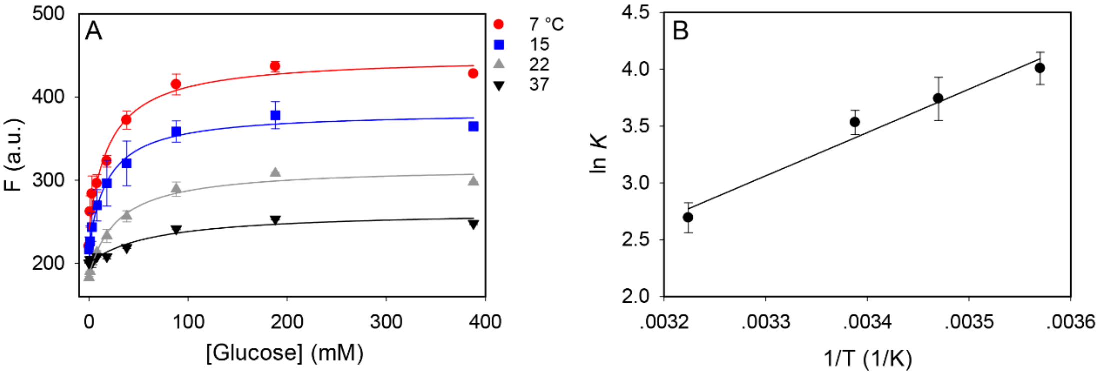

Figure 5. Glucose-dependent 2AP-labeled aptamer fluorescence change in different temperatures in buffer A. (B) The van't Hoff plot of the binding reactions to extract the thermodynamic parameters.

Selectivity and detection limit.

To evaluate the selectivity of the aptamer, a few mono- and disaccharides including galactose, fructose, trehalose, and sucrose were tested (Figure 6A). At below 50 mM, galactose showed a moderate binding, whereas fructose did not; both monosaccharides saturated at a low fluorescence intensity (Figure 6B), indicating that they could not induce the same binding as glucose. The slightly stronger galactose binding was consistent with the original aptamer selection paper.[17]

The binding of disaccharides was not previously reported for this aptamer. We noticed that although sucrose and trehalose both had weaker binding (K_d >150 mM), they reached a similar final fluorescence as glucose, suggesting that they can arrive at a final binding structure similar to glucose. Sucrose has a fructose joined to a glucose, while trehalose is joint by two glucose molecules. Thus, it is not surprising that both can bind to this aptamer. Based on the structure of sucrose, it is likely that the binding does not involve the C1 carbon of glucose. In blood, only glucose is expected to be present at a high concentration, while the rest of the sugars are not.[42] Therefore, this aptamer has sufficient selectivity for glucose.

We then sensed glucose using the 2AP-labeled aptamer in an artificial interstitial fluid (ISF) and compared it to that obtained in the selection buffer. Figure 6C shows glucose-dependent increase of the 2AP fluorescence signal under the two conditions, and the response in the selection buffer has a linear range between 0 and 8 mM glucose with a detection limit of 0.9 mM (Figure 6D). In the artificial ISF, a linear range between 0 and 88 mM glucose was observed with a detection limit of 6.0 mM. The detection limit was calculated using $3\sigma/S$, with σ and S representing the absolute standard deviation at the intercept and the slope of the calibration curve, respectively. The lower Na^+ concentration in the ISF is the main reason for its lower sensitivity.

It needs to be pointed out that 2AP fluorescence is not an ideal way of sensing and the main goal of this work is to study glucose binding. The UV excitation and emission of 2AP suffer from strong light scattering and absorption in real samples. Environment-sensitive fluorophores in the visible region are more suitable for biosensors.[43-44] In the original paper where this aptamer was selected, a field-effect transistor based biosensor was fabricated and it reached a middle point of response below 1 mM glucose in diluted whole blood.[17]

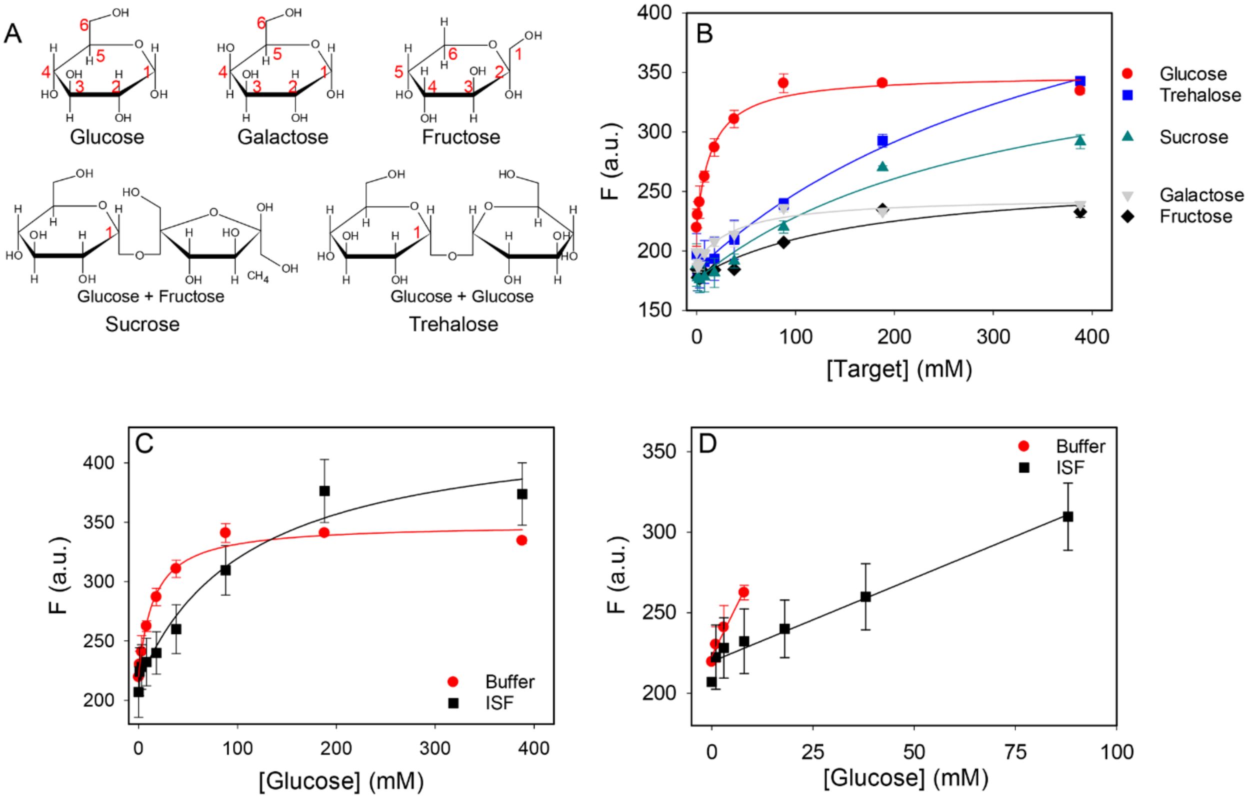

Figure 6. (A) The structures of the sugar molecules tested. (B) Fluorescence responses indicating the selectivity of the 2AP-labeled glucose aptamer in the presence of various sugars. (C) The fluorescence enhancement of 2AP-labeled aptamer for glucose detection in the selection buffer and artificial ISF. (D) The linear range in the selection buffer and ISF for the data in (C). The composition of the artificial ISF: 2.5 mM $CaCl_2$, 10 mM HEPES, 3.5 mM KCl, 0.7 mM $MgSO_4$, 123 mM NaCl, 1.5 mM NaH_2PO_4, 7.4 mM sucrose, pH 7.6.

Conclusions

In this work, we used 2AP as a fluorescent probe to study glucose binding by a DNA aptamer. 2AP is a useful probe here since this aptamer has high K_d around 10 mM, making it difficult for other binding assays to be used. Nevertheless, this affinity is relevant to the physiological concentration of glucose. Our study indicated that this aptamer requires two Na^+ ions, which cannot be replaced by Li^+ or K^+. The role of Na^+ can be partially replaced by Mg^{2+}. Na^+ might contribute to the binding of glucose by metal-bridged interactions. From the temperature-dependent studies, the thermodynamic constants of this binding reaction were extracted from the van't Hoff equation, indicating that it is an enthalpy-driven binding reaction. From the selectivity study, glucose has the highest binding affinity, and the aptamer is better at discriminating against monosaccharides compared to glucose-containing disaccharides. Finally, we measured the figures of merit of this DNA probe as a biosensor for detection in both the selection buffer and in an artificial ISF. The lower Na^+ concentration in the latter can explain its lower sensitivity. Overall, 2AP is a useful probe for biochemical binding assays but not an ideal one for biosensors since its excitation and emission are both in the UV region. Other environmentally sensitive probes with emissions in the visible region and even larger fluorescence changes can be tested in future work.

Experimental Section

Chemicals. The DNA samples used in this work were purchased from Integrated DNA Technologies (Coralville, IA, USA). The sequence of the 2AP glucose aptamer is 5′-ACG ACC GT TGT GTT GCT CTG TAA CAG TGT CC/2AP/T TGT CGT-3′, and the two scrambled controls are DNA1: 5′-GTC ACG AGT CAC TAT/2AP/GGA AGA TGG CGA AA-3′, and DNA2: 5′-GAC GAC GAT TGT GGT CTA TTC /2AP/TA GGC GTC CGC TGA GTC GTC-3′. Sodium chloride, potassium chloride, lithium chloride, lithium hydroxide, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), and sodium phosphate monobasic anhydrous (NaH_2PO_4) were from Mandel Scientific (Guelph, ON, Canada). Magnesium chloride, magnesium sulfate, calcium chloride, fructose, trehalose, sucrose and galactose were from Sigma-Aldrich. Milli-Q water was used for making all the buffers and solutions.

2AP fluorescence spectroscopy. All of the fluorescence spectra were recorded on a Varian Eclipse fluorometer. The 2AP was excited at 310 nm and its emission was recorded from 360 to 450 nm. 500 μL of the 2AP-modified aptamer (1 μM) in buffer A, the selection buffer (1 M NaCl, 10 mM $MgCl_2$, 5 mM KCl, 20 mM HEPES, pH 7.6) was heated to 95 °C for 1 min and then cooled to room temperature. The DNA sample was put in a 1×1 cm quartz fluorescence cuvette, and then different concentrations of glucose were added for fluorescence measurement. The peak intensity at 370 nm was used for quantification. For one site binding, the K_d value was calculated based on: $F = F_0 + a[G]/(K_d + [G])$, where F_0 and F are the fluorescence intensity before and after adding glucose, respectively; [G] is the glucose concentration; and *a* is the fluorescence change when [G] is infinitely high. In some cases, the data were also fitted using the Hill equation, where logΘ/(1-Θ) was plotted against log[G], log[Na^+] or log[Mg^{2+}], and the slope of the fitted line is the Hill coefficient. Θ is the fraction of bound aptamer. Measurements in this work were carried out in triplicate and the standard deviations were plotted as error bars. The dilution factor was corrected for the fluorescence intensity. All the experiments were performed at room temperature (~22 °C) unless otherwise indicated.

Glucose detection in ISF. To prepare artificial interstitial fluids (ISF), 2.5 mM $CaCl_2$, 10 mM HEPES, 3.5 mM KCl, 0.7 mM $MgSO_4$, 123 mM NaCl, 1.5 mM NaH_2PO_4, and 7.4 mM sucrose were mixed,[45] and the solution was adjusted to pH 7.6. Then the fluorescence spectra of the aptamer with different glucose concentrations in the ISF were measured.

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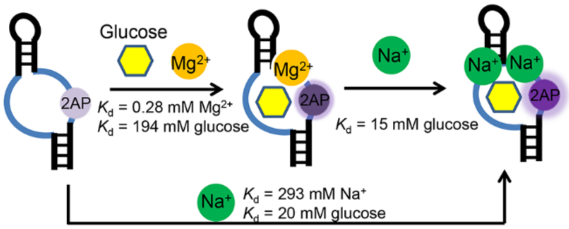
For Graphic abstract



Using 2-aminopurine fluorescence spectroscopy, the glucose binding DNA aptamer showed binding to glucose and glucose containing disaccharides. This enthalpy-driven binding reaction requires two Na^+ ions or one Mg^{2+} ion, and the Na^+ binding state has a higher affinity for glucose.

Twitter handles of Juewen Liu: @bionaloo

Chemistry of University of Waterloo: @uWaterlooChem