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DNA-Glucose Interaction: Thermodynamic Study

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ABSTRACT: Here we report the thermodynamic analysis of glucose interaction on conformational transitions on DNA duplex with different base compositions and lengths by using Circular Dichroism, UV thermal melting and native gel electrophoresis. CD results revealed that three duplexes Duplex 1 "D1" and Duplex 2 "D2" folded into B-form while Duplex 3 "D3" folded into mixed A and B like forms in the presence of different cations and glucose. We monitor the interaction between DNA duplex and glucose over a range of temperatures. Thermal melting data revealed that these duplexes are more stable without glucose under all cationic (Na^+, K^+, $Na^+ + Mg^{++}$ and $K^+ + Mg^{++}$) conditions tested and calculated the enthalpy "ΔH", entropy "ΔS" and free energy "ΔG". Moreover, these duplexes are marginally destabilized at high concentration of glucose. In this study, glucose was considered as an essential model for understanding interactions between interfacial water molecules at hydrophilic surfaces of DNA duplexes.

KEYWORDS: DNA duplex; effect of cation on DNA duplex; Thermodynamics**.**

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I INTRODUCTION

Three different forms of duplex nucleic acid (A, B or Z DNA) have been described in living system. The most common form of DNA at neutral pH and physiological ionic concentrations is right-handed double helical structure is B-form. The B-form DNA duplex structure is involved in the regulation of fundamental cellular functions, such as transcription or its regulation. Many physical factors affect the B-form DNA duplex structure like water, metal ions, pH, temperature etc. Nucleic acid hydration is crucially important for the conformation and utility of DNA. The double helix can take number of conformations with differing hydration. The predominant B-form DNA has a wide and deep major groove and a narrow and deep minor groove and is highly hydrated form. Previous report suggests that short dodecamer is surrounded by a spine of hydration in its minor groove [1]. High resolution data of the same oligonucleotides showed that water hexagons layers forms the spine of hydration [2].

On the other hand, metabolic activities of living organisms depend on metal ions to carry out many vital processes of cell functioning. Hydration free energy of the cation, its valency and the coordination geometry regulates the specific binding of a particular cation with nucleic acids by electrostatic interactions. In aqueous solutions, cations are surrounded by at least three hydration layers and X-ray diffraction showed that metal ions interact with DNA in their first solvation shell of water molecules by charge-dipole interaction, and therefore fully hydrated ions are treated as excellent hydrogen bond donors [3]. It is very well documented that the monovalent ions (K^+, $Na^{+)}$ and divalent ions (Mg^{2+}, Ca^{2+}) binds to the negatively charged phosphate groups on the DNA backbone and hence reducing the replusion between the phosphodiester backbones and stabilizes the DNA structure and its conformation [4]. Structural studies using solution NMR, X-ray Crystallography and Molecular dynamic (MD) simulations studies reported that various monovalant cations like Na^+, K^+, Cs^+ and Rb^+ release their coordinated water and directly interact with AT rich sequences through its minor groove [4,5]. High ionic strength of the divalent alkaline earth metals makes them more reactive than alkali metals. Two positive charges of Mg^{2+} ions and hexa-coordinate geometry of water allow the formation of hydrogen bonds to bind with DNA molecule. The structure and function of physiological form of DNA depends on the nucleotide sequence and environmental conditions. Several reports in past few years suggest the fundamental reactivity of glucose derived from plant biomass in various chemical environments. However, the molecular-level interactions of alkali metal ions and glucose are unknown. These interactions play important physiological role of cation--glucose cotransport [6].

The present article explores the interaction of glucose with duplex structure of two designed DNA sequences (37-mer and 21-mer) as well as the behaviors of G-rich and C-rich human telomere DNAs, as a model molecule and glucose as hydrophilic molecule in the presence of different monovalent (NaCl or KCl) and divalent ($MgCl_2$) cations. For alkaline earth metals, smaller atomic numbers seemed to correlate better with the duplex stabilization. $MgCl_2$ was the most effective for duplex stabilization [4-5].

The structures of oligonucleotides were analyzed by using circular dichroism (CD) spectroscopy, nondenaturating polyacrylamide gel electrophoresis (PAGE), and thermal melting analysis as followed by UV spectroscopy. We found that both 37-mer oligonucleotide and human telomere oligonucleotide formed a stable B-form duplex while 21-mer oligonucleotide formed a mixture of both A- and B- like forms of duplex, in the absence (1:0) and presence of DNA: glucose (1:0.1, 1:100 and 1:10000) with major changes in CD spectra at 200 nm to 240 nm. These findings suggest that the glucose may bind to the oilgonucleotides non-specifically through sugar-phosphate backbone through the hydration shell of DNA. We also measured the thermodynamics of oligonucleotides and compared their stability in the absence and presence of glucose. Following this, we explored the structure and interaction of structural changes in complexes of glucose with different cations on the structure and stability of three DNA duplexes. Such interactions are abundantly present in nature. Understanding the interactions of these salts and glucose with DNA provides a foundation for future mechanistic studies of how these salts affect reactions and biological function.

II MATERIALS AND METHODS

DNA Sequences

PAGE purified DNA sequences (Table 1) were purchased from Life Technologies (Delhi, India). Single-strand concentrations of DNA sequences were determined by measuring absorbance at 260 nm at a high temperature using a spectrophotometer (1800; Shimadzu, Tokyo, Japan) connected to a thermo-programmer. Single-strand extinction coefficients were calculated from mononucleotide and dinucleotide data using the nearest neighbor approximation [5, 7-8].

Circular Dichroism (CD) Measurements

CD experiments utilizing a spectropolarimeter (J-815; Jasco) were measured at room temperture in a 0.1 cm path length cuvette for 1 μM total strand concentration of DNA. Samples were prepared in 30 mM MES buffer (pH 7.0) containing 100 mM NaCl, 100 mM KCl with or without 1 mM $MgCl_2$ and 0.5 mM EDTA at DNA: glucose (1:0.1, 1:100 and 1:10000) ratios. The CD spectrum was averaged of at least three scans from 200 to 350 nm. The temperature of the cell holder was regulated by a temperature controller (PTC-348, Jasco), and the cuvette-holding chamber was flushed with a constant stream of dry N_2 gas to avoid condensation of water on the cuvette exterior. Before measurement, the sample was heated to 95^{o}C, gently cooled at a rate of 0.5^{o}C min^{-1} and incubated at 4^{o}C overnight.

UV Melting Analysis

UV absorbance was measured with a Shimadzu 1800 spectrophotometer equipped with the temperature controller. Melting curves of DNA structures were obtained by measuring the UV absorbance at 260 nm. Samples were prepared in 30 mM MES buffer (pH 7.0) containing 100 mM NaCl, 100 mM KCl with or without 1 mM $MgCl_2$ and 0.5 mM EDTA at DNA:glucose (1:0.1, 1:100 and 1:10000). Before measurement, the samples were heated to 95^{o}C containing different cations, glucose and gently cooled at a rate of 0.5^{o}C min^{-1}, and incubated at 4^{o}C for several hours. Measurement was performed using a 1 cm path length cuvette. The melting temperature (*T*m) values for DNA structures were obtained from the UV melting curves as described previously [9]. The heating rate was 0.5^{o}C min^{-1}. The thermodynamic parameters were obtained from the UV melting curves using the following equation [7].

$$Tm^{-1}=[R \ln (Ct/4) + \Delta S^o]/ \Delta H^o$$

where ΔS^o and ΔH^o are calculated entropy and enthalpy changes for the duplex formation, respectively, R is the gas constant, and *C*t is the total strand concentration. The free energy change at 25^{o}C (ΔG^o_{25}) was evaluated using the following equation:

$$\Delta G^o_{25} = \Delta H^o - T\Delta S^o$$

where T = 298.15 K (25^{o}C).

Nondenaturing Gel Electrophoresis

Nondenaturing gel electrophoresis was carried out with 15% polyacrylamide gels (19:1 acrylamide/bisacrylamide). DNA samples of 8 μM were mixed with ice-cold loading buffer. Samples were prepared in 30 mM MES buffer (pH 7.0) at DNA:Glucose (1:10000) and without glucose containing 100 mM NaCl, 100 mM KCl in the absence and presence of 1 mM $MgCl_2$ and 0.5 mM EDTA respectively. Finally, 8 μL aliquot of the mixed solution was loaded and ran at 5 V cm^{-1} at 4^{o}C. The gel was stained using ethidium bromide and imaged using Gel-Doc (Invitrogen).

III RESULTS AND DISCUSSION

Sequence Design. We carried out the structural analysis of three duplexes that differs from each other with respect to the length and base composition named as Duplex 1 (D1), Duplex 2 (D2) and Duplex 3 (D3). To

compare the effect of length, we have taken Duplex 1(D1-37 mer) and Duplex 2 (D2) or Duplex 3 (D3) of similar length *i.e.* - 21 mer. Moreover, these duplexes differ from each other with respect to base composition. Duplex 1 (D1) has almost equal G.C and A.T content while the Duplex 2 (D2) is more G.C rich and Duplex 3 (D3) is more A.T-rich.

Structural Analysis of DNA Sequences

First, we studied the structure of D1 using CD spectroscopy. CD spectra of 1 μM D1 in Tris-HCl buffer (pH 7.0) in the presence of 100 mM NaCl, KCl, with and without $MgCl_2$ at room temperature were recorded. CD spectra of D1 had a positive peak at 275 nm and negative peaks at 210 nm and 240 nm under all conditions in the absence of glucose Figure 1 (a-d). It has been reported that B-form DNA displays characteristic positive peaks between 250 nm and 280 nm and two negative peaks at 210 nm and 240 nm [10]. This indicates that D1 folds into B-form duplex. There were no significant changes in the CD spectra and intensity with different monovalent cation species indicating that the structure of D1 is mostly independent of the type of cation. CD spectra of 1 μM D2 showed similar signatures suggesting the formation of B-form duplex under all conditions Figure S1 (a-d). CD spectra of 1 μM D3 was observed to be highly polymorphic. The CD spectra had a broad positive peak covering the region from 260 nm to 290 nm along with negative peaks at 210 nm and 240 nm Figure S2 (a-d). It has been reported that the A-form duplex is mostly defined by a positive peak at 260 nm and negative peaks at 210 nm and 240 nm [11]. This suggests that D3 folded into a mixture of A- and B- form duplexes. The mixed duplex structure of D3 is consistent with a previous report on the self-complementary G-rich sequence d(GGGGCCCC) [12]. It has been shown that the CD spectrum of d(GGGGCCCC) suggests an A-form duplex with stacking of half of the bases, whereas the other half stack in a B-form like fashion.

Next, we studied the effect of glucose on the structure of D1, D2 and D3. CD spectra of D1 in the presence of NaCl and glucose without (Figure 1a) and with $MgCl_2$ (Figure 1b) displayed decrease in ellipticity at 270 nm. There was a shift of positive peak at 215 nm to 221 nm at low concentrations of glucose but as the concentration increases, a large positive band develops at 221 nm [13]. Previous reports suggest that the addition of glucose reduces the number of water free OH groups (not involved in H bonds), glucose molecules strongly interact with water and the strength of water-water and water-glucose interactions are of similar extent. Therefore, we propose that at low concentration binding of glucose with DNA is mediated through the water molecules around sugar-phosphate backbone of the DNA while the high concentration of glucose enhancement of CD signal at 221 nm demonstrates the binding of glucose to the groove of the DNA consistent with the previous reports [14]. The CD spectra of D1 in the presence of NaCl and $MgCl_2$ showed similar signatures except at high glucose concentration there was no enhancement of CD intensity at 221 nm (Figure 1b). The CD spectra of D1 in the presence of K^+ without (Figure 1c) and with $MgCl_2$ (Figure 1d) and glucose were almost identical indicating that the structure of D1 is mostly independent of the type of cation.

Structural Analysis of Human Telomeric Sequences (D2) in the presence of glucose

Next, we studied the effect of glucose on the conformation of human telomeric duplex (1:1 mixture of G-rich and C-rich sequences) named as D2 duplex with and without glucose. Figure. S1 (a-d) shows the CD spectra of 1μM D2 in the absence and presence of glucose with different cations. The CD spectrum of 1:1 mixture of G-rich and C-rich sequences in the absence of glucose has a positive peak around 266 nm and a negative one near 240 nm, which is the characteristic of DNA duplex [15]. These results are consistent with previous reports in which the duplex is the predominant at neutral pH [16]. However, minor variation in CD intensity at 266 nm and major variation in CD signals between 200 nm to 240 nm, suggests the binding of glucose with D2 duplex mainly around sugar-phosphate backbone at low concentration of glucose and with grooves of DNA at high concentration of glucose. It is possible that during glucose-water interaction three types of hydrogen bonds are formed: intramolecular hydrogen bonds in glucose molecules, intermolecular hydrogen bonds between glucose molecules and hydrogen bonds between glucose and water molecules. Raman findings on other disaccharides and recent molecular dynamics (MD) simulation results suggest that the glucose acts as a weak structure breaker on the tetrahedral water network. Conversely, the capability of glucose in enhancing the tetrahedral H-bond structure of water has been discussed earlier [17-18]. The destructive effect on the tetrahedral water is partially compensated by the formation of new H bonds within the sugar hydration shell [19-20]. Therefore, it could be possible that similar major changes in CD spectra of the D2 duplex in all ionic conditions in the presence of glucose is due to hydration of the glucose itself around sugar-phosphate backbone of DNA.

In contrast to D1 and D2, CD spectra of D3 at pH 7.0 in the presence of glucose were observed to be polymorphic depending on the nature of cation Figure S2 (a-d). However, in the presence of NaCl, $MgCl_2$ and glucose, the CD spectrum had a broad positive peak covering the region from 260 nm to 290 nm along with negative peaks at 210 nm and 245 nm suggesting that the D3 folded into mixed form of duplex. In addition, presence of similar CD signatures between 200 nm to 240 nm demonstrates the similar binding pattern of

glucose around D3. Interestingly, in the presence of KCl with glucose (Figure S2c), sharp positive CD peak at 280 nm decrease at high glucose concentration, while the addition of $MgCl_2$ (Figure S2d) undergoes a structural conversion from an antiparallel to more parallel duplex induced by the binding of glucose in the presence of these cations. Our CD results are consistent with previous report suggesting that the salt strongly affects the structure of glucose and the sugar– salt–water ratio is a useful parameter in bringing about controlled ion–sugar contacts [21]. The water and the sugar seem to act synergistically in this respect; therefore differences in CD spectra under similar glucose concentrations may be due to the differences in sugar-salt and sugar-salt-water complex around DNA duplex [21].

Structural status of the D1, D2 and D3 in the absence and presence of glucose

We further investigated the structure of D1 using Non-danaturating PAGE. Figure 2 shows the migration of duplex 1 (D1) (lane 1-2) in the presence of 100 mM NaCl without and with DNA:glucose (1:10000), (lane 3-4) in the presence of 100 mM KCl without and with DNA:glucose (1:10000), (lane 5-6) in the presence of 100 mM NaCl and 1 mM $MgCl_2$ without and with DNA:glucose (1:10000), (lane 7-8) in the presence of 100 mM KCl and 1 mM $MgCl_2$ without and with DNA:glucose (1:10000) along with 10 bp ladder as size-marker. We found that D1 showed one major band around the 40 bp DNA marker with different cations in the absence and presence of DNA: glucose (1:10000). These results indicate that the major structure of D1 is a duplex, consistent with our CD data in the absence and presence of high concentration of duplex. In all the lanes, band intensities did not vary depending on the coexisting cations and high concentration of glucose, further suggesting that the structure of D1 was duplex under all conditions.

Thermal Stability of DNA duplexes in the presence of Glucose

To investigate the effect of glucose as hydrophilic molecule on the stability of DNA duplexes, UV melting method was used [7]. We examined the effect of concentration of glucose on the T_m of duplexes (D1, D2, and D3). UV melting curves of duplexes prepared at 1 μM concentration were obtained at 260 nm in the absence and presence of glucose in a buffer containing 30 mM Tris-HCl (pH 7.0), 100 mM NaCl, KCl, and 1 mM EDTA Figure 2 (a-d), Figure S3 (a-d), Figure S4 (a-d). The T_m of the duplex 1 (D1) was marginally descends from 75 ^oC to 74 ^oC in the presence of Na^+ and from 74.5 ^oC to 73.5 ^oC in the presence of K^+ at DNA: glucose (1:0.1, 1:100 and 1:10000) (Table S1). However, T_m of the (D1) duplex was increased by 2^oC in the presence of 100 mM NaCl or 100 mM KCl and 1 mM $MgCl_2$, while there was negligible change in T_m under all concentrations of glucose. These results are consistent with the previous reports that suggest that both divalent and monovalent cations bind to nucleic acid molecules and affect their physical properties [19]. Pioneering experiments done over 40 years ago showed that magnesium ions stabilize DNA duplexes significantly more than the same concentrations of monovalent ions [22]. For example, 10 mM $MgCl_2$ stabilizes a six base pair duplex nearly as much as a 1 M NaCl solution [9] which is substantially more than what would be expected from the magnesium ion contribution to the ionic strength of the solution. Here, glucose is used as relatively simple sugar and hydrophilic molecule. Therefore, glucose may be regarded as a convenient model of more complex biological systems for the study of basic structural and dynamical properties of aqueous media and contrasting results were obtained on the stability of DNA duplex. Based on the experimental results and in view of earlier reports, the differential effect on the structure and stability of the Watson-Crick bonded DNA duplex was observed in the presence of glucose.

UV melting curves of duplex (D2) were also acquired at 260 nm with and without glucose under similar ionic conditions. The T_m of the duplex 2 (D2) was marginally descends from 67^oC to 65^oC in the presence of 100 mM NaCl, from 66.5 ^oC to 65 ^oC in the presence of 100 mM KCl at DNA: Glucose (1:0.1 to 1:10000) (Figure S3a, S3c and Table S2). However, T_m of the (D2) duplex was increased by 2^oC in the presence of 100 mM NaCl or 100 mM KCl and 1 mM $MgCl_2$ (Figure S3b, S3d).

UV melting curves of duplex 3 (D3) were also acquired at 260 nm with and without glucose under similar ionic conditions. The trend of destabilization of the Duplex 3 (D3) was almost similar to D1 and D2. The T_m of the duplex 3 (D3) was marginally descends from 46^oC to 44.5^oC in the presence of 100 mM NaCl. There was negligible change in T_m from 45.5 ^oC to 45 ^oC in the presence of 100 mM KCl, at DNA:glucose (1:0.1 to 1:10000) (Figure S4a, S4c and Table S3). However, T_m of the (D3) duplex was increased by 2^oC and 3^oC in the presence of 100 mM NaCl or 100 mM KCl and 1 mM $MgCl_2$ in the absence of glucose respectively (Figure S4c, S4d).

Effect of glucose on the Thermodynamic Parameters of Nucleic Acid Structure formation

From the UV melting curves, we calculated thermodynamic parameters as described in the Experimental section (Equations 1 and 2). The values of $\Delta H°$, $T\Delta S°$, and $\Delta G°$ at 25 °C ($\Delta G°25$) for all the duplexes studied here are shown in Tables S1, S2, and S3. Duplex 1 (D1) in a 30 mM Tris-HCl (pH 7.0) containing 100 mM NaCl or 100 mM KCl in the absence and presence of glucose, $\Delta H°$ increased from (-39.9 to

-34.5), (-42.5 to -37.6), (-38.3 to -37.4) and (-39.9 to -38.1) kcal/mol, $T\Delta S°$ increased from (-26.4 to -22.1), (-28.8 to -25.2), (-25.9 to -25.2), (-27.6 to -26.5) kcal/mol and the value of $\Delta G°$ at 37 °C calculated from melting curves were estimated as (-13.56 to -12.47), (-13.76 to -12.47), (-12.46 to –11.96) and (-12.36 to -11.55) kcal/mol respectively (Table S1). Next, we calculated the thermodynamic parameters for duplex 2 (D2) under similar conditions, $\Delta H°$ increased from (-70.1 to -61.1), (-69.5 to -65.1), (-62.1 to -59.1) and (-62.9 to -59.3), kcal/mol, $T\Delta S°$ increased from (-56.6 to -48.7), (-56.5 to -53.7), (-49.2 to -47.1), (-50.6 to -48.3) kcal/mol and the value of $\Delta G°$ at 37 °C calculated from melting curves were estimated as (-13.6 to -12.4), (-12.9 to -11.5), (-12.9 to –12.0) and (-12.3 to -11.0) kcal/mol respectively (Table S2). Under similar conditions, the thermodynamic parameters for duplex 3 (D3) were also calculated, $\Delta H°$ increased from (-99.9 to -96.6), (-101.1 to -98.7), (-95.2 to -93.1), (-98.9 to -97.1) kcal/mol $T\Delta S°$ increased from (-89.9 to -88.1), (-89.4 to -89.5), (-85.3 to -83.9), (-89.3 to -88.4) kcal/mol and the value of $\Delta G°$ at 37 °C calculated from melting curves were estimated as (-10.1 to -8.5), (-11.8 to -9.2), (-9.9 to –9.2) and (-9.7 to -8.7) kcal/mol respectively (Table S3). Therefore, UV melting results and negligible change in $\Delta G°$ in all three duplexes in the presence of glucose supports the conclusion obtained from the CD data that the formation of the DNA duplex was least affected in the presence of glucose. This indicates that water-water and water-glucose H bonds are of similar magnitude confirming that very stable hydration shells may be formed around the carbohydrates [23-27].

IV CONCLUSION

Here, we have used the glucose a simple sugar molecule which is hydrophilic in nature to see the effect of interaction of hydroxyl groups of glucose structure with shell of hydration and their overall effect on the structure and stability on the hydrogen bonded DNA duplex. The data presented here emphasize some important points. Firstly, all three duplexes were found to be more stable in the absence of glucose and marginally destabilized even at high concentration of glucose. This may be a consequence of the common mode of interaction of glucose with water molecules present around DNA molecules in the presence of different cations. Our data suggests that the properties of sugar–water–salt systems are an important parameter to understand the interaction between hydrophilic groups and interfacial water molecules and its effect on the structure and stability of DNA duplex. This can open the new strategy not only in understanding the folding of DNA duplex in the presence of hydrophilic groups but also to design the small ligands based on the similarity in the structure of natural molecules and to develop drugs or DNA nanodevices. We are currently working to determine the effect of similar molecules on Hoogsteen bonded structures like G-quadruplex and other higher order structures like i-motif etc.

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Table-1 Sequences of DNA used in this study*

Abbreviation	Sequences
D1	5'-ATGTCCTAGCAATGTAATCGTCAAGCTTTATGCCGTT- 3' 5'-CAACGGCATAAAGCTTGACGATTACATTGCTAGGACAT-3'
D2	5'- GGGTTAGGGTTAGGGTTAGGG-3' 5'- CCCAATCCCAATCCCAATCCC -3'
D3	5'-TAATTTAAAATTTTTAAAAAA - 3' 5'-TTTTTTAAAAATTTTAAATTA - 3'

* Duplex (D1), Duplex (D2) and Duplex (D3)

Figure 1

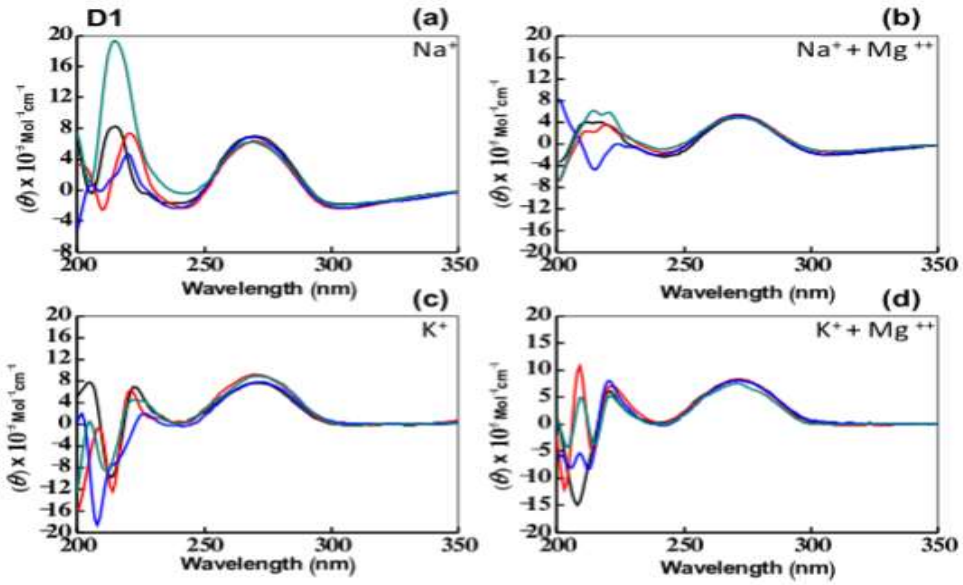

Figure 1. CD spectra of 1 mM D1 in 30 mM Tris-HCl buffer (pH 7.0) containing 0.5 mM EDTA and 100 mM NaCl (a), 100 mM NaCl + 1 mM MgCl2 (b), 100 mM KCl (c), 100 mM KCl + 1 mM MgCl2 (d) with DNA : glucose as 1: 0 (black), 1: 0.1 (red), 1:100 (blue), 1:10000 (green) respectively.

Figure 2

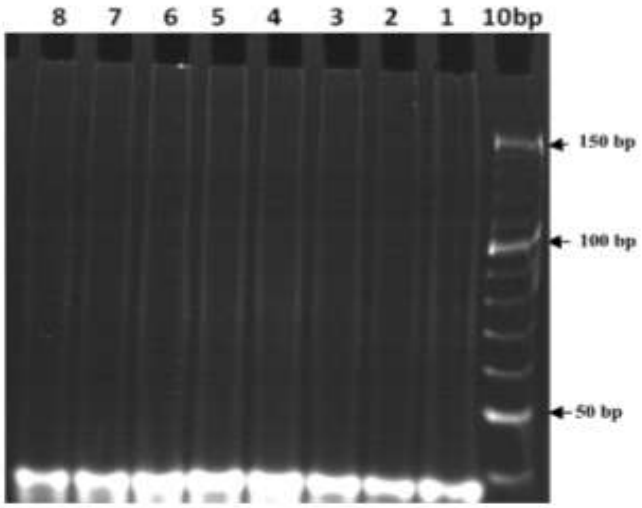

Figure 3

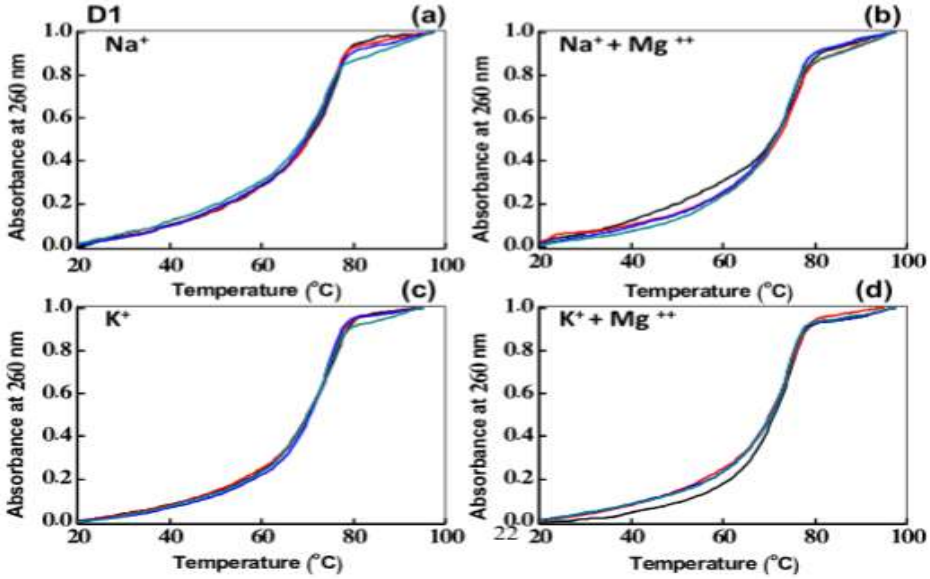

Figure 3. Thermal melting spectra of 1mM D1 in 30 mM Tris-HCl buffer (pH 7.0) containing

Graphical Abstract

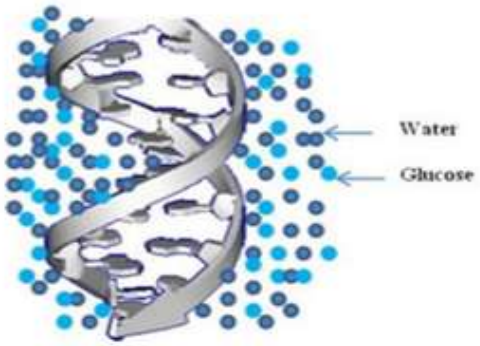

Figure S1.

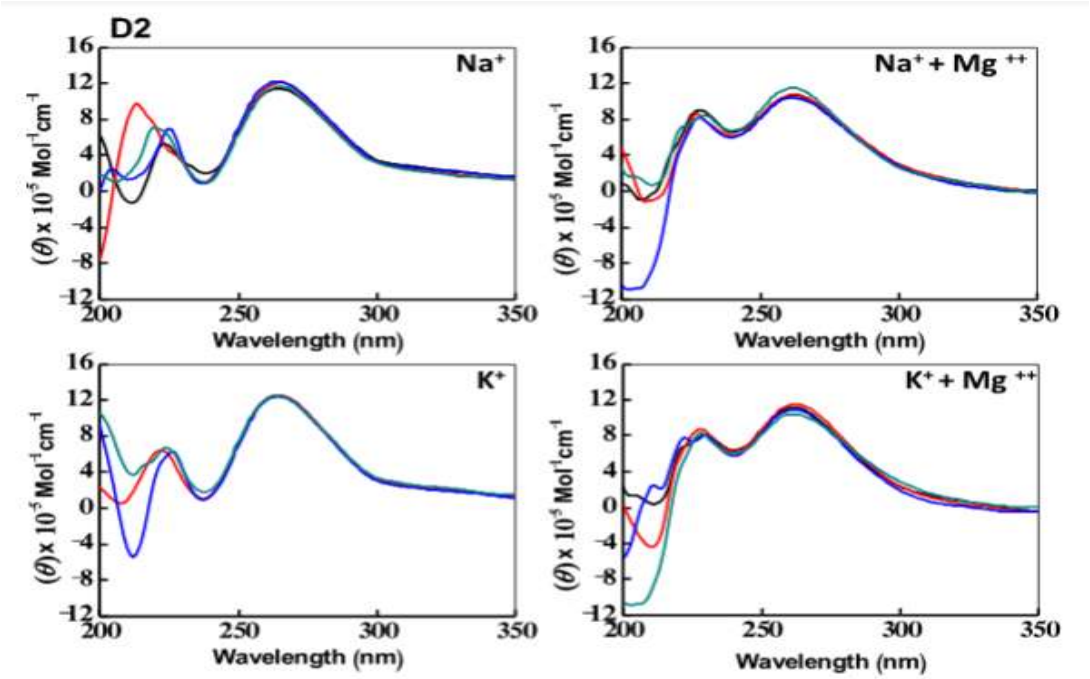

Figure S1. CD spectra of 1mM D2 in 30 mM MES buffer (pH 7.0) containing 0.5 mM EDTA and 100 mM NaCl (a), 100 mM NaCl + 1 mM MgCl2 (b), 100 mM KCl (c), 100 mM KCl + 1 mM MgCl2 (d) with DNA : glucose as 1: 0 (black), 1: 0.1 (red), 1:100 (blue), 1:10000 (green) respectively.

Figure S2.

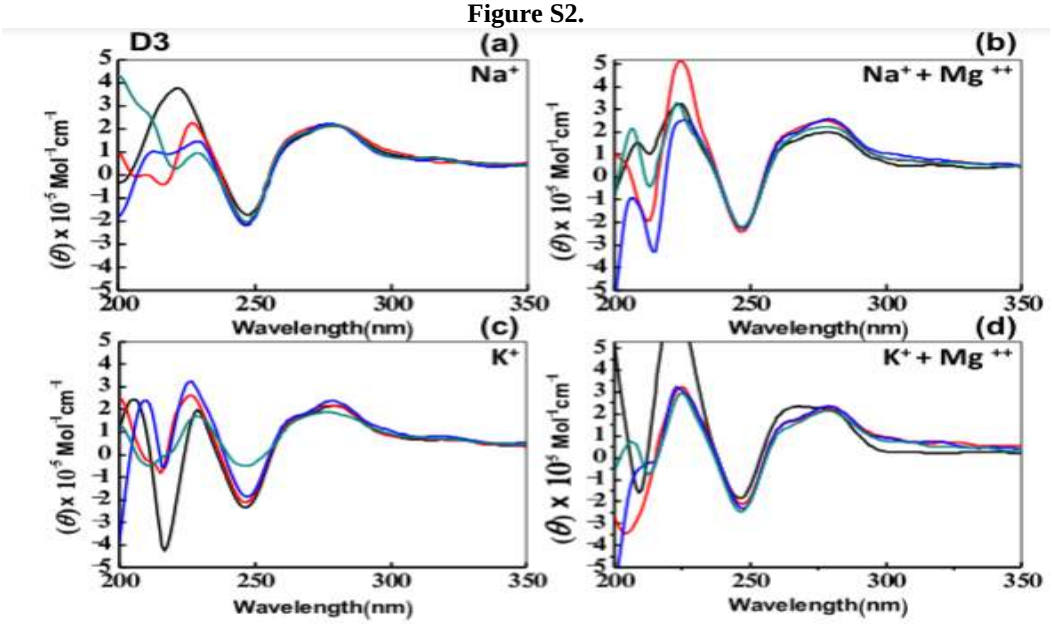

Figure S2. CD spectra of 1mM D3. Measurements were carried out at 4oC in 30 mM Tris-HCl buffer (pH 7.0) containing 0.5 mM EDTA and 100 mM NaCl (a), 100 mM NaCl + 1 mM MgCl2 (b), 100 mM KCl (c), 100 mM KCl + 1 mM MgCl2 (d) with DNA : glucose as 1: 0 (black), 1: 0.1 (red), 1:100 (blue), 1:10000 (green) respectively.

Figure S3.

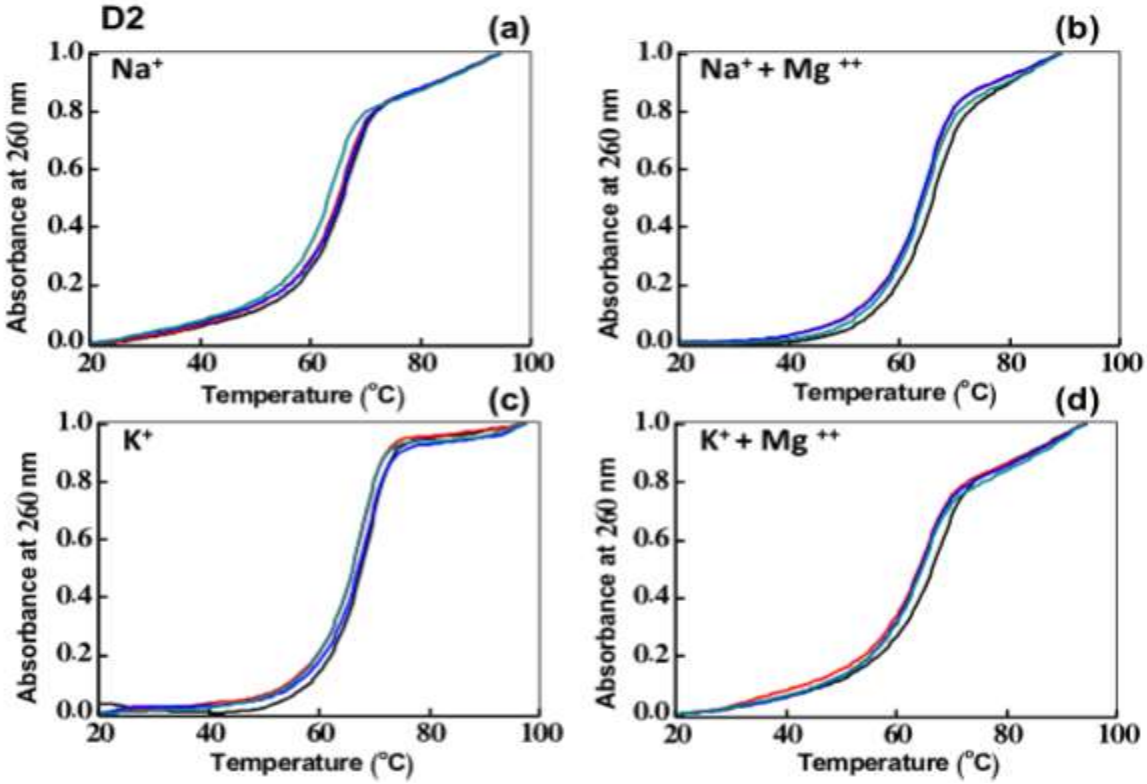

Figure S3. Thermal melting spectra of 1mM D2 in 30 mM MES buffer (pH 7.0) containing 0.5 mM EDTA and 100 mM NaCl (a), 100 mM NaCl + 1 mM MgCl 2 (b), 100 mM KCl (c), 100 mM KCl + 1 mM MgCl 2 (d) with DNA : glucose as 1: 0 (black), 1: 0.1 (red), 1:100 (blue), 1:10000 (green) respectively.

Figure S4.

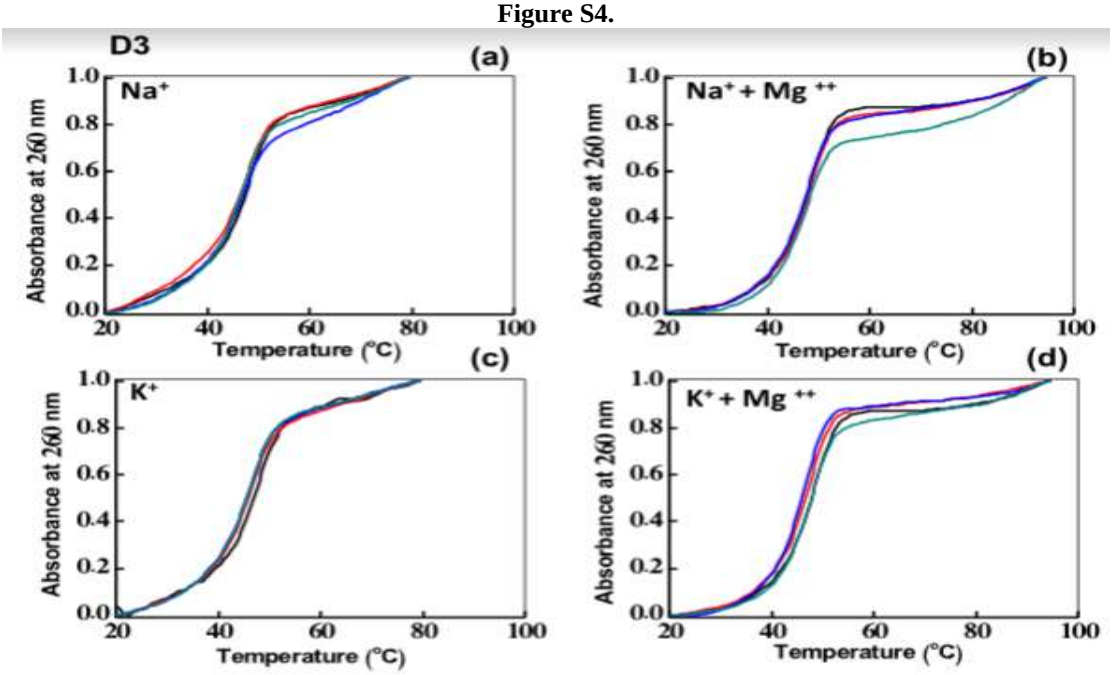

Figure S4. Thermal melting spectra of 1mM D3 in 30 mM MES buffer (pH 7.0) containing 0.5 mM EDTA and 100 mM NaCl (a), 100 mM NaCl + 1 mM MgCl 2 (b), 100 mM KCl (c), 100 mM KCl + 1 mM MgCl 2 (d) with DNA : glucose as 1: 0 (black), 1: 0.1 (red), 1:100 (blue), 1:10000 (green) respectively.

Table -S1. Thermodynamic Parameters for the interaction of Duplex (D1) with glucose

Duplex (D1)	ΔH° (kcal mol^{-1})	$T\Delta S^{\circ}$ (kcal mol^{-1})	ΔG°_{25}(kcal mol^{-1})	T_m (°C)
D1-Na	-39.92 ± 1.2	-26.36 ± 1.3	-13.56 ± 0.3	75.0
D1-Na (1:0.1G)	-39.17 ± 2.6	-25.65 ± 2.1	-13.52 ± 0.2	74.5
D1-Na (1:100G)	-38.01 ± 2.9	-25.02 ± 2.1	-12.99 ± 0.3	74.5
D1-Na (1:10000G)	-34.59 ± 1.2	-22.12 ± 1.3	-12.47 ± 0.3	74.0
D1-K	-42.52 ± 1.3	-28.76 ± 1.4	-13.76 ± 0.2	74.5
D1-K (1:0.1G)	-40.64 ± 2.2	-27.25 ± 2.0	-13.39 ± 0.3	74.0
D1-K (1:100G)	-39.72 ± 2.7	-26.53 ± 2.0	-13.19 ± 0.4	73.5
D1-K (1:10000G)	-37.58 ± 1.1	-25.21 ± 1.5	-12.37 ± 0.2	73.5
D1-NaM	-38.32 ± 1.4	-25.86 ± 1.2	-12.46 ± 0.1	76.0
D1-NaM (1:0.1G)	-38.29 ± 1.8	-25.84 ± 1.8	-12.45 ± 0.2	76.0
D1-NaM (1:100G)	-38.15 ± 2.8	-25.79 ± 1.5	-12.36 ± 0.3	76.0
D1-NaM (1:10000G)	-37.48 ± 1.1	-25.52 ± 1.4	-11.96 ± 0.3	75.8
D1-KM	-39.99 ± 1.1	-27.63 ± 1.3	-12.36 ± 0.2	76.5
D1-KM (1:0.1G)	-39.89 ± 1.3	-27.44 ± 1.5	-12.35 ± 0.1	76.0
D1-KM (1:100G)	-39.55 ± 1.8	-27.29 ± 1.9	-12.26 ± 0.2	76.0
D1-KM (1:10000G)	-38.07 ± 2.1	-26.52 ± 1.4	-11.55 ± 0.3	76.0

Table- S2. Thermodynamic Parameters for the interaction of Duplex (D2) with glucose

Duplex (D2)	ΔH° (kcal mol^{-1})	$T\Delta S^{\circ}$ (kcal mol^{-1})	ΔG°_{37} (kcal mol^{-1})	T_m (°C)
D2-Na	-70.14 ± 1.0	-56.57 ± 3.1	-13.57 ± 0.2	67.0
D2-Na (1:0.1G)	-64.63 ± 1.1	-51.11 ± 2.5	-13.52 ± 0.1	66.5
D2-Na (1:100G)	-62.98 ± 1.2	-50.05 ± 2.1	-12.93 ± 0.3	66.0
D2-Na (1:10000G)	-61.11 ± 1.0	-48.74 ± 1.2	-12.37 ± 0.2	65.0
D2-K	-69.46 ± 1.3	-56.53 ± 1.4	-12.93 ± 0.2	66.5
D2-K (1:0.1G)	-66.95 ± 2.2	-54.16 ± 2.0	-12.79 ± 0.3	65.0
D2-K (1:100G)	-65.88 ± 2.7	-53.98 ± 2.0	-11.90 ± 0.4	65.0
D2-K (1:10000G)	-65.12 ± 1.1	-53.65 ± 1.5	-11.47 ± 0.2	65.0
D2-NaM	-62.09 ± 1.4	-49.23 ± 1.2	-12.86 ± 0.1	69.0
D2-NaM (1:0.1G)	-61.95 ± 1.8	-49.26 ± 1.8	-12.69 ± 0.2	68.5
D2-NaM (1:100G)	-60.15 ± 2.8	-47.79 ± 1.5	-12.36 ± 0.3	68.0
D2-NaM (1:10000G)	-59.11 ± 1.1	-47.13 ± 1.4	-11.98 ± 0.3	67.5
D2-KM	-62.96 ± 1.0	-50.66 ± 1.3	-12.30 ± 0.1	68.0
D2-KM (1:0.1G)	-60.72 ± 1.4	-49.80 ± 1.5	-10.92 ± 0.3	66.5
D2-KM (1:100G)	-60.25 ± 1.1	-49.02 ± 1.8	-11.23 ± 0.2	66.5
D2-KM (1:10000G)	-59.27 ± 1.1	-48.26 ± 1.3	-11.01 ± 0.4	66.5

Table- S3. Thermodynamic Parameters for the interaction of Duplex (D3) with glucose

Duplex (D3)	ΔH° (kcal mol^{-1})	$T\Delta S^{\circ}$ (kcal mol^{-1})	ΔG°_{37} (kcal mol^{-1})	T_m (°C)
D3-Na	-99.92 ± 1.2	-89.97 ± 1.3	-10.05 ± 0.3	46.0
D3-Na (1:0.1G)	-98.87 ± 2.6	-89.20 ± 2.1	-9.67 ± 0.2	45.5
D3-Na (1:100G)	-98.01 ± 2.9	-88.82 ± 2.1	-9.19 ± 0.3	45.0
D3-Na (1:10000G)	-96.59 ± 1.2	-88.12 ± 1.3	-8.47 ± 0.3	44.5
D3-K	-101.14 ± 1.3	-89.38 ± 1.4	-11.76 ± 0.2	45.5
D3-K (1:0.1G)	-99.87 ± 2.2	-89.48 ± 2.0	-10.39 ± 0.3	45.0
D3-K (1:100G)	-99.34 ± 2.7	-89.15 ± 2.0	-10.19 ± 0.4	45.0
D3-K (1:10000G)	-98.68 ± 1.1	-89.51 ± 1.5	-9.17 ± 0.2	45.0
D3-NaM	-95.23 ± 1.4	-85.32 ± 1.2	-9.91 ± 0.1	48.0
D3-NaM (1:0.1G)	-94.21 ± 1.8	-84.42 ± 1.8	-9.79 ± 0.2	48.0
D3-NaM (1:100G)	-93.35 ± 2.8	-83.99 ± 1.5	-9.36 ± 0.3	47.5
D3-NaM (1:10000G)	-93.08 ± 1.1	-83.92 ± 1.4	-9.16 ± 0.3	47.5
D3-KM	-98.99 ± 1.1	-89.29 ± 1.3	-9.70 ± 0.2	48.5
D3-KM (1:0.1G)	-98.74 ± 1.3	-89.11 ± 1.5	-9.63 ± 0.1	48.0
D3-KM (1:100G)	-97.63 ± 1.8	-88.44 ± 1.9	-9.19 ± 0.2	47.5
D3-KM (1:10000G)	-97.07 ± 2.1	-88.41 ± 1.4	-8.66 ± 0.3	47.5

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