

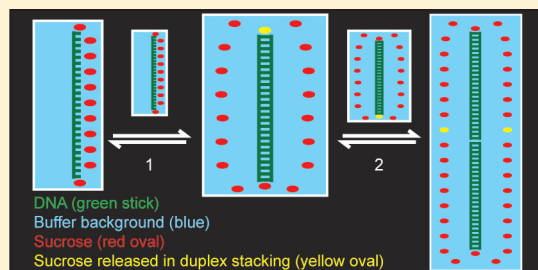
Effects of a Protecting Osmolyte on the Ion Atmosphere Surrounding DNA Duplexes

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S Supporting Information

ABSTRACT: Osmolytes are small, chemically diverse, organic solutes that function as an essential component of cellular stress response. Protecting osmolytes enhance protein stability via preferential exclusion, and nonprotecting osmolytes, such as urea, destabilize protein structures. Although much is known about osmolyte effects on proteins, less is understood about osmolyte effects on nucleic acids and their counterion atmospheres. Nonprotecting osmolytes destabilize nucleic acid structures, but effects of protecting osmolytes depend on numerous factors including the type of nucleic acid and the complexity of the functional fold. To begin quantifying protecting osmolyte effects on nucleic acid interactions, we used small-angle X-ray scattering (SAXS) techniques to monitor DNA duplexes in the presence of sucrose. This protecting osmolyte is a commonly used contrast matching agent in SAXS studies of protein–nucleic acid complexes; thus, it is important to characterize interaction changes induced by sucrose. Measurements of interactions between duplexes showed no dependence on the presence of up to 30% sucrose, except under high Mg^{2+} conditions where stacking interactions were disfavored. The number of excess ions associated with DNA duplexes, reported by anomalous small-angle X-ray scattering (ASAXS) experiments, was sucrose independent. Although protecting osmolytes can destabilize secondary structures, our results suggest that ion atmospheres of individual duplexes remain unperturbed by sucrose.



Osmolytes are small intracellular organic solutes that function as a vital component of cellular stress response.^{1–4} They are chemically diverse and include polyols, sugars, amino acids, and methylamines. Organisms preferentially utilize subsets of these molecules.^{1,3,4} For example, under conditions of increased salinity, plants accumulate a variety of osmolytes such as amino acids (proline and valine) and sugars (glucose and sucrose); however, *Saccharomyces cerevisiae* utilizes glycerol as an osmolyte in a near-exclusive fashion.⁴ Despite these differences in chemical structure and stress specificity, protecting or compatible osmolytes enhance protein stability by the same chemical mechanism: preferential exclusion.^{2,5,6} The protecting osmolyte is excluded from the surface of the protein, making denaturation less energetically favorable than in an aqueous environment. Thus, the ratios of protecting to nonprotecting osmolytes as well as protecting osmolytes to ions must be carefully maintained to preserve protein structure and protein–nucleic acid interactions.^{7–9}

In contrast to osmolyte effects on protein stability, structure, and function, the effects of osmolytes on nucleic acids are generally less well understood. Nonprotecting osmolytes such as urea can destabilize nucleic acid structures and are commonly utilized in folding studies.^{10–16} However, the effects of protecting osmolytes on nucleic acid structure depend on the type of nucleic acid, its structural complexity, and folding interactions. Although most protecting osmolytes destabilize secondary structure, their effects on tertiary or higher order structure formation rely on various complex interactions. Changes in nucleic acid hydration and nucleic acid–osmolyte

interactions must be balanced against the impact of molecular crowding and changes in excluded volume.^{17–30} Moreover, the effects of osmolytes on ion atmospheres of nucleic acids have not been fully characterized. The complementary or sometimes opposing effects of ions and osmolytes as well as attenuation of osmolyte– Mg^{2+} stabilization of nucleic acid structure are beginning to be studied,^{21,27} but important structural properties such as the number of bound ions have so far been determined by indirect measurement.²³ Here we describe results of experiments designed to probe the effects of osmolytes on nucleic acid electrostatics and the ion atmosphere. We examined the interactions of a 25-base-pair (bp) DNA duplex in the presence and absence of sucrose, a protecting osmolyte, using small-angle X-ray scattering (SAXS) techniques. Sucrose is an important contrast matching agent in SAXS studies of protein–nucleic acid complexes. At elevated concentrations, sucrose's electron density matches that of protein, rendering it (mostly) indistinguishable from the solvent. Nucleic acids have higher electron densities than proteins, so are not contrast matched even at these increased sucrose concentrations. Contrast matching studies have been used to selectively probe the nucleic acid portion of complexes including the ribosome and nucleosome core particles.^{31–34} Although sucrose can destabilize nucleic acid secondary structures,^{21,22} we find

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that electrostatic interactions between duplexes remained unaffected even in 30% sucrose, except under high salt conditions where the duplexes can interact via intermolecular stacking. In addition, anomalous small-angle X-ray scattering (ASAXS) experiments report no change in the number of ions around the duplexes as the sucrose content of the solution increases from 0% to 30%. The shape of the anomalous scattering signal reports the ion correlation to the nucleic acid, and it is not modified by added sucrose, suggesting that the spatial distribution of the ions is unaffected by the presence of this osmolyte. Taken together, these results suggest that both electrostatic interactions between duplexes and the ion atmospheres around individual duplexes remain unperturbed by sucrose. Moreover, this approach is easily extended to elucidate the role of other osmolytes in modifying nucleic acid interactions.

MATERIALS AND METHODS

DNA Duplex Preparation. The desalted and purified single-strand components of the 25-bp DNA were purchased from Integrated DNA Technologies (IDT, Coralville, IA). The DNA sequence GCATCTGGGCTATAAAAGGGCGTCG is the same as in previous studies.^{35–39} Lyophilized samples were reconstituted in an annealing buffer of 10 mM (millimolar) TRIS, 1 mM EDTA, and 50 mM NaCl at pH 8.0. Concentrations of the single strands were calculated using UV absorbance at 260 nm, and equimolar amounts of the complementary strands were mixed and annealed at 95 °C for 2 min before the solutions were allowed to slowly cool to room temperature. For SAXS experiments, the duplex strands were then dialyzed extensively against bulk 1 mM Na⁺ MOPS solutions (pH 7) containing MgCl₂ using Amicon Ultra centrifugal filter units (Millipore Corp., Billerica, MA) with a 10 000 nominal molecular weight limit. Sucrose was added to samples postdialysis for 0.32 and 1.25 *m* concentrations which we report as the more familiar 10 and 30 wt %, respectively. DNA duplexes were prepared for final concentrations of approximately 0.15 and 0.5 mM duplex for “low” and “high” concentration samples, respectively. The concentrations were determined from absorbance at 260 nm, and the duplex extinction coefficient was calculated from extinction coefficients of the single strands as previously described.⁴⁰ DNA duplex samples for ASAXS measurements were prepared in similar fashion to those used for SAXS, except that these samples were dialyzed against 1 mM Na⁺ MOPS solutions (pH 7) containing either rubidium acetate (Rb⁺ acetate) for ASAXS samples or sodium acetate (Na⁺ acetate) for the control samples. Rb is used for ASAXS experiments because of its readily accessible absorption edge near 15 keV. The energy of the comparable edge for Na is inaccessible.

SAXS Experiments and Determination of Second Virial Coefficients. All SAXS and ASAXS experiments were performed at the Cornell High Energy Synchrotron Source (CHESS) C1 station. SAXS profiles effectively report on interactions between DNA duplexes in solution.^{35,36,38,41} The concentration normalized scattering intensity $I(q)$ is given by eq 1.

$$I(q) = P(q)S(c, q) \quad (1)$$

Here, c is the concentration of molecules and q is the momentum transfer ($4\pi/\lambda$) $\sin \theta$, where λ is the X-ray wavelength and 2θ is the scattering angle. $P(q)$ (form factor) is the spherically averaged scattering from an individual molecule,

and $S(c, q)$ (effective structure factor) is related to the spatial arrangement of the molecules: $S(c, q)$ describes intermolecular interactions and accounts for short-range order in the ensemble. This effective structure factor relies on a spherical approximation, which is valid for short duplexes.⁴¹ The second virial coefficient (A_2) provides a measure of intermolecular interaction potential and can be derived from a linear fit to the following expression:^{35,36,38,41}

$$\frac{1}{S(c, q=0)} = 1 + 2MA_2c \quad (2)$$

In eq 2, $S(c, q=0)$ is the effective structure factor extrapolated to $q = 0$, M is the molecular weight of the molecule, and q and c are the same as in eq 1. To use eq 2, $P(q)$ must be known either from experiment or calculation.^{35,36,38,41} However, the following expression is obtained by substituting the reciprocal of eq 1 into eq 2:

$$\frac{1}{I(q=0)} = \frac{2MA_2c}{P(q=0)} + \frac{1}{P(q=0)} \quad (3)$$

This equation defines a strategy for finding A_2 without knowledge of $P(q)$: divide the slope ($2MA_2/P(q=0)$) by the intercept ($1/P(q=0)$). This approach is useful when accurate measurements of $P(q)$ may be difficult to obtain experimentally due to high scattering of the osmolyte in solution or subtle osmolyte-induced contrast variation effects in multicomponent systems. Reference 41 outlines a detailed procedure for extracting A_2 from SAXS data.

ASAXS Experiments and Counting Ions around DNA Duplexes. ASAXS experiments, which exploit the energy dependence of scattering factors of ions around the DNA duplex, were performed to measure the spatial distribution of associated monovalent cations (100 mM Rb⁺ acetate, 100 mM Na⁺ acetate controls). Both the number and distributions of ions surrounding the 25-bp DNA duplex were obtained in the presence of 0, 10, and 30% sucrose. The ASAXS technique was recently reviewed, and important experimental details were described including the beamline setup, background subtraction, and fluorescence correction.^{37,38,41,42} In addition, absolute calibration of the ASAXS signals enables measurement of the number of excess ions in solution due to the presence of the nucleic acid (N_{ions}).³⁹ Excess ions are the charge-compensating ions present in the DNA-containing solution, relative to the bulk solvent. The number of excess ions is easily computed using scattering profiles measured at two X-ray energies:³⁹

$$N_{\text{ions}} = \sqrt{\frac{\alpha}{S(c, q=0)}} \times \frac{\sqrt{I(q=0, E_1)} - \sqrt{I(q=0, E_2)}}{f'(E_1) - f'(E_2)} \quad (4)$$

In eq 4, $I(q=0)$ is the concentration normalized scattering intensity extrapolated to $q = 0$, and $S(c, q=0)$ is as in eq 1. Alpha (α), the scale factor for absolute intensity calibration, is determined using the procedure discussed in ref 37. E_1 and E_2 are X-ray energies 1 and 2 and the f' are (sucrose-independent) ion scattering factors at X-ray energies 1 and 2, respectively. For these experiments, $E_1 = 15.093$ keV, $E_2 = 15.193$ keV, $f'(E_1) = -4.09$ electrons, and $f'(E_2) = -7.02$ electrons.

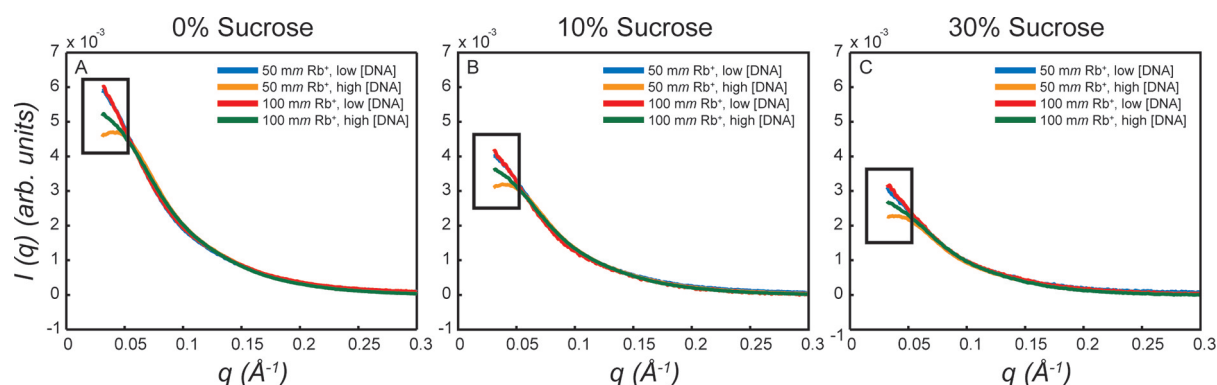


Figure 1. Concentration normalized scattering intensity profiles of 25-bp duplex DNA in Rb^+ acetate and sucrose. Panels A–C show $I(q)$ vs q for low and high DNA concentrations at 50 and 100 mM Rb^+ acetate with 0%, 10%, and 30% sucrose, respectively. All curves shown are the average of 16–32 scattering images, with a standard deviation of $\sim 1\%$. The overall decrease in signal from panel A to panel C is due to contrast variation effects of the sucrose. However, the same trends in the data are observed. The curves from the 50 mM Rb^+ samples fall below those from the 100 mM Rb^+ samples in the low q region, consistent with greater repulsion in lower ionic strength. In addition, more repulsion is observed at higher DNA concentration, signaled by a more pronounced downturn in the intensity profile at low q .

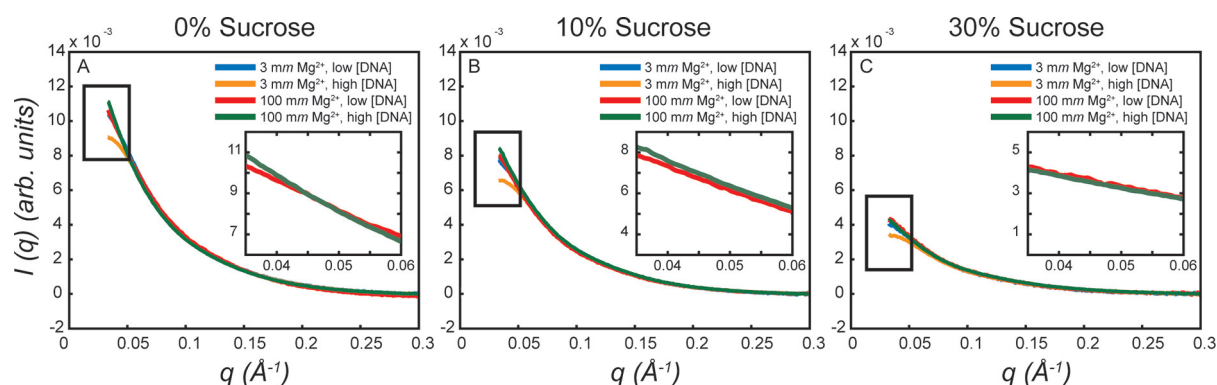


Figure 2. Concentration normalized scattering intensity profiles of 25-bp duplex DNA in MgCl_2 and sucrose. Panels A–C show $I(q)$ vs q for low and high DNA concentrations at 3 and 100 mM MgCl_2 with 0%, 10%, and 30% sucrose, respectively. All curves shown are the average of 16–32 scattering images, with a standard deviation of $\sim 1\%$. The overall decrease in signal from panel A to panel C is due to contrast matching effects of the sucrose. Note that the same trends in the 3 mM Mg^{2+} data are observed. The curves from the 3 mM Mg^{2+} samples generally fall below those from the 100 mM Mg^{2+} samples in the low q region consistent with repulsion in 3 mM Mg^{2+} , and more repulsion is observed for the high DNA concentration samples in 3 mM Mg^{2+} signaled by the downturn in the intensity profile at low q compared to the low concentration profiles. However, there is a difference in the 100 mM Mg^{2+} samples with increasing sucrose. In no sucrose (A) the high concentration profile (green) falls above the low (red) concentration profile consistent with end-to-end stacking of the DNA duplexes. This difference is qualitatively less with increasing sucrose (B, C), suggesting less end-to-end stacking in sucrose. The insets are zoomed to $0.035 < q < 0.06$ to better show the change in trend at 100 mM Mg^{2+} with increasing sucrose.

UV Melting Experiments. DNA duplex melt samples were prepared by diluting aliquots of the SAXS samples to $\sim 5 \mu\text{M}$ duplex with the same buffers and sucrose solutions as used in the SAXS experiments. Because of the high stability of duplexes in 100 mM MgCl_2 , melts were not possible. UV melts used a Cary 50 UV–vis spectrophotometer (Varian Inc., Australia) with a data point acquired every 0.5°C at a wavelength of 260 nm. The DNA was thermally denatured and refolded over temperature ranges of 15–95 and 95–15 $^\circ\text{C}$. The rate of temperature changes was $1^\circ\text{C}/\text{min}$ and provided data consistent with reversibility of the two-state folding transition. Absorbance curves were fit to determine thermodynamic parameters using MeltWin 3.5 software.⁴³

RESULTS AND DISCUSSION

Sucrose Does Not Affect Interduplex Interactions Except at High Mg^{2+} Concentrations. Past studies of this 25-bp duplex^{35,36,38} have quantified the interactions of monovalent and divalent cations with DNA as well as ion-

induced interactions between DNA. Here, we address changes in interduplex interactions following variation of a third parameter: sucrose concentrations between 0 and 30%. To enable comparison with past studies, Rb^+ acetate was selected as the monovalent salt. Importantly, Rb^+ is the lightest monovalent counterion compatible with ASAXS. Studies were carried out at both 50 and 100 mM Rb^+ acetate, where repulsive interactions between duplexes dominate. SAXS studies on divalent ions were restricted to MgCl_2 at concentrations of 3 and 100 mM MgCl_2 . In 0% sucrose, repulsive interactions between DNAs are measured at low Mg^{2+} concentrations, and end-to-end stacking of helices is observed at higher Mg^{2+} concentrations.^{35,36,38,44} A discussion of the uniqueness of this interpretation can be found in the Supporting Information for ref 38. Lastly, the DNA samples were prepared at two different concentrations, approximately 0.15 mM and 0.5 mM duplex, respectively, to probe interparticle interactions and enable application of eq 2 or 3.^{35,36,38}

Concentration normalized SAXS scattering profiles for DNA in Rb^+ are shown in Figure 1. Panels A–C of Figure 1 show Rb –DNA in the presence of 0, 10, and 30% sucrose. At 0% sucrose (Figure 1A) trends are identical to those reported previously.^{35,36,38} For a given concentration of Rb^+ acetate (e.g., 50 mM Rb^+) increasing DNA concentration (blue to orange curves) causes a decrease in intensity at low q , $q < 0.05$, consistent with greater repulsion between helices and increased variation of $S(c, q)$. Additionally, scattering profiles acquired at a fixed DNA concentration display more repulsion at lower ionic strength; e.g., the low q scattering profile is further depressed in 50 mM compared with 100 mM Rb^+ . This effect becomes more pronounced at higher DNA concentration, e.g., compare the orange and green curves in Figure 1A. As the sucrose concentration increases to 10% (Figure 1B) and then to 30% (Figure 1C), the overall signal strength drops due to the decreasing contrast with electron dense sucrose. Despite this overall decrease, the trends observed at 0% sucrose persist: the enhancement of repulsive forces resulting from a decrease in salt concentration and/or an increase in DNA concentrations is manifested as a decrease in the low q scattering intensity. Qualitatively, the data of Figure 1 suggest that sucrose neither enhances nor disrupts interduplex interactions in monovalent salt.

Sucrose-dependent SAXS scattering profiles for the 25-bp DNA duplex in the presence of MgCl_2 are shown in Figure 2. In the absence of sucrose, these data agree with previous measurements: at 3 mM Mg^{2+} data (blue and orange curves), increasing DNA concentration results in a decrease in scattering intensity at low q , reflecting greater repulsion between duplexes.^{35,36,38} In contrast, when solutions contain 100 mM Mg^{2+} (red and green curves), an increase in DNA concentration results in increased low q intensity, consistent with the stacking of DNA duplexes into extended helical structures.^{35,36,38,44} This ionic strength dependence follows previously established trends: repulsion is enhanced as ionic strength decreases. As a function of increasing sucrose (Figure 2A–C), the intensity of each curve decreases due to the contrast matching effects of the osmolyte, as observed in the Rb^+ data (Figure 1A–C). However, whereas the inter-DNA repulsion measured at 3 mM Mg^{2+} persists at all sucrose concentrations (orange and blue curves, all panels Figure 2), Mg^{2+} -induced association of helices depends on sucrose. The enhanced scattering intensity at low q with increasing DNA concentration, indicative of end-to-end stacking of duplexes, vanishes at 30% sucrose (Figure 2C). There is a measurable decrease in the population of end-to-end stacked duplexes. Thus, the data of Figure 2 suggest that addition of sucrose does not modify the repulsive forces present at low concentrations of divalent salts, but sucrose hinders formation of superstructure in the presence of high concentration of divalent salt.

To quantify the strength of the repulsive interactions, we computed the second virial coefficient, A_2 , for both the Rb^+ and Mg^{2+} containing samples (Figure 3). In general, $A_2 > 0$ signifies net repulsion and $A_2 < 0$ (Figure 3, shaded region) implies net attraction between biomolecules. For both 50 and 100 mM Rb^+ A_2 is positive. In accord with measurements at 0% sucrose, the A_2 values are larger at 50 mM than 100 mM Rb^+ . Moreover, the A_2 values for 0, 10, and 30% sucrose samples (circle, square, and triangle, respectively) are the same within error for a given Rb^+ concentration. Thus, the A_2 data demonstrate that duplex interactions in monovalent salt are unperturbed by added sucrose.

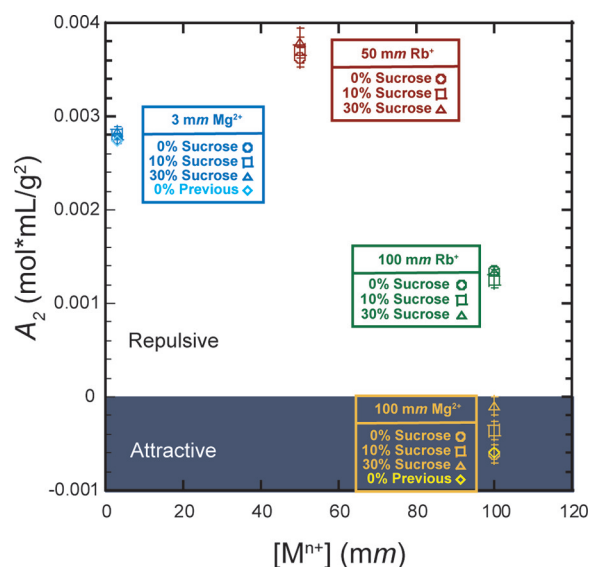


Figure 3. A_2 vs monovalent or divalent cation concentration. Shows A_2 for 50 and 100 mM Rb^+ as well as 3 and 100 mM Mg^{2+} . For all of the repulsive conditions ($A_2 > 0$) the interaction potential among DNA duplexes remains unchanged within error with sucrose, and 0% sucrose data agrees with previously published values for the 3 mM Mg^{2+} data.³⁸ The A_2 value for 100 mM Mg^{2+} at 0% sucrose also agrees with previously published values,³⁸ but the interaction potential decreases with increasing sucrose, suggesting disruption of end-to-end duplex stacking. Error bars shown are propagated from linear fits from eq 2 or 3.

Unlike the Rb^+ data, the A_2 values for the Mg^{2+} containing samples show different trends with increasing sucrose depending on the Mg^{2+} concentration. At 0% sucrose, A_2 is consistent with previously published values.³⁸ In the presence of 3 mM Mg^{2+} , A_2 is positive and, as with the monovalent ion data, independent of sucrose concentration up to 30%. In contrast, in 100 mM Mg^{2+} , the magnitude of A_2 decreases with added sucrose, reflecting weaker interparticle association. This perturbation of interduplex interactions suggests interplay between the osmolyte and the helical stacking.

In summary, the scattering profiles and A_2 values measured as a function of sucrose suggest that the osmolyte does not affect interduplex interactions in the presence of monovalent and low concentrations of divalent counterions where only secondary structure is present. However, in the presence of high concentrations of divalent counterions, sucrose disrupts the formation of higher order structure.

Sucrose Does Not Change the Distribution or Number of Ions around DNA Duplexes. To probe the ion atmosphere of the 25 bp DNA, ASAXS was used to count the number of excess ions around the duplex in the presence of 0, 10, and 30% sucrose. ASAXS scattering profiles are sensitive to counterion–DNA distances⁴² and thus also provide the spatial correlation of these ions to the duplex. The anomalous scattering profiles for both the 100 mM Rb^+ acetate samples and the 100 mM Na^+ acetate controls are shown in Figure 4A. As expected, the controls show near zero scattering intensity. Since no resonant elements are present, no variation of scattering with energy is expected for these samples. As sucrose is added, the overall size of the anomalous signal decreases, which reflects the changing contrast. However, the shape of the anomalous scattering profile is sucrose independent. Thus, the quantity of interest, e.g., the spatial distribution of ions around

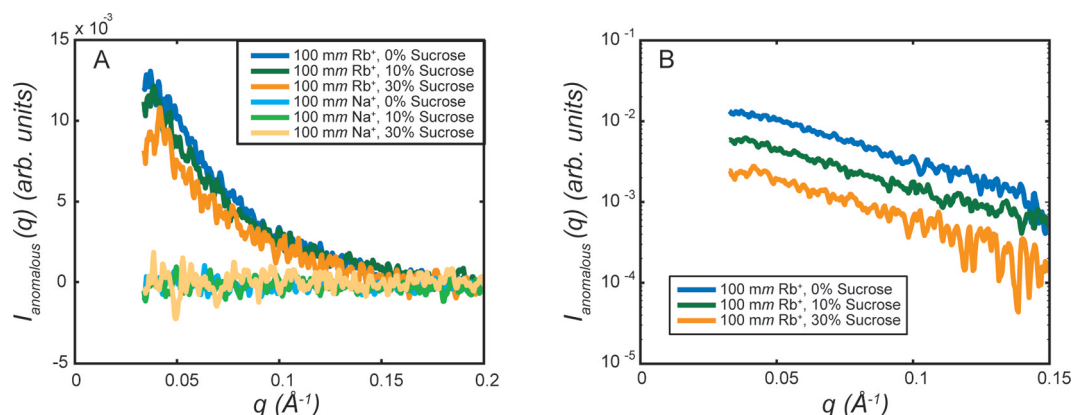


Figure 4. Concentration normalized ASAXS profiles for 100 mM Rb^+ acetate samples and 100 mM Na^+ acetate controls. (A) ASAXS data for 0, 10, and 30% sucrose for Rb^+ and Na^+ controls. The small differences in intensity may be from contrast matching effects of sucrose, and Na^+ controls show near zero anomalous scattering intensity. (B) Rb^+ anomalous scattering intensity as in panel A, but on a logarithmic scale (data offset by a multiplicative factor) to highlight the similarities in the shape of the curve decay with increasing sucrose. This shape similarity suggests the distribution of ions around the duplex is not changed by increasing sucrose concentration.

the DNA, does not change with sucrose. Figure 4B displays the data on a logarithmic scale and highlights the similar shape of these profiles. From these data we conclude that sucrose does not alter the distribution of ions around the duplex.³⁸

The number of excess ions around the duplex was calculated using eq 4 (Materials and Methods), and N_{ions} is shown in Figure 5. In 0% sucrose N_{ions} equals 34 ± 3 , in good agreement

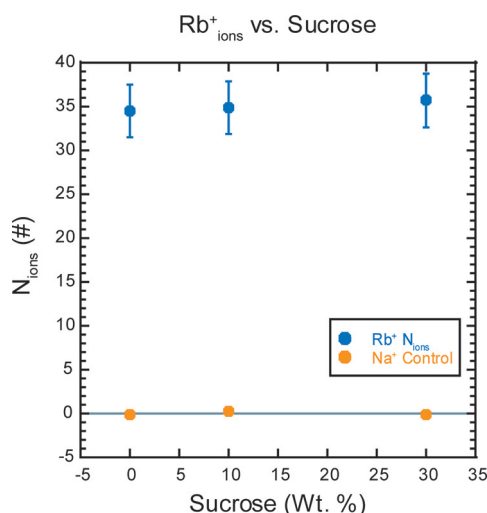


Figure 5. N_{ions} vs sucrose. Shows N_{ions} for Rb^+ and Na^+ controls at 0%, 10%, and 30% sucrose. Note that N_{ions} is unchanged within error with increasing sucrose and agrees with previous results in 0% sucrose.³⁹ Controls are also unchanging with sucrose and near zero since no resonant elements are present in the ASAXS measurement. Error bars shown are $\sim 10\%$ for both Rb^+ and Na^+ with the latter being small than the size of the data points.

with previous counting experiments and NLPB calculations.³⁹ This value remains constant (within error) with increasing sucrose, reinforcing the conclusions drawn from the SAXS data: electrostatic interactions are minimally dependent on sucrose concentration. Thus, both the distribution and number of monovalent ions around DNA appear independent of sucrose; sucrose does not perturb nucleic acid ion atmospheres in monovalent salt background.

Sucrose Destabilizes DNA Duplexes. The SAXS and ASAXS data presented above suggest that sucrose does not alter interduplex interactions or the duplex ion atmosphere in 50 and 100 mM Rb^+ acetate or in 3 mM MgCl_2 . To determine any other effects of sucrose on duplex structure, we examined duplex stability via UV melts of SAXS samples for these three ionic conditions in 0 and 30% sucrose. Thermal denaturation of the 100 mM Mg^{2+} sample was not attempted due to the high stability of the duplex. Typical melting curves are shown in Figure 6; the thermodynamic parameters extracted from

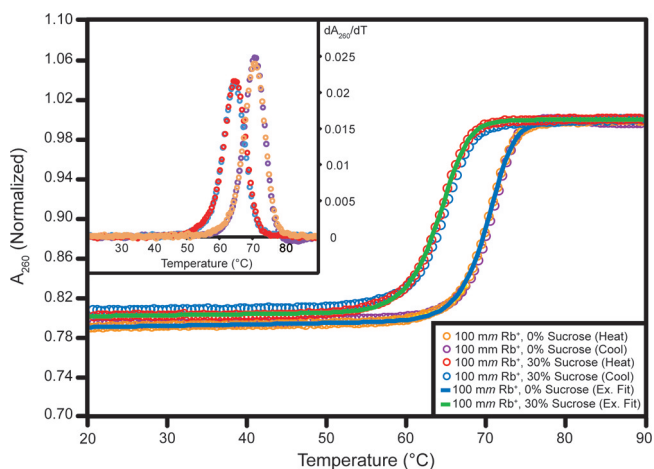


Figure 6. Absorbance vs temperature curves from thermal denaturations. Shows representative heating and cooling melt curves for 100 mM Rb^+ data in 0% (orange, purple) and 30% (red, blue) sucrose. Curves have been corrected for buffer absorbance before fitting, and example fits from Meltwin 3.5 are also shown for the heating curves. Fits of cooling data are similar, but omitted here for figure clarity. The inset shows the derivative of the melting curves with temperature. These data were smoothed using an 11-pt window.

analysis of the curves are provided in Table 1. For all ionic conditions studied, the duplex was destabilized by sucrose, consistent with reports that sucrose destabilizes other nucleic secondary structures.^{21,22} The destabilization was larger for samples containing monovalent ions and compares well in magnitude with previous measurements of similarly sized poly(ethylene glycol)s and comparable length DNA duplexes.¹⁹

Table 1. Thermodynamic Parameters for Folding of 25-bp DNA Duplex

samples	$\Delta H^{\circ a}$ (kcal/mol)	$\Delta S^{\circ a}$ (eu)	$\Delta G_{37}^{\circ a}$ (kcal/mol)	$\Delta \Delta G_{37}^{\circ a}$ (kcal/mol)	T_M^a (°C)	ΔT_M^a (°C)
100 mM Rb ⁺ acetate, 0% sucrose	-201.3 ± 7.4	-558 ± 22	-28.14 ± 0.75		70.5 ± 0.4	
100 mM Rb ⁺ acetate, 30% sucrose	-183.0 ± 5.9	-516 ± 17	-23.06 ± 0.53	5.08 ± 0.91^b	64.5 ± 0.2	-6.0 ± 0.4^b
50 mM Rb ⁺ acetate, 0% sucrose	-200.6 ± 8.2	-563 ± 28	-26.02 ± 0.49		65.8 ± 0.5	
50 mM Rb ⁺ acetate, 30% sucrose	-179.1 ± 4.0	-510 ± 12	-20.75 ± 0.36	5.27 ± 0.61^c	60.5 ± 0.6	-5.3 ± 0.8^c
3 mM MgCl ₂ , 0% sucrose	-214.2 ± 5.1	-592 ± 15	-30.60 ± 0.62		72.9 ± 0.2	
3 mM MgCl ₂ , 30% sucrose	-203.7 ± 9.4	-569 ± 27	-27.16 ± 1.14	3.44 ± 1.30^d	68.3 ± 0.5	-4.6 ± 0.5^d

^aErrors are standard deviations of melting curves of at least two independent samples. An extra significant figure is included to eliminate rounding errors. ^bValues are from comparison to 100 mM Rb⁺ acetate, 0% sucrose. ^cValues are from comparison to 50 mM Rb⁺ acetate, 0% sucrose. ^dValues are from comparison to 3 mM MgCl₂, 0% sucrose.

Although the observed change in folding free energy with increasing sucrose is significant, all X-ray scattering experiments were conducted at room temperature (~ 25 °C) well below the melting temperatures (T_M) for the observed unfolding transitions. Thus, although sucrose does destabilize the duplex as suggested from osmolyte literature, it does not significantly affect the folded populations in our scattering experiments.

End-to-End Interhelical Stacking Is Destabilized by Sucrose. DNA duplex interactions as well as the number and distribution of ions around the duplex remained unaltered under most experimental conditions, yet in the presence of 100 mM MgCl₂, sucrose modifies interparticle interactions (the magnitude of A_2 decreases). To reconcile these observations, we proposed an interaction scheme (Figure 7) similar to a

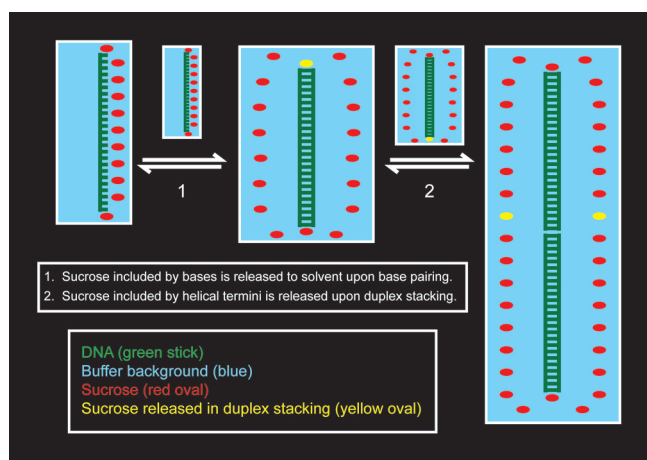


Figure 7. Sucrose influence on folding and end-to-end stacking in 25-bp DNA duplexes. Shown above in step one, sucrose accumulated by DNA bases in the single-stranded state is released upon duplex formation. In step two, end-to-end stacking cause the release of sucrose included at the helix ends. With increasing sucrose this step is more unfavorable and less stacking is observed.

model for general osmolyte effects previously presented to describe folding of RNA secondary and tertiary structures.²¹ For the purposes of this model no sucrose-induced changes to the ion atmosphere are considered, consistent with the ASAXS characterization presented above. In addition, we do not consider changes in excluded volume commonly associated with the molecular crowding phenomena. Excluded volume effects can be significant to the stabilization and function of nucleic acids,²⁶ and large molecular weight crowding agents have been shown to stabilize duplexes.^{17,19,22,30} However, the

destabilization of secondary structure in the presence of smaller cosolutes such as most osmolytes,^{17,19–23,30} and the sucrose destabilization of the 25 bp DNA duplex herein is opposite the stabilization observed when excluded volume effects are dominant. Therefore, excluded volume effects are unlikely to play a significant role in explaining our experimental results. The proposed interaction scheme does not include changes in ion activity due to the presence of sucrose. Thirty percent sucrose can increase the activity coefficient of 100 mM NaCl from 0.778⁴⁵ to 0.799,⁴⁶ which is measurable but small, and it is unlikely that Rb⁺–sucrose interactions are substantially larger than those for Na⁺–sucrose. These small changes are below the sensitivity of the ASAXS data. In contrast, there is a paucity of literature concerning the effects of sucrose on divalent ion activity. However, we used 8-hydroxyquinoline-5-sulfonic acid, a dye that reports Mg²⁺ activity in RNA studies,⁴⁷ to measure Mg²⁺ activity in sucrose-containing solutions. Binding curves of the Mg²⁺–HQS complex in 0% and 30% sucrose are nearly identical, suggesting that the presence of sucrose does not greatly impact Mg²⁺ activity (Figure S1). Since the measured changes in A_2 observed in 30% sucrose would require an $\sim 10\times$ change in Mg²⁺ activity, we conclude that the effects of sucrose on Mg²⁺ activity are small compared to its effects on end-to-end stacking.

Within the constraints described above, the interaction scheme of Figure 7 summarizes our observations. In the single-stranded state the bases are solvent exposed, and sucrose accumulates around the bases (first panel). This condition applies to all experimental conditions. When the single strands fold to form the duplex (second panel), bases become buried in the helix via stacking and inaccessible to sucrose. This step is consistent with thermodynamic studies on DNA folding and sucrose which suggest significant changes in water and sucrose organization as well as inaccessibility of sucrose to interior base pairs formed in duplex hybridization.^{17,19,22}

In high concentrations of Mg²⁺, efficient charge screening of the duplex by divalent ions allows for end-to-end stacking, shown in the second transition in Figure 7. This stacking is disrupted when sucrose is added. This effect can be understood by accounting for the preferential interaction of sucrose with the nonstacked bases at the ends of the helix. In contrast to the bases within the interior of the duplex, osmolyte can accumulate around these solvent-exposed ends. Duplex stacking requires release of this included sucrose to the solvent (yellow molecules in panel). Thus, end-to-end stacking is disfavored at 30% sucrose relative to 0% sucrose, reflected by a decrease in the magnitude of A_2 . This destabilization of super-secondary structure differs from the stabilizing effect of other osmolytes

such as trimethylamine, which is excluded from phosphate groups and stabilizes structures which bury the nucleic acid backbone.²⁷ These differences in osmolyte–nucleic acid interactions suggest that the identity, concentration, and ratios of osmolytes as well as the types of surface area buried in forming super-secondary and folding tertiary structures all play critical roles in determining the stability and population of functional nucleic acids as cells react to stresses and adapt to changing cellular environments.

CONCLUSION

As interest in the role of osmolytes in cellular processes continues to expand, it is critically important to understand the physiochemical mechanisms of their effects on the structure and stability of functional nucleic acids. Experiments described here employed both SAXS and ASAXS to monitor ion–DNA as well as inter-DNA interactions as a function of increasing sucrose concentration. The presence of even large concentrations of sucrose does not perturb electrostatic interactions when monovalent ions or low concentrations of divalent cations are present in the buffer. The number of excess ions remains constant, as does the second virial coefficient reporting interduple interactions. Sucrose-induced changes were observed only when divalent cations were present at concentrations that enabled end-to-end stacking of duplexes. We propose that sucrose alters these interactions by disfavoring interhelical stacking. To describe these results, we invoke an interaction scheme that considers the burial of base surfaces from sucrose upon folding or stacking of the duplexes. Thus, inclusion of sucrose by DNA bases is a model for osmolyte effects on the DNA duplexes. This model of inclusion and solvation is similar in principle to that proposed for osmolyte–protein interactions. We note that protecting osmolytes may destabilize or stabilize nucleic acid structures depending on the types of moieties (phosphate backbone vs bases) exposed or made inaccessible during folding transitions, and for smaller cosolutes such as sucrose, preferential interactions are likely to dominate the nucleic acid interactions rather than excluded volume effects or changes to counterion atmosphere. Therefore, our observations suggest that sucrose's use as a contrast matching agent in SAXS experiments does not alter ion atmosphere around short DNA duplexes and underscores the utility of ASAXS as a probe of osmolyte effects on nucleic acid interactions.

ASSOCIATED CONTENT

Supporting Information

Plots of relative fluorescence of HQS–Mg²⁺ complex with increasing Mg²⁺ in 0 and 30% sucrose. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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ABBREVIATIONS

ASAXS, anomalous small-angle X-ray scattering; bp, base pair; MOPS, 3-(*N*-morpholino)propanesulfonic acid; SAXS, small-angle X-ray scattering; *T*_M, melting temperature.

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