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Complex Interactions between Molecular Ions in Solution and Their Effect on Protein Stability

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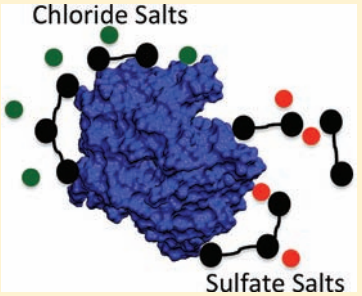
Supporting Information

ABSTRACT: Protein stability in ionic solutions depends on the delicate balance between protein−ion and ion−ion interactions. For molecular ions containing multiple charged groups, the role of ion−ion interactions is particularly important. In this study, we show how the interplay between homo- and heteroion pairing influences protein stability using polyarginine salts as a model system. For the chloride salts, protein thermostability decreases as the size of the peptide increases, indicating enhanced binding to the protein. Moreover, it indicates reduced homoion pairing between Gdm^+ and carboxylate groups that is largely responsible for aggregation suppression, rather than denaturation, in monomeric arginine solutions. However, for the sulfate salts, strong heteroion pairing between the Gdm^+ groups and the sulfate counterions compensates for the loss of homoion pairing and, in return, leads to enhanced thermostability and a dramatically reduced (up to 10−30 times) rate of protein aggregation. Molecular dynamics simulations reveal how this ion pairing enhances conformational stability and, at the same time, reduces protein association. This study provides insight into complex ion effects on protein stability and serves as an example of how these intrasolvent interactions can be leveraged to enhance protein stability.



INTRODUCTION

Small molecule osmolytes are frequently used to enhance stability and reduce aggregation of protein drug formulations[1] and by various organisms to counter biochemical stresses.[2] Interactions between ions and proteins in solution and their subsequent effect on protein stability have been studied extensively in the past century.[2−7] In order to describe protein stability effects, salts are often treated as a single component despite the presence of two or more ions in solution.[8] Not only can the cation and anion differ widely in terms of their interaction with proteins, but their effect on protein stability is typically nonadditive.[9−11] Recently, several studies have highlighted the role of ion−ion interactions in solution and how such interactions affect protein stability.[9,12−16] This is of particular importance in explaining the diverse effects of guanidinium (Gdm^+) salts on protein stability. Gdm^+ is a unique compound, in that it can form hydrogen and electrostatic bonds with proteins that in return can destabilize and unfold the protein molecule. Likewise though, it can form similar bonds with hydrogen bond accepting anions (e.g., sulfate, phosphate, citrate, carbonate, etc.).[12,13,17] Such attractive interactions seem to cause Gdm^+ salts to form clusters in solution and inhibit the Gdm^+ ion from binding to proteins. Heteroion pairing in Gdm_2SO_4 solutions has been shown to be responsible for inhibiting the denaturing power of Gdm^+ to such an extent that this guanidinium salt has no effect on the conformational stability of most proteins.[12]

Complex molecular ions have multiple charged groups, and different groups can have contrasting effects on protein stability. Furthermore, the presence of multiple charged groups provides an opportunity for self-interaction between molecular ions.[9,16−18] For example, we have recently shown that, for arginine, an attractive interaction between the Gdm^+ and carboxylate groups (homoion pairing) limits the binding of the Gdm^+ group to protein surfaces. This contributes significantly to the inhibition of protein aggregation by arginine despite the presence of a functional group that typically denatures proteins.[9,14] Furthermore, we found a connection between the extent of heteroion pairing between Gdm^+ and its counterion in various arginine salts and the extent of aggregation suppression, with stronger heteroion pairing leading to enhanced aggregation suppression.[9]

Molecules containing multiple Gdm^+ groups interact strongly with proteins and have been used as adhesive molecules (e.g., paclitaxel, an anticancer drug) to stabilize microtubules against depolymerization by holding together the tubulin heterodimers.[18] For large molecular ions, the polyvalent interaction with a protein also depends on the structural flexibility of the molecule.[19] An arginine nonamer (a molecule with nine Gdm^+ groups) has been shown to interact strongly with BSA ($K_{assoc} = 5.8 \times 10^4\ M^{-1}$) due to the flexible anchoring of the molecule to the oxyanionic groups of BSA.[18] The presence of multiple Gdm^+ groups leads to a greatly enhanced avidity between the molecules and proteins, as compared to the interaction induced by a single Gdm^+ group. However, there can be contrasting effects of this avidity on protein stability. Strong binding should lead to a reduction of attractive protein−protein interactions, but it can also lead to a

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reduction of conformational stability.[9,19] On the other hand, weak interactions (i.e., exclusion from protein surface) should enhance conformational stability but also induce protein association. The complex ion interactions between proteins and polyvalent (containing multiple Gdm^+ groups) molecules and their effect on protein stability can be thoroughly investigated using polyarginine salt solutions as a model system. Hetero- and homoion pairing, coupled with the structural flexibility of polyarginine peptides, should significantly affect the interaction of the multiple functional groups with protein surfaces.

In this study, we report the effect of chloride and sulfate salts of arginine *n*-mers ($n = 1-4$) on the stability of the proteins α-Chymotrypsinogen A (aCgn) and Concanavalin A (Con A). In particular, we show that the chloride salts of arginine *n*-mers ($n > 1$) inhibit aggregation at low concentrations but accelerate it at moderate to high concentrations, whereas the sulfate salts reduce aggregation at all concentrations. These results are surprising given that monomeric arginine (chloride salt) is a widely used aggregation suppressor. Changes in the denaturation midpoint temperature of aCgn show that the chloride salts reduce conformational stability, whereas the sulfate salts show an apparent stability enhancement. Moreover, this enhanced stability is greater than that induced by sodium sulfate, suggesting a more complex mechanism than just stabilization by sulfate ions. Using molecular dynamics simulations and vapor pressure osmometry measurements, we quantify the effect of homo- and heteroion pairing on protein stability and association in polyarginine solutions.

RESULTS AND DISCUSSION

Aggregation Suppression. Figure 1a shows the loss of protein with time due to aggregation. The figure depicts the concentration of the aCgn monomer, as determined by size exclusion HPLC, relative to the initial concentration versus time when in the presence of a cosolute and incubated at an elevated temperature, in this case 52.5 °C. The rate of monomer loss in the presence of an arginine chloride dimer (RR-Cl_2) is slower at low concentrations (0.1 M) when compared to the reference solution containing no cosolute. This aggregation suppression is comparable to monomeric arginine hydrochloride (R-Cl) of the same concentration; however, as the peptide concentration increases, the aggregation rate reduction decreases until, ultimately, the rate of aggregation is increased. For concentrations above 0.25 M, aggregation is significantly increased. Likewise, larger polyarginine chloride peptides, also at a concentration of 0.25 M, rapidly induce aggregation of the protein, as shown in the Figure 1a. These results demonstrate that the attractive interaction these compounds have for proteins disrupts attractive protein−protein interactions at low concentrations, when conformation destabilization is minimal, given that aggregation is inhibited despite their destabilizing effect. However, at high concentrations, the attractive protein−peptide interaction is likely too strong, leading to significant conformational destabilization that enhances aggregation to an extent that cannot be compensated for by a reduction of protein association.

These results also raise questions about why arginine peptides induce aggregation, whereas monomeric arginine inhibits it. As previously mentioned, arginine peptides are expected to interact with strong attraction with protein surfaces due to (a) the presence of multiple Gdm^+ groups and the intrinsic flexibility

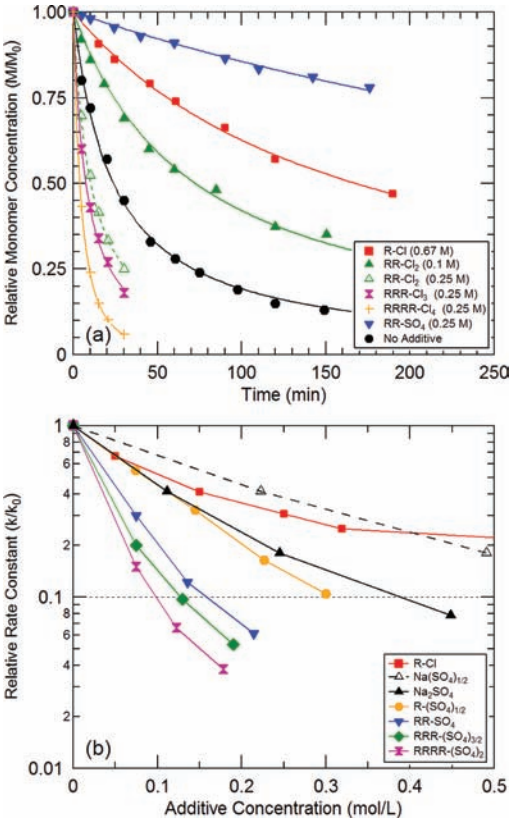

Figure 1. Influence of polyarginine peptides, monomeric arginine salts, and sodium sulfate on aCgn monomer loss due to aggregation at 52.5 °C. For all experiments, the initial monomer concentration, M_0, was 10 mg/mL prepared in a 20 mM sodium citrate pH 5 buffer and all rate loss profiles were fitted to a second-order rate law. (a) Monomer loss profiles for solutions containing the chloride salt form of each arginine peptide compared to a solution containing the arginine dimer with a sulfate counterion. (b) Rate constant, *k*, for aCgn monomer loss relative to the rate constant for no additive, k_0, versus additive concentration.

of the molecule (which facilitates the interaction with distant anionic pockets on the protein surface) and (b) the reduction of the ratio of hydrogen bond accepting to donating groups (which reduces to $1/(n+1)$ for an arginine peptide with *n* residues), thereby reducing homoion pairing (or the interaction between the Gdm^+ and carboxylate groups). Both of these results enhance the attractive interaction between Gdm^+ and proteins relative to monomeric arginine, thereby perturbing conformational stability.

When the chloride ion is exchanged with sulfate, it improves the aggregation suppression ability of not only monomeric arginine but also higher order peptides as well. The monomer loss profile (shown in Figure 1a) of RR-SO_4 at a concentration of 0.25 M exhibits an aggregation rate lower than that for a high concentration of R-Cl. This indicates that exchanging the chloride ion for an anion that can form a stronger hydrogen bond with Gdm^+ reduces the denaturing effect of the compound and improves its aggregation suppression ability. This superior aggregation suppression improves monotonically with concentration and peptide size for that salt form, as shown in Figure 1b, which depicts a sharp reduction in the rate of aggregation as compared to R-Cl. The aggregation suppression depicted in Figure 1b suggests that the arginine sulfate peptides perform equally when

Table 1. aCgn Denaturation Midpoint Temperature Increment[a] and Protein Solution Shelf-Life Extension[b] Resulting from Aggregation Suppression Induced by Polyarginine Peptides[c]

Additive	$dT_m/d[3]$ K·L/mol	Concn mM	aCgn $t_{95}/t_{95,0}$	Con A $t_{95}/t_{95,0}$
Sucrose	5.6[20−22]	280	1.9	1.5
Na_2SO_4	6.6	140	3.1	1.1
R-Cl	0.0	170	3.3	0.4
RR-Cl_2	−0.8	–	–	–
R-$(SO_4)_{1/2}$	5.4	260	7.3	–
RR-(SO_4)	10.4	190	13.7	–
RRR-$(SO_4)_{3/2}$	–	130	10.3	20.0
RRRR-$(SO_4)_2$	–	90	9.5	33.3

[a] As determined by DSC. [b] At accelerated conditions (52.5 °C for aCgn and 37 °C for Con A). [c] Formulated at isotonic concentrations.

compared on a per monomer unit basis, indicating that larger polyarginine sulfate salts will be potent aggregation suppressors at low molar concentrations.

The aggregation suppression results are not limited to high temperature incubations or aCgn aggregation. Table 1 depicts the factor by which the shelf life of aCgn and Con A at accelerated conditions is extended when formulated with the polyarginine peptides. This shelf life extension factor was determined by comparing the length of time for a 5% loss of protein (t_{95}) when in the presence of the compounds to the original length of time ($t_{95,0}$) for a 5% loss in a buffer only solution, both at an elevated temperature of 52.5 °C for aCgn and 37 °C for Con A. For comparison purposes, isotonic concentrations of the compounds were used. These results are also observed at temperatures as low as 37 °C for aCgn (data not shown). Polyarginine peptides, in the form of a sulfate salt, significantly improve the shelf life of these two proteins, at either high (52.5 °C) or moderate temperatures (37 °C). The sulfate form of the polyarginine trimer and tetramer can extend the shelf life of Con A by factors of 20 and 33, respectively, which is 10 to 20 times longer than other commonly used cosolutes (some of which are shown in Table 1).

Conformation Stability. Table 1 also shows the denaturation midpoint temperature increment, $dT_m/d[3]$, defined as the slope of T_m with respect to the molar concentration of the cosolute (component 3), as determined by DSC. The sulfate form of the arginine dimer increases the melting temperature of the protein at a rate of 10.4 °C·M^{-1}, which is nearly double that for monomeric arginine sulfate. Moreover, the chloride salt form of the arginine dimer decreases the melting temperature of the protein at a rate of 0.8 °C·M^{-1}, which is in contradiction to the negligible effect of monomeric arginine hydrochloride. These qualitative results seem to indicate that the sulfate salt forms significantly stabilize the native structure of the protein and inhibit the formation of partially unfolded species that contribute to nonnative aggregation. However, the unfolding of aCgn under these conditions is irreversible; thus these results could also indicate a reduction in the rate of aggregation during the scan.

At first glance it may appear that the reduced aggregation is the mere result of the sulfate ion stabilizing the conformation of the protein. Figure 1b presents aggregation suppression data for sodium sulfate, with concentrations computed for either Na-$(SO_4)_{1/2}$ or Na_2SO_4 for direct comparison to either R-$(SO_4)_{1/2}$ or RR-SO_4, respectively. On a molar basis of SO_4^{2-}, the arginine and diarginine salts reduce the rate of aggregation 3−4 times more than sodium sulfate, demonstrating the impact of the aggregation suppression effect of arginine and arginine peptides. It is unclear whether this observed aggregation suppression is purely from enhanced conformational stability resulting from ion−ion interactions or a combination of enhanced stability and reduced protein association. The $dT_m/d[3]$ values for aCgn in the presence of sucrose, other sugars, and sodium sulfate lie in a range of approximately 5−7 °C·M^{-1} for a concentration range of 0.2−1.0 mol/L, which is similar to the values reported for the polyarginine peptides.[21−23] On a molar basis of sulfate, $dT_m/d[3]$ values for arginine sulfate and diarginine sulfate are slightly larger than that for sodium sulfate, suggesting enhanced thermostability over the contribution provided by the sulfate ion. Molecular level insight into how the arginine peptides interact with the protein is required for it to be argued that the peptides affect the overall stability of the protein by another mechanism (i.e., protein association suppression) that complements conformational stabilization.

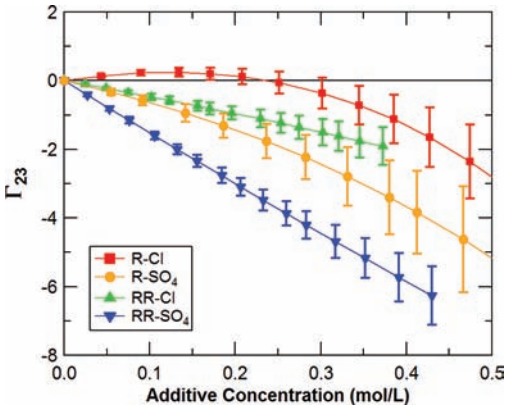

Figure 2. Preferential interaction coefficient, Γ_{23}, values versus additive concentration for the interaction between arginine *n*-mers, as either a chloride or sulfate salt, and aCgn, as determined from VPO measurements. All solutions were prepared in a 20 mM sodium citrate pH 5 buffer.

Preferential Interaction Coefficient. To gain insight into how the arginine peptides may inhibit attractive protein−protein interactions, the preferential interaction coefficient, Γ_{23}, at various concentrations was determined both experimentally, via vapor pressure osmometry (VPO) measurements,[24−27] and computationally, via MD simulations.[14,28−31] The preferential interaction coefficient is a measure of the excess number of molecules in the local domain of the protein as compared to the bulk solution. The experimental results for the interaction between the arginine dimers and aCgn are depicted in Figure 2. The first thing to note is that the net preferential interaction coefficient values for both the chloride and sulfate salt forms are negative at all concentrations, which indicates that the salts are excluded from the surface of the protein in its native state. When the chloride ion is exchanged with sulfate, the salts become even more excluded from the native state. The simulated Γ_{23} values (at a concentration of 0.5 mol/kg) for the salts agree with the experimental results (see Table 2). However, the MD simulations have the benefit of computed preferential interaction coefficient values for each individual ion (see Table 2), which shows that the counterions contribute significantly to the overall exclusion of the salts.

Table 2. Experimental and Simulated Preferential Interaction Coefficient Values for aCgn in 0.5 mol/kg Polyarginine Salt Solutions[a]

Additive	Γ^{VPO}	Γ^{MD}	$\Gamma^{MD,+}$	$\Gamma^{MD,-}$
R-Cl	-2.6 ± 0.3	-2.5	-1	-4
R-$(SO_4)_{1/2}$	-5.2 ± 1.7	-4.5	-4	-3
RR-Cl_2	-2.4 ± 0.8	-3.7	-3	-9
RR-SO_4	-7.0 ± 1.0	-6.5	-5	-8
RRR-Cl_3	–	-4.8	-5	-14

[a] The error bars on the Γ_{23} values are on the order of ± 1.

The concentration of peptides near the protein surface, without taking the counterion into consideration, is also less than the bulk ($\Gamma^{MD,+} < 0$), which is atypical for most denaturing compounds. Therefore, the data suggest that the peptides are excluded from the native state due to their large size and repulsive electrostatic interactions with the positively charged aCgn molecule. However, upon unfolding, when more guanidinium binding sites are exposed and the density of positively charged surface groups is reduced, the polyarginine chloride salts can bind to the protein, thus shifting the folding equilibrium toward the unfolded state. However, ΔT_m results indicate that the same is not true for the sulfate peptides. Given that sulfate salts typically stabilize proteins, it can be argued that the stabilization might be the result of the nonspecific exclusion of the sulfate ions from the unfolded state. However, nonspecific exclusion is simply proportional to the surface area of the protein, and the simulated results suggest that sulfate is less excluded than the chloride ions. Moreover, the individual preferential interaction coefficient values for the peptide molecules indicate that the peptide molecules become more excluded in the presence of sulfate, while the sulfate ions themselves become less excluded than the chloride ions. These two results indicate that sulfate−peptide interactions inhibit the binding of the peptide molecules to the protein surface, in either the native or unfolded states, and, at the same time, pull sulfate molecules from the bulk solution into the local domain of the protein. However, the significant reduction of aggregation suggests that polyarginine sulfate salts are significantly reducing attractive protein−protein interactions.

Intrasolvent Interactions. The reason why the polyarginine sulfate salts inhibit protein association might be that the peptide molecules that are present in the local domain might be interacting with the protein, even though the peptide concentration in the region is less than the concentration in the bulk. Therefore, we calculated the number of R groups near the protein surface and the hydrogen bonds (H-bonds) formed between them and the protein. Counting the number of R groups also provides the effective concentration of Gdm^+ groups around the protein and it makes the comparison between different peptides easier. Similarly, for the ease of comparison, the number of H-bonds formed between peptides and the protein are reported after dividing it by the number of residues in the peptide. The number of R groups per number of peptide residues near the protein surface (within 0.6 nm) increases with peptide size for both the chloride and sulfate salts (Figure 3a), indicating enhanced interaction with the protein as the size of the peptide increases. Furthermore, the difference between the chloride and sulfate salts of the same peptide is not significant. However, the number of H-bonds (Figure 3b) formed between the R groups and the protein surface shows a different trend for the chloride and sulfate salts. The

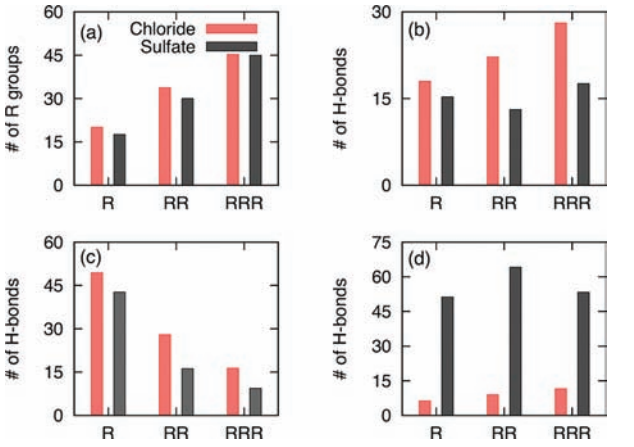

Figure 3. For aCgn MD simulations in 0.5 mol/kg polyarginine solutions, (a) the number of R-groups per number of peptide residues around the protein surface and (b) the number of H-bonds per number of peptide residues formed between the protein and the peptides near the protein surface. For aqueous 0.5 mol/kg polyarginine solutions (without protein) (c) the number of H-bonds between Gdm^+ and COO^- groups and (d) the number of H-bonds between Gdm^+ and the counterions.

number of H-bonds for the chloride salts increases with the number of R groups near the surface, indicating an increase in the interaction between the Gdm^+ groups and the protein. This increase in the interaction with the protein is coupled with a decrease in the number of H-bonds formed (per Gdm^+ group) between Gdm^+ and COO^- groups (Figure 3c). There is an absence of homoion pairing between peptides (as compared to monomeric R), which would otherwise limit the binding of Gdm^+ to the protein. Therefore, the chloride salts of the arginine dimer and trimer can form a strong attractive interaction with protein surfaces, thereby denaturing proteins when the interaction is enhanced upon unfolding, which is consistent with our experimental results.

For the sulfate salts, the number of R groups near the surface increases (Figure 3a) but the number of H-bonds between the R groups and the protein remains the same (Figure 3b), indicating that an increase in the number of R groups near the protein surface does not lead to an increase in the interaction between the Gdm^+ groups and the protein. Therefore, there is enhanced crowding around the protein in the sulfate salt solutions without enhancement in the overall interaction between the protein and the peptides. This leads to both protein association suppression and conformational stabilization, respectively. The heteroion pairing between Gdm^+ and sulfate ions in aqueous polyarginine salt solutions (without protein) explains this enhanced crowding around the protein. For both salt types, homoion pairing between peptides decreases as peptide size increases (Figure 3c). This is a trivial consequence of losing a COO^- for each additional arginine residue, but the consequence is significant for homoion paring. The decrease for the sulfate salts is more than that for the chloride salts because the sulfate ion competes with the COO^- group for binding to the Gdm^+ groups. However, the decrease is more than compensated for by the subsequent increase in heteroion pairing between Gdm^+ and sulfate ions (Figure 3d). The sulfate ion limits the binding of Gdm^+ to the protein surface due to heteroion pairing, which is in contrast to the near absence of heteroion pairing and the strong interaction between Gdm^+ and the protein for the chloride salts.

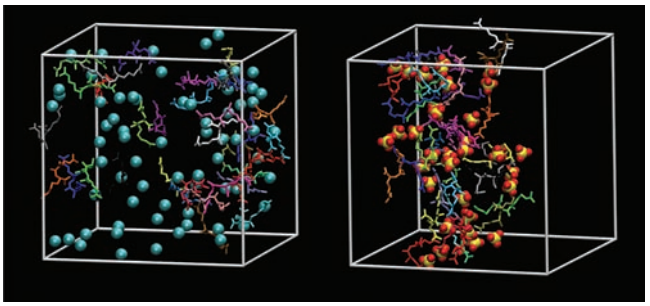

Figure 4. Snapshots of aqueous diarginine salt solutions obtained from MD simulations with the counterions chloride (left) and sulfate (right). The diarginine molecules are shown in licorice style, and counterions are shown as VdW spheres. The hydrogen atoms and water molecules are not shown to improve clarity.

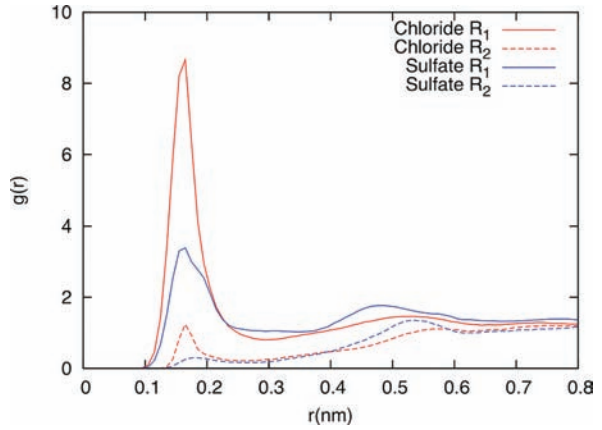

Figure 5. RDFs of the Gdm^+ groups in RR peptides (chloride and sulfate salts) with respect to the protein surface. The R groups are labeled 1−2 depending on their distance from the protein surface, with 1 denoting the closest Gdm^+ group.

Much of what has been discussed and quantified previously regarding ion pairing can easily be visualized in the simulation snapshots shown in Figure 4. For the polyarginine chloride salts, there is an absence of homoion pairing that is observed for monomeric arginine[9,14] as well as a lack of heteroion pairing that is observed for the sulfate salts. This leads to the chloride salts being randomly distributed, whereas the sulfate salts form clusters due to sulfate ions bridging together Gdm^+ groups. This has the consequence of the chloride salt forms being free to interact strongly with the protein surface, opposite of monomeric arginine, and the sulfate salt forms experiencing competition when interacting with the protein surface and enhanced crowding due to clustering.

Radial distribution functions (RDFs) of the Gdm^+ groups in the peptides with respect to the protein surface (see Figure 5) can corroborate the physical picture suggested by the H-bond data and visualized in the simulation boxes. It can be seen that, for the arginine dimer, the Gdm^+ group closest to the protein surface (R_1) interacts strongly with the protein, as indicated by the height of the peak in the chloride salt. Furthermore, the RDF for the second closest arm indicates a level of cooperative binding. For the sulfate salt, the peak height is almost half for the closest Gdm^+ but there is a second peak at 0.5 nm, which is formed due to the presence of a sulfate ion between the Gdm^+ group and the protein. Similarly, the second Gdm^+ group in RR-SO_4 shows no peak at the protein surface (0.15 nm) but it has a small peak at around 0.5 nm. These results demonstrate that the sulfate ion limits the binding of Gdm^+ groups to the protein surface by interacting directly with the Gdm^+ groups.

CONCLUSIONS

In conclusion, we have shown that complex interactions among ions in solution and with protein surfaces significantly alter protein conformational stability and aggregation propensity. This is most significant for molecules with multiple oppositely and like charged groups, which provide opportunities for both hetero- and homoion pairing in solution. For polyarginine chloride salts, reduced homoion pairing relative to monomeric arginine and a lack of heteroion pairing enable a strong attractive interaction with proteins. For the sulfate salts, heteroion pairing with the sulfate counterion limits the binding of the peptide to proteins and leads to an enhanced crowding around the protein without a concomitant increase in the interaction with the protein that often leads to destabilization. These ion-pairing effects on the interactions between ions and proteins are dependent not only on ion concentration but also on the number and type of charged groups present in the molecular ion. From the viewpoint of stabilizing protein formulations, this study highlights the often overlooked but important role played by ion−ion interactions in altering protein−protein interactions, not to mention, revealing that arginine peptides exhibit enhanced aggregation suppression abilities.

MATERIALS AND METHODS

Materials. Custom peptides were ordered from GenScript Corporation. Dimers, trimers, and tetramers, containing only arginine residues (in the form of trifluoroacetate salts), were synthesized by GenScript with a specified purity greater than 98%. For alternate salt forms, the counterions were exchanged using Amberlite IRA 400 anion exchange resin loaded using the appropriate sodium salt. Bovine α-chymotrypsinogen A type II (C4879) and jack bean Concanavalin A (C2010) were obtained from Sigma-Aldrich (St. Louis, MO). All other reagents were obtained from Sigma-Aldrich in the highest available grade. The concentrations of aCgn and Con A were determined spectrophotometrically with a PerkinElmer Lambda 35 UV/vis spectrometer using extinction coefficients of 1.97 $mL \cdot mg^{-1}cm^{-1}$ at 282 nm and 1.37 $mL \cdot mg^{-1}cm^{-1}$ at 282 nm, respectively.

Accelerated Aggregation. The aggregations of aCgn and Con A were accelerated by incubating samples at an elevated temperature in a Bio-Rad MyCycler thermal cycler. Aggregate formation and monomer loss was monitored using an Agilent 1200 series HPLC, equipped with a Zorbax GF-250 (4.6 mm × 250 mm, 4 μm) size exclusion column and a UV−vis detector.

Differential Scanning Calorimetry. The thermodynamic stability of aCgn in the presence of the arginine peptides was determined by differential scanning calorimetry (DSC). A Microcal VP-Differential Scanning Calorimeter was utilized, and each reading began with a minimum of three buffer−buffer up and down scans (in this case, the buffer also contains the cosolute of interest) to establish a reproducible thermal history followed by a single protein−buffer up scan. aCgn was analyzed at a concentration of 1 mg/mL in a 20 mM sodium citrate pH 5 buffer containing the cosolute of interest and a scan rate of 90 °C/h. The data were analyzed in the MicroCal Origin plotting software. The denaturation midpoint temperature, T_m, was taken as the temperature at the peak of the unfolding event. For each peptide salt form, three

concentrations (including zero concentration) were tested and T_m values with respect to cosolute concentration were fitted to a linear trend.

Molecular Dynamics Simulations. Molecular dynamics (MD) simulations of aqueous solutions of the polyarginine peptide salts with and without aCgn (PDB Id: 2CGA) were performed using NAMD 2.7,[32] with the CHARMM27[33] force field and the TIP3P[34] water model. The force field parameters for the counterions were taken from the literature,[35,36] and the force field parameters for the peptides were taken from the CHARMM force field parameters for arginine, C-Terminal and N-terminal groups.

Preferential Interaction Coefficient. Theoretical preferential interaction coefficient (Γ_{23}) values were calculated using a statistical mechanical method applied to an all-atom model with no adjustable parameters.[37,38] Experimental values were obtained from changes in water activity as determined by vapor pressure osmometry.[39]

■ ASSOCIATED CONTENT

Supporting Information. Experimental and computational details, including peptide synthesis, characterization, and procedure used for measuring the preferential interaction coefficient using VPO and MD simulations. This material is available free of charge via the Internet at http://pubs.acs.org.

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