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Protein-Associated Cation Clusters in Aqueous Arginine Solutions and Their Effects on Protein Stability and Size

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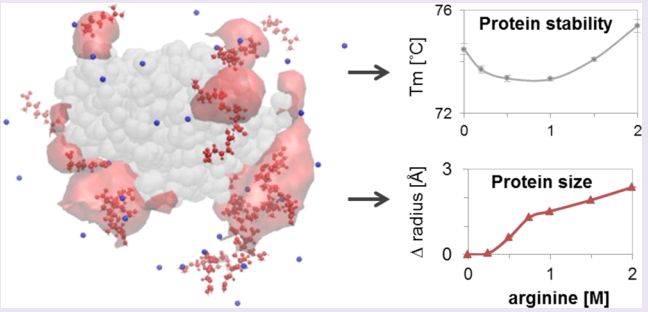
Supporting Information

ABSTRACT: Arginine is one of the most prominent residues in protein interactions, and arginine hydrochloride is widely used as an additive in protein solutions because of its exceptional effects on protein association and folding. The molecular origins of arginine effects on protein processes remain, however, controversial, and little is known about the molecular interactions between arginine cations and protein surfaces in aqueous arginine solutions. In this study, we report a unique biochemical phenomenon whereby clusters of arginine cations (Arg^+) are associated with a protein surface. The formation of protein-associated Arg^+ clusters is initiated by Arg^+ ions that associate with specific protein surface loci through cooperative interactions with protein guanidinium and carboxyl groups. Molecular dynamics simulations indicate that protein-associated Arg^+ ions subsequently attract other Arg^+ ions and form dynamic cation clusters that extend further than 10 Å from the protein surface. The effects of arginine on the thermal stability and size of lysozyme and ovalbumin are measured over a wide concentration range (0 to 2 M), and we find that the formation of protein-associated Arg^+ clusters consistently explains the complex effects of arginine on protein stability and size. This study elucidates the molecular mechanisms and implications of cluster formation of Arg^+ ions at a protein surface, and the findings of this study may be used to manipulate synthetic and biological systems through arginine-derived groups.



Arginine plays a ubiquitous role in nature where it is one of the most prominent residues in protein interactions.[1−3] Arginine-based synthetic groups are often incorporated in drug-like and biomimetic molecules.[4] Furthermore, arginine hydrochloride, commonly referred to as arginine in brief, is widely used as an additive in protein solutions for increasing protein solubility[5−8] and improving protein refolding yield.[9−12] Arginine also enhances protein elution characteristics from various chromatographic materials[13−15] and decreases the viscosity of high-concentration protein formulations.[8,16] The beneficial effects of arginine on protein processes often surpass those of other additives, and several studies have started to elucidate key aspects of the molecular origins of its exceptional behavior.[8,17,18] Yet much remains unknown, and selection of arginine for a particular protein process is generally based on trial-and-error.[18] In particular, little is known about the molecular interactions between arginine and protein surfaces.

Beneficial effects of arginine on protein processes are often attributed to its ability to weaken hydrophobic interactions, which is evidenced by the solubilizing effect of arginine on small hydrophobic compounds[19−22] and proteins with hydrophobic surface patches.[6,23] Weakening of hydrophobic interactions appears to be a thermodynamic consequence of the preferential interactions of arginine with hydrophobic surfaces and, in particular, with aromatic groups through van der Waals and cation−π interactions with the guanidinium group (Gdm^+) of arginine.[21,22,24−26] The importance of the Gdm^+ group for arginine−protein interactions is also evidenced by the similarity of the interactions of arginine and GdmCl with almost all of the naturally occurring amino acids.[20] However, the similarity of preferential interactions with GdmCl and arginine for individual amino acids does not apply to proteins.[10] In fact, GdmCl denatures proteins, whereas arginine generally does not affect protein structure.[27] Moreover, weakening of hydrophobic interactions is not a unique feature of arginine.[28] As a consequence, arginine effects on protein process cannot merely be attributed to weakening of hydrophobic interactions or protein interactions of the Gdm^+ group alone.

Arginine interacts with proteins through a number of interaction mechanisms including hydrogen bonding, electrostatic, van der Waals, and cation−π interactions.[17,29] Protein binding modes of drug-like molecules indicate the preference of

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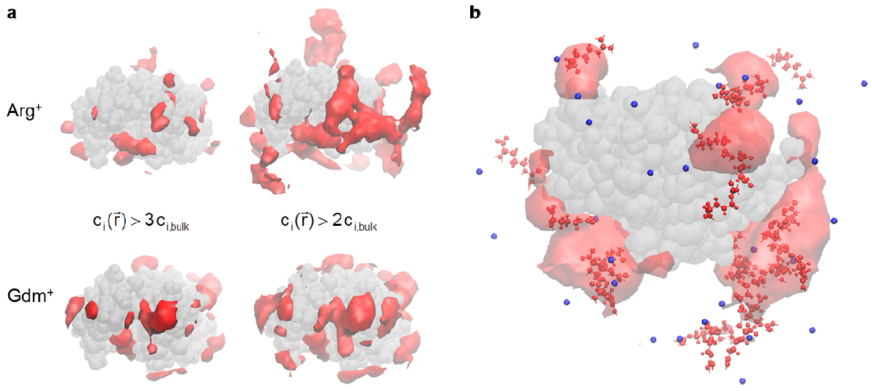

Figure 1. Protein-associated Arg^+ clusters. (a) Local concentration maps of Arg^+ and Gdm^+ ions at the surface of lysozyme. Solvent regions with local concentrations larger than 3 or 2 times the respective bulk concentration, $c_{i,bulk}$, are highlighted in red. (b) Arg^+ and Cl^- ions at the surface of lysozyme in 0.2 M arginine. Arg^+ and Cl^- ions are colored in red and blue ball-and-stick models, respectively, and solvent regions with high local Arg^+ concentrations ($c_i(\vec{r}) > 3c_{i,bulk}$) are colored in transparent red.

Gdm^+ groups for protein carboxyl groups, and the preference of protein arginine side-chains for oxygen-containing groups, including carboxyl groups.[30,31] The strong interaction energy between Gdm^+ and carboxyl groups [32] is also evident from the self-association of arginine ions (Arg^+) in aqueous solutions by head-to-tail hydrogen bonding.[25] Association of Arg^+ ions also occurs through pairing of Gdm^+ groups in a staggered geometry, which optimizes quadrupole−quadrupole and dispersion interactions and reduces electrostatic repulsion and solvent exclusion.[25,33] The extent to which interactions of Gdm^+ and carboxyl groups influence preferential interactions of Arg^+ ions with proteins remains, however, unknown.

Preferential interaction behavior of arginine with proteins appears complex.[34,35] At low arginine concentrations (<0.5 M), preferential interactions of arginine are nearly neutral, and arginine effects on protein association are comparable to that of a neutral crowder.[18,36] However, unlike neutral crowders, local preferential contacts of Arg^+ ions vary considerably for different protein residues, even for residues of the same amino acid type.[25,37] Preferential interactions of arginine with proteins are further complicated by cluster formation of Arg^+ ions in the bulk solvent.[19,25] Clustering of Arg^+ ions strongly depends on the type of counterions, and arginine effects on protein processes vary widely for different arginine salts.[38−40] It remains unclear how Arg^+ clusters interact with protein surfaces and how counterions and physicochemical protein properties determine preferential interactions of arginine with the protein surface.

In this study, we employ a newly established methodology for characterizing local protein solvation to study the interactions of arginine with the surface of lysozyme.[41] Unexpectedly, we find that arginine cations (Arg^+) form clusters that are associated with the protein surface. Unlike Arg^+ clusters in the bulk solvent, the formation of Arg^+ clusters at the protein surface is initiated by Arg^+ ions that associate with specific protein surface loci through cooperative interactions of Gdm^+ and carboxyl groups. We show that protein-associated Arg^+ clusters determine the complex effects of arginine on protein stability, and we demonstrate that, contrary to the prevalent belief that arginine weakens hydrophobic interactions, arginine strengthens interactions of proteins with hydrophobic chromatographic materials. Using size-exclusion chromatography, we establish that arginine increases the apparent protein size, and we explain this phenomenon based on protein-associated Arg^+ clusters.

RESULTS AND DISCUSSION

We first investigate the molecular interactions of arginine with proteins by applying a newly established methodology for characterizing local solvent−protein interactions over the entire protein surface.[41] Interactions of distinct protein surface loci with Arg^+ ions and their carboxyl and guanidinium moieties are analyzed at gradually increasing resolution, and we observe that Arg^+ ions form clusters at the protein surface. Preferential interactions of arginine with the protein surface are investigated over a wide concentration range, and we present evidence that protein-associated Arg^+ clusters determine the unusual preferential interaction behavior of arginine. We then perform a series of experiment to determine arginine effects on protein stability and size, and we show that the formation of protein-associated Arg^+ clusters consistently explains the complex effects of arginine on protein stability and size.

Protein-Associated Arg^+ Clusters. Figure 1a shows the surface of lysozyme surrounded by solvent regions with high local concentrations of arginine cations (Arg^+) and guanidinium cations (Gdm^+). Similar to other cosolvents,[42] Gdm^+ regions are located within 6 Å from the protein surface. In contrast, Arg^+ regions appear as clusters that originate at specific protein surface loci and extend further than 10 Å from the protein surface (Figure 1a). Cluster-like Arg^+ regions occur over a wide range of arginine (ArgHCl) concentrations (Supplementary Figure S4) and encompass Arg^+ ions resolved in X-ray crystal structures of lysozyme [29] (Supplementary Figure S5).

Whereas Cl^- ions appear randomly distributed around the protein, Arg^+ ions tend to form clusters at the protein surface (Figure 1b). Interactions between Cl^- ions and Arg^+ ions are weak,[39] and cluster-like Arg^+ regions appear independent of Cl^- ions (Supplementary Figure S6). Arg^+ clusters continuously form and disintegrate as Arg^+ ions diffuse to-and-from the protein surface (Supplementary Movie 1). The formation of Arg^+ clusters primarily takes place near specific loci of the protein surfac and is initiated by the association of one or more Arg^+ ions with these loci (Supplementary Movie 1). Protein-associated Arg^+ ions interact on their turn with other Arg^+ ions in the solvent. This results in the formation of dynamic clusters of Arg^+ ions that are associated with the protein surface through one or more protein-associated Arg^+ ions (Figure 1b).

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About half of the Arg^+ ions at the protein surface has a characteristic residence time of ~7 ns, and the other half has a characteristic residence time of ~50 ns (Table 1). They are hereafter referred to as class I and class II Arg^+ ions, respectively. Class II Arg^+ ions typically associate with the same protein surface locus for tens of nanoseconds and serve as anchors for Arg^+ clusters that repeatedly engage and disengage protein-associated class II Arg^+ ions (Supplementary Movie 1). Protein-associated Arg^+ clusters include practically all Arg^+ ions near the protein surface (Table 1) and may also include several Arg^+ ions further away from the protein surface ($r > 6$ Å) (Figure 1b).

Table 1. Characteristic Residence Times and the Corresponding Numbers of Arg^+ Ions at the Surface of Lysozyme[a]

ArgHCl (M)	n_1^{+b}	τ_1^{+b} (ns)	n_2^{+c}	τ_2^{+c} (ns)	n_{tot}^{+d}
0.2	3.6	7	3.3	77	7.6
0.5	4.8	7.1	5.5	41	12.7
1	9.5	7.1	9.8	61	22.0
2	14.6	6	20.9	64	39.4

[a]Parameters obtained by fitting Arg^+ survival functions to eq 1. [b]n_1^+ is the number of class I Arg^+ ions with characteristic residence time τ_1^+. [c]n_2^+ is the number of class II Arg^+ ions with characteristic residence time τ_2^+. [d]n_{tot}^+ is the total number of Arg^+ ions at the protein surface ($r < 6$ Å).

For arginine concentrations up to 1 M, the number of class I and class II Arg^+ ions are nearly equal (Table 1). This implies that, in this concentration range, protein-associated Arg^+ clusters comprise on average one class I Arg^+ ion per class II Arg^+ ion. At 2 M ArgHCl, there are substantially fewer class I than class II Arg^+ ions (Table 1), indicating that clustering of class I Arg^+ ions at protein-associated class II Arg^+ ions becomes less favorable at higher arginine concentrations (>1 M). Furthermore, the sublinear increase of the number of class II Arg^+ ions indicates that several protein surface loci become saturated at arginine concentrations above 0.5 M (Supplementary Figure S7).

Association of Arg^+ Ions with the Protein Surface. To identify features of the protein surface that enable association to Arg^+ clusters, local concentration maps of Arg^+, Gdm^+, and glycine are superimposed (Figure 2). High Gdm^+ concentrations occur near protein carboxyl groups, and high glycine concentrations primarily occur near protein Gdm^+ groups , and, to a lesser extent, near primary amine groups. High Arg^+ concentrations occur only near protein surface loci that comprise at least one carboxyl group and one Gdm^+ or amine group, and all Arg^+ regions overlap to a certain extent with both Gdm^+ regions and glycine regions (Figure 2). This indicates that cooperation of protein carboxyl and Gdm^+ groups is essential for the association of Arg^+ ions with the protein surface.

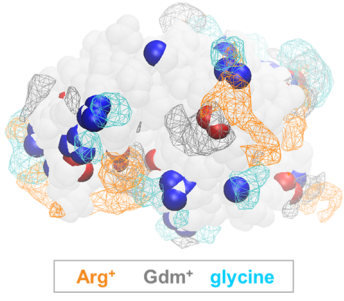

Figure 2. Superimposition of local concentration maps of Arg^+, Gdm^+, and glycine at the surface of lysozyme. Oxygen atoms of protein carboxyl groups are colored red, and nitrogen atoms of protein Gdm^+ and primary amine groups are colored dark blue. Data obtained from simulations of lysozyme with 1 M ArgHCl, GdmCl, and glycine, respectively.

Protein carboxyl and Gdm^+/amine groups cooperatively interact with class II Arg^+ ions so that the Gdm^+ group of Arg^+ contacts a protein carboxyl group, and the carboxyl group of Arg^+ contacts a protein Gdm^+/amine group (Figure 3a). Rather than being locked in such a double-associated orientation, protein association of class II Arg^+ ions occurs primarily through one of its groups (either Gdm^+ or carboxyl), whereas the other group repeatedly engages and disengages the protein surface (Supplementary Movie 2). As a result, class II Arg^+ ions repeatedly adapt single-associated orientations whereby their Gdm^+ or carboxyl group points toward the bulk solvent (Figure 3b). Class II Arg^+ ions in single-associated orientations are able to interact with Arg^+ ions in the bulk solvent and with class I Arg^+ ions at the protein surface (Figure 3b). These interactions appear similar to Arg^+–Arg^+ interactions in the bulk solvent;[25] yet, instead of forming Arg^+ clusters that diffuse freely in the solvent, interactions with class II Arg^+ ions result in Arg^+ clusters associated with specific loci of the protein surface.

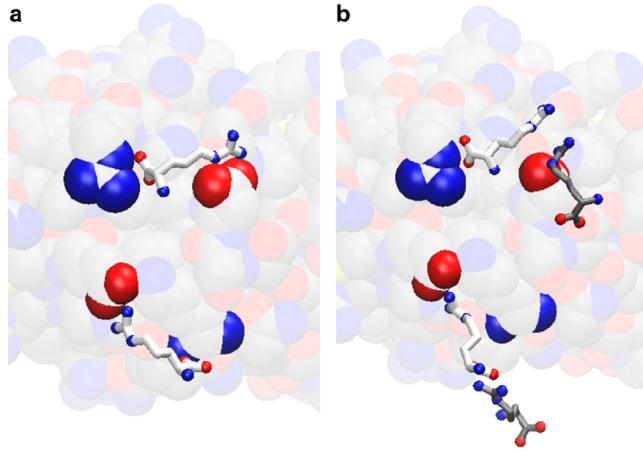

Figure 3. Interaction modes of Arg^+ ions with the protein surface. (a) Double-associated class II Arg^+ ions (stick model) interact simultaneously with protein carboxyl and Gdm^+/amine groups (highlighted van der Waals spheres). (b) Single-associated class II Arg^+ ions (white stick model) interacting with either one protein carboxyl or one protein Gdm^+/amine group, and with other Arg^+ ions (gray stick model). Oxygen atoms are colored red and nitrogen atoms blue.

Association of Arg^+ clusters with the protein surface occurs at protein surface loci that comprise both carboxyl and Gdm^+/amine groups. Although not all protein surface loci with proximate carboxyl and Gdm^+/amine groups favor association with arginine (Figure 2), we find that even a small protein like lysozyme contains multiple specific surface loci that associate to Arg^+ clusters (Figure 1b). The formation of protein-associated Arg^+ clusters is therefore expected to be a general phenomenon for proteins in aqueous solutions.

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Implications of Protein-Associated Arg^+ Clusters on Preferential Interactions. At 0.2 M ArgHCl, several surface loci of lysozyme become occupied by class II Arg^+ ions for almost the entire simulation time (Supplementary Movie 2). The relatively high affinity of these loci for Arg^+ is manifested in the radial distribution function of Arg^+, which exhibits a first distinct peak at 1.5 Å, corresponding with double-associated class II Arg^+ ions, and a broad second peak around 4.0 Å, corresponding with single-associated class II Arg^+ ions and class I Arg^+ ions clustered around class II Arg^+ ions (Figure 4a).

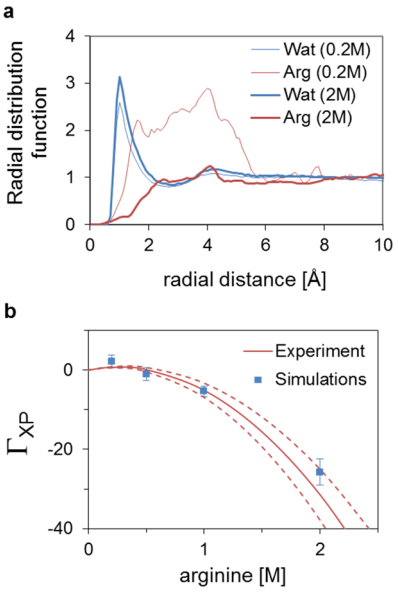

Figure 4. Radial distribution and preferential interactions of arginine with lysozyme. (a) Radial distribution function of water and Arg^+ with respect to the protein surface in 0.2 and 2 M arginine. (b) Preferential interaction coefficient Γ_{XP} for lysozyme at various arginine concentrations. Curves are extrapolated from experimental data up to 0.7 m.[35]

Strong preferential interactions at protein-associated Arg^+ clusters prevail over preferential exclusion of Arg^+ at the rest of the protein surface, and the preferential interaction coefficient Γ_{XP} of lysozyme is marginally positive at 0.2 M ArgHCl (Figure 4b).

At higher arginine concentrations (>0.5 M), Γ_{XP} of lysozyme decreases and arginine becomes preferentially excluded from the protein surface (Figure 4b). Schneider et al. [35] hypothesized that the decrease of Γ_{XP} results from the protein surface becoming saturated with arginine at about 0.5 M. Our simulations show that saturation at low arginine concentrations (<0.5 M) effectively occurs at specific protein surface loci that associate with Arg^+ clusters (Supplementary Movie 2). As a result, the number of Arg^+ clusters increases at a slower rate than the arginine concentration in the bulk solvent (Supplementary Figure S7), and Γ_{XP} decreases as arginine concentrations increase.

As pointed out earlier, cluster formation of class I Arg^+ ions with protein-associated class II Arg^+ ions becomes less favorable at 2 M ArgHCl. At such high ArgHCl concentrations, the density of Arg^+ ions becomes so high that almost all Arg^+ ions in the bulk solvent are engaged in clusters,[25] and solvent regions with high local Arg^+ concentrations appear further than 8 Å from the protein surface (Supplementary Figure S8). These Arg^+ regions form layers of extended Arg^+ clusters that avoid contact with most protein surface regions. Extended Arg^+ clusters layered around the protein surface compete with protein-associated Arg^+ clusters by drawing Arg^+ ions away from the protein surface,[37] and the solvent region between 5 and 8 Å from the protein surface becomes moderately depleted from Arg^+ ions (Figure 4). This results in an additional decrease of Γ_{XP} at ArgHCl concentrations >1 M (Figure 4b).

Implications of Protein-Associated Arg^+ Clusters on Protein Stability. Since strong preferential interactions at protein-associated Arg^+ clusters explain the complex preferential interaction behavior of arginine, we hypothesized that protein-associated Arg^+ clusters play a major role in the effects of arginine on protein stability. To investigate this hypothesis, we measured the melting temperature of lysozyme and ovalbumin at 0−2 M arginine by differential scanning calorimetry. The melting temperature T_m of lysozyme decreases at arginine concentrations <0.5 M, but increases at arginine concentrations >1 M (Figure 5a). Similar effects of arginine on the T_m of lysozyme occur at different buffer conditions (Figure S9), and the thermal transitions of lysozyme are reversible (Supporting Information). The decrease and subsequent increase of T_m in function of arginine concentration indicate therefore that the conformational stability of lysozyme decreases at low arginine concentrations but increases at higher arginine concentrations. Similar effects of arginine on protein stability are observed for ovalbumin (Figure 5a), suggesting that protein destabilization at lower arginine concentrations and protein stabilization at higher arginine concentrations is a general phenomenon.

A revealing observation is made when comparing Γ_{XP} (Figure 4b) and T_m for lysozyme (Figure 5a): the respective curves mirror each other over the entire concentration range. This remarkable correlation explains the effects of arginine on protein stability according to the thermodynamic principles of preferential interactions:[43] at lower concentrations (<0.5 M), arginine destabilizes proteins because of preferential interactions of arginine to the protein surface, but at higher concentrations (>1 M), arginine incrementally stabilizes proteins because it is preferentially excluded from the protein surface.

Arginine Effects on Protein Size and Hydrophobic Interactions. The effects of arginine on the hydrodynamic protein radius are measured by size-exclusion chromatography (SEC) experiments. SEC elution times of lysozyme and ovalbumin decrease at higher arginine concentrations, and the corresponding protein radii apparently increase by about 2 Å at 2 M ArgHCl (Figure 5b). In contrast, apparent protein radii decrease in the presence of NaCl. Similar observations were made for other proteins (Supplementary Tables S2, S3, and S5). The opposite effects of ArgHCl and NaCl on apparent protein radii cannot be attributed to well-known salt effects such as charge screening, which would lead to shorter elution times and smaller apparent protein radii for both salts, or preferential exclusion, which would lead to longer elution times and smaller apparent protein radii since ArgHCl and NaCl are both excluded at higher concentrations.[34,35,44] We further investigated the effects of ArgHCl and NaCl on the chromatographic matrix (Supplementary Table S6) and found that the effects of ArgHCl and NaCl on protein elution times cannot be attributed to solvent-induced changes of the chromatographic matrix (Supporting Information). Hence, alternative mechanisms must be responsible for the opposite effects of ArgHCl and NaCl on the apparent protein radius.

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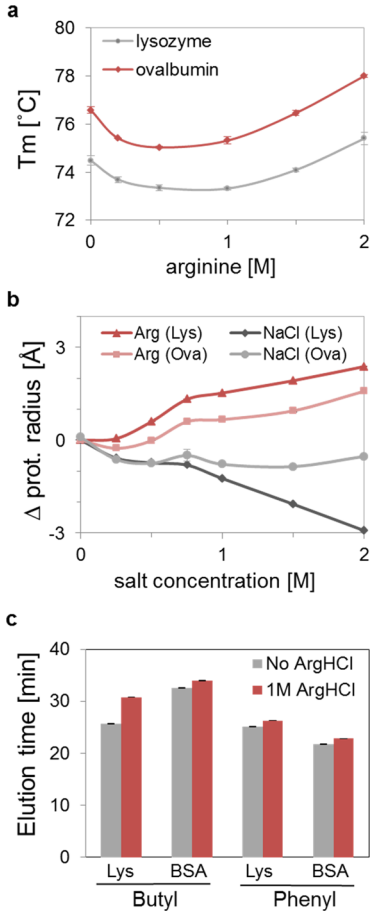

Figure 5. Effects of arginine on protein conformational stability, protein size, and hydrophobic interactions. (a) Effects of arginine on the conformational stability of lysozyme and ovalbumin. Protein melting temperatures T_m in buffer A were measured by differential scanning calorimetry. (b) Effects of arginine on apparent protein size estimated from changes of protein elution times measured by size-exclusion chromatography. Arginine, but not NaCl, increases the apparent protein radius of lysozyme (Lys) and ovalbumin (Ova). (c) Effects of arginine on protein interactions with hydrophobic chromatographic materials. The elution times of lysozyme (Lys) and bovine serum albumin (BSA) from butyl and phenyl resins are longer in the presence of 1 M arginine, indicating strengthening of hydrophobic interactions by arginine.

Arakawa and co-workers attributed opposing effects of ArgHCl and NaCl on protein elution times measured by SEC to the weakening and strengthening of hydrophobic interactions by ArgHCl and NaCl, respectively.[15,45] To test this hypothesis, the effect of ArgHCl on hydrophobic interactions is measured by hydrophobic interaction chromatography (HIC) (Figure 5c). Similar to NaCl,[46] ArgHCl increases protein elution times on HIC. This indicates that, contrary to the above hypothesis, arginine strengthens interactions between proteins and hydrophobic materials.

Strengthening of protein interactions with hydrophobic materials seems to contradict the solubilizing effect of arginine on hydrophobic compounds.[6,19−23] This apparent contradiction can be explained considering that, unlike hydrophobic compounds and (partially) unfolded proteins, native proteins generally do not expose extended hydrophobic surfaces that preferentially interact with arginine. Indeed, our simulations show that Arg^+ ions preferentially interact only with Gdm^+ and carboxyl groups but are preferentially excluded from the rest of the protein surface (Figure 2). Similar observations were made for glycerol, which preferentially interacts with extended hydrophobic surfaces but is preferentially excluded from hydrophobic surface regions of a native protein due to the interference of polar protein atoms adjacent to the hydrophobic surface regions.[28] We therefore attribute the arginine-induced increase of protein elution times on HIC to the preferential exclusion of arginine from noncharged surface regions in native proteins. Similarly, the slight decrease of the colloidal stability of lysozyme by arginine[47] can be attributed to preferential exclusion of arginine from noncharged surface regions, whereas the arginine-induced increase of the colloidal stability of proteins in GdmCl solutions[47,48] can be attributed to preferential interactions of arginine with extended hydrophobic surface regions in partially unfolded proteins.

Could the observed increase of apparent protein radius by arginine be attributed to protein-associated Arg^+ clusters? To answer this question, we first consider protein-associated class II Arg^+ ions, which reside at the same protein surface site for about 50 ns (Table 1). A residence time of ∼50 ns not only greatly exceeds typical lifetimes of nonspecific protein encounter complexes[49] but also is the time it takes for a protein to diffuse over a distance at which intermolecular interactions vanish.[50] Thus, as far as it concerns hydrodynamic protein properties and intermolecular interactions, protein-associated Arg^+ ions can be considered as an extension of the protein surface. A simple model that takes into account the occupancy of the protein surface by Arg^+ ions indicates that extension of the protein surface by protein-associated Arg^+ ions accounts for a major fraction of the observed increase of the apparent protein radius (Supplementary Table S11). The remaining part of the observed increase of the protein radius could then be attributed to clustering of Arg^+ ions near protein-associated Arg^+ ions.

CONCLUSIONS

This study reports cluster formation of arginine cations (Arg^+) at the surface of a protein. Cluster formation is initiated by the association of Arg^+ ions with specific protein surface loci through cooperative interactions of protein Gdm^+ and carboxyl groups. Protein-associated Arg^+ ions have unusually long residence times (∼50 ns) during which they repeatedly adapt orientations whereby either their Gdm^+ or carboxyl group is free to interact with other Arg^+ ions in the solvent. Such interactions lead to the formation of dynamic protein-associated Arg^+ clusters that affect the average local solvent structure further than 10 Å from the protein surface.

The effects of arginine on protein conformational stability are complex: arginine destabilizes proteins at lower arginine concentrations (<0.5 M) but incrementally stabilizes proteins at high arginine concentrations (>1 M). This complex behavior results from strong preferential interactions at protein-associated Arg^+ clusters and the preferential exclusion of arginine from the rest of the protein surface. Arginine was also found to strengthen protein interactions with hydrophobic chromatographic materials. This implies that, even though arginine generally solubilizes hydrophobic compounds, it may weaken the colloidal stability of native proteins.

Because of their long residence times, protein-associated Arg^+ ions can be considered as an extension of the protein surface. Extension of the protein surface by protein-associated Arg^+ ions may significantly affect local and global protein

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surface properties. This is demonstrated for the apparent protein radius measured by size-exclusion chromatography, which significantly increases in the presence of arginine. Protein-associated Arg^+ clusters are also expected to affect the apparent charge properties and hydrophobicity of the protein surface. It appears therefore that the unique effects of arginine on many protein processes involving protein association and folding originate from an equally unique phenomenon, the formation of protein-associated cation clusters in aqueous arginine solutions.

METHODS

Size-Exclusion Chromatography. Size-exclusion chromatography (SEC) experiments were carried out on a Shimadzu Class-VP HPLC system connected to a Superdex 75 PC 3.2/30 column (GE Healthcare). Protein elution times were measured at increasing concentrations of ArgHCl or NaCl in buffer A (50 mM MES, pH 6.5, 5 mM EDTA, and 0.05% sodium azide). At each salt concentration, the apparent protein radius was calculated from the elution time using a standard curve of 5 differently sized proteins in buffer A without arginine. Further details are in the Supporting Information.

Hydrophobic Interaction Chromatography. Hydrophobic interaction chromatography experiments were performed for lysozyme and bovine serum albumin (BSA) on two different hydrophobic resins (toyopearl butyl 600 M and phenyl 650 M, Tosoh Bioscience) in 50 mM phosphate, pH 7.2, with 0 and 1 M ArgHCl, respectively. Proteins were bound to the chromatographic supports in the presence of 2.3 M ammonium sulfate and eluted by a linear gradient to 0 M ammonium sulfate. Further details are in the Supporting Information.

Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) measurements were performed using a VP-Capillary DSC system (Microcal Inc.). Protein samples were scanned from 30 to 100 °C at 1 °C min^{-1} in the presence of various concentrations of ArgHCl (0–2 M) in buffer A (50 mM MES, pH 6.5, 5 mM EDTA, and 0.05% sodium azide), and the melting temperature T_m was determined by the Origin 7.0 software (OriginLab Corporation). Further details are in the Supporting Information.

Characterization of Local Protein Solvation. Local protein solvation in arginine solutions was characterized by the methodological analysis of extended molecular dynamics simulations.[41] All-atom molecular dynamics simulations were performed for lysozyme in aqueous solutions of ArgHCl, GdmCl, and glycine, respectively. Local concentrations of solvent ions at the protein surface were calculated based on the atom occupancy of a three-dimensional grid. Solvent regions with local solvent concentrations greater than n times the respective bulk solvent concentrations, i.e., $c_i(\vec{r}) > nc_{i,bulk}$, were visualized in local concentration maps. Residence times of Arg^+ ions at the protein surface ($r < 6$ Å) were recorded, and characteristic residence times of Arg^+ ions were obtained by fitting survival functions to a biexponential decay function:

$$N_{Arg^+}(t) \cong n_1^+ \, e^{-t/\tau_1^+} + n_2^+ \, e^{-t/\tau_2^+} \quad (1)$$

In the above equation, n_1^+ and n_2^+ are the average numbers of Arg^+ ions at the protein surface with characteristic residence times τ_1^+ and τ_2^+, respectively. Solvent molecules were counted at increasing radial distances from the protein surface, and preferential interaction coefficients Γ_{XP} were calculated based on the average numbers of water and cosolvent molecules near the protein surface.[51] Further details are in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

Experimental and computational details, figures of local concentration maps, and movies of Arg^+ and Cl^- ions at the protein surface. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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