Guanidinium Cations Pair with Positively Charged Arginine Side Chains in Water

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Guanidinium cations pair with positively charged arginine side chains in water

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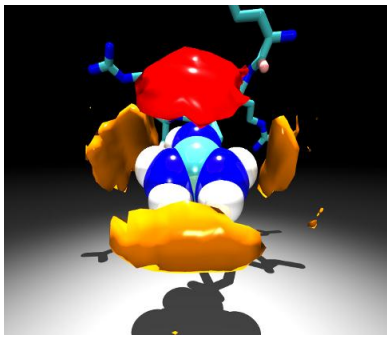
ABSTRACT: Using capillary electrophoresis and molecular dynamics simulations we directly demonstrate that guanidinium cations exhibit an affinity for the positively charged side chains of arginine-containing peptides in aqueous solutions, while no such effect is observed for sodium cations and/or lysine. Such a counter-intuitive pairing between these two positively charged moieties has implications for destabilization and aggregation of arginine-rich proteins in guanidinium salt solutions.



Keywords: guanidinium, arginine, ion-pairing, electrophoresis, molecular dynamics.

Guanidinium (Gdm^+) is the most common and versatile ionic protein denaturant.[1-4] Its versatility is due to its ability to form contacts with various chemical moieties present in proteins, from hydrophobic (particularly aromatic) to polar and negatively charged groups.[5] In addition, there is strong indication from calculations that guanidinium cations can also pair with each other in water despite the apparent electrostatic repulsion between them, due to a combination of cavitation effects and quadrupole-quadrupole and dispersion inetractions.[6-9] Analogous side chain pairing of arginines has been invoked in rationalizing the unusual abundance of close guanidinium-guanidinium contacts in structures of proteins and protein dimers.[9, 10] These findings imply that there could also be appreciable pairing between dissolved Gdm^+ and arginine side chains, which may have structural consequences in the denaturation process, however, such pairing has not been directly observed yet. Here, we demonstrate the existence of guanidinium-arginine side chain pairing by a combination of capillary electrophoresis and molecular dynamics (MD) simulations of *tetra*-arginine in aqueous guanidinium chloride solutions. In control experiments and simulations we also show that no such ion - side chain pairing exists when Gdm^+ is replaced by Na^+ and/or *tetra*-arginine by *tetra*-lysine, which underlines the specific properties of the guanidinium group.

Capillary electrophoresis experiments were carried out on a 7100 CE system (Agilent Technologies, Waldbronn, Germany) using a 75-μm-i.d. fused-silica capillary coated with hydrophilic polymer (CEP) purchased from Agilent Technologies. The capillary was cut to 80.0-cm length (71.5 cm to the detection window). The diode-array detector was operated at 200 nm. Temperature was maintained at 25 °C using air cooling. Standard method for electroosmotic flow measurement[11] was modified for measuring electrophoretic mobilities of peptides.[12] The method used was identical as in our previous work[12], where detailed description is provided Migration of peptides was driven by a voltage of 10 kV applied for 10 min. Mobilities were calculated from the distance between zones of the peptide and of a neutral marker (thiourea). Concentration of either of the two peptides was 2 mM. All measurements were carried out in triplicates. *Tetra*-arginine (trifluoroacetate salt), and *tetra*-lysine (acetate salt), were purchased from Bachem (Bubendorf, Switzetland), GdmCl and all other chemicals were obtained from Sigma (St. Louis, MO, USA).

Tetra-arginine or *tetra*-lysine (described with the parm99sb force field[13]) in aqueous 1 M GdmCl or NaCl solution were simulated using periodic boundary conditions with 2104 SPC/E water molecules,[14] 42 Cl^-,and 38 Gdm^+ or Na^+ ions[8, 15, 16] per unit cell. This relatively high concentration was chosen to obtain better statistics. The temperature and pressure were kept at 300 K and 1 atm, respectively, using the Berendsen thermostat and the analogous barrostat.[17] After 0.5 ns of equilibration, the MD trajectories were propagated for 15 ns, using a time step of 1 fs. The MD simulations were carried out using the AMBER 10 program package.[18]

The results of electrophoresis of *tetra*-arginine in 50 mM mixed aqueous salt solutions of GdmCl and NaCl are presented in Figure 1. As the relative amount of Gdm^+ grows from 0 to 100 %, the electrophoretic mobility of *tetra*-arginine also increases, from 31.9 to 34.1 $\times$ 10^{-9} m^2 V^{-1} s^{-1}. This is a remarkable increase (well above the experimental error of <0.1 $\times$ 10^{-9} m^2 V^{-1} s^{-1} within the present setup or <0.5 $\times$ 10^{-9} m^2 V^{-1} s^{-1} if compared to previous measurements[12]), which can be rationalized by a larger affinity of Gdm^+ than Na^+ to *tetra*-arginine. The attraction of Gdm^+ to *tetra*-arginine then leads to an effectively higher positive charge on the peptide resulting in an increase in electrophoretic mobility. This increase is not strictly linear but exhibits onset of saturation at higher Gdm^+ relative concentrations (Figure 1), such a behavior being consistent with a weak binding of the ion to the peptide.

In contrast to *tetra*-arginine, *tetra*-lysine does not exhibit any measurable change in electrophoretic mobility when moving from NaCl to GdmCl (see Table 1). This is an important control experiment for two reasons. First, it shows that the observed changes of mobility of *tetra*-arginine are not due to different bulk properties of NaCl vs GdmCl solutions. Second, it demonstrates that Gdm^+ exhibits affinity specifically for the guanidinium moiety in the arginine side chain, since this is the only group that is different when comparing the two peptides.

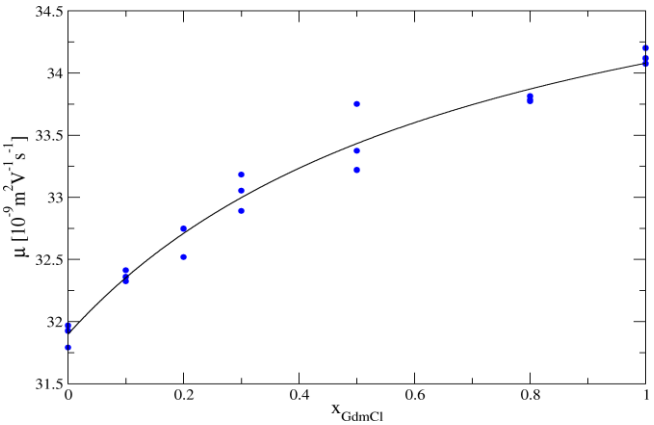


Figure 1. Electrophoretic mobilities μ (× 10^{-9} m^2 V^{-1} s^{-1}) of *tetra*-arginine in 50 mM mixed aqueous salt solutions of GdmCl and NaCl.

Table 1. Electrophoretic mobilities μ (x 10^{-9} m^2 V^{-1} s^{-1}) in 50 mM GdmCl and NaCl aqueous salt solutions.

	GdmCl	NaCl
tetra-arginine	34.13 ± 0.06	31.89 ± 0.09
tetra-lysine	35.31 ± 0.10	35.27 ± 0.06

MD simulations provide a microscopic picture of the specific interaction of Gdm^+ with arginine side chains. Figure 2 shows distributions of salt ions around side chains of *tetra*-arginine and *tetra*-lysine, averaged over the whole trajectories. In all cases Cl^- exhibits affinity for the positively charged side chains and, not surprisingly, Na^+ is repelled from them. In contrast, the behavior of Gdm^+ is not so intuitive – it is repelled from the side chains of *tetra*-lysine but not *tetra*-arginine, where it actually accumulates.

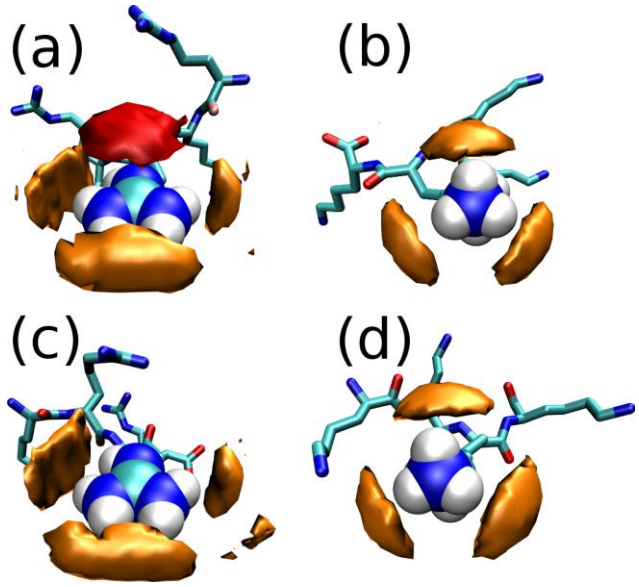


Figure 2 Distribution of guanidinium cations (red), sodium cations (green, but not present), and chloride anions (gold) around the side chains of (a) *tetra*-arginine in GdmCl(aq), (b) *tetra*-lysine in GdmCl(aq), (c) *tetra*-arginine in NaCl(aq), and (d) *tetra*-lysine in NaCl(aq).

This special ability of Gdm^+ to pair with the guanidinium moiety of arginine is quantified in Figure 3 which shows all the radial distribution functions between the salt ions and positively charged side chains. As expected, Cl^- peaks near both positively charged side chains, irrespective of the nature of the counter-cation (Na^+ or Gdm^+). No accumulation near the positively charged *tetra*-peptides is observed for the salt cations except for Gdm^+ next to the side chain of *tetra*-arginine, where the corresponding radial distribution function peaks at a value of >2 (with value of 1 corresponding to bulk ion concentration).

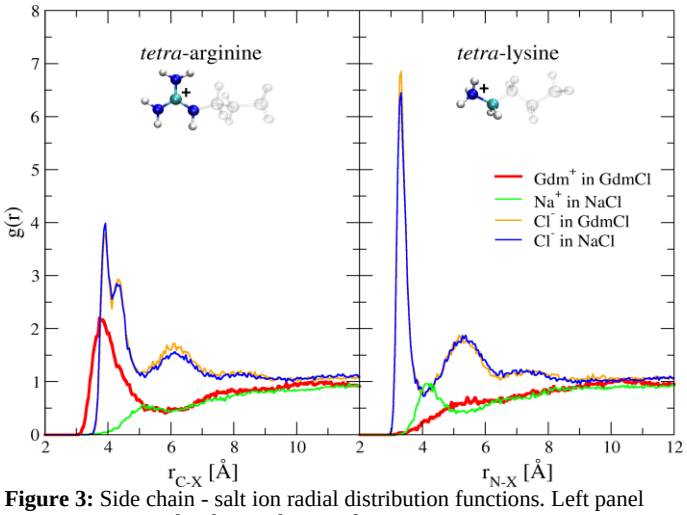


Figure 3: Side chain - salt ion radial distribution functions. Left panel - *tetra*-arginine and right panel - *tetra*-lysine.

In summary, electrophoretic measurements and MD simulations clearly point to a specific attractive interaction between guanidinium cations and positively charged side chains of arginine in aqueous solutions. In capillary electrophoresis, this attraction between two cationic groups leads to an increased electrophoretic mobility of *tetra*-arginine in GdmCl compared to NaCl, with no such enhancement observed in a control experiment for *tetra*-lysine in these two salts. The corresponding MD simulations support this observation and confirm that it is due to formation contact pairs between Gdm^+ and the guanidinium moieties in the side chains of *tetra*-arginine. The present study thus provides a strong experimental and computational indication for the counter-intuitive pairing between Gdm^+ and positively charged side chains of arginine. Such pairing has direct implications for the molecular mechanisms of aggregation and/or destabilization of arginine-rich peptides and proteins in aqueous solutions of guanidinium salts, which may be more complex than previously anticipated.[9, 10, 19, 20]

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