RESEARCH ARTICLE

Why do GroEL Ions Exhibit Two Gas Phase Conformers?

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Abstract

The reasons for the existence of two gas phase conformers for electrosprayed ions of the large tetradecameric protein complex GroEL are considered. Key features are that: (1) both conformers extrapolate to very similar cross sections in the limit of zero charge; (2) both conformers supercharge above the maximum value permitted by the Rayleigh criterion; and (3) one of the conformers supercharges substantially more than the other. We hypothesize that the supercharging observed is associated to the approximately cylindrical shape of GroEL in aqueous solution, enabling accumulation of extra charge on either one or two of its bases when they intersect the evaporating drop surface. The two conformers would then correspond to cases when either one or the two bases of the cylinder carry extra charge. Apparently, the conformer symmetrically (doubly) supercharged on both ends is Coulombically stretched, therefore exhibiting a mobility different from the conformer asymmetrically supercharged only on one end. Several general consequences follow. First, non-spherical proteins may generally be charged above the usual Rayleigh limit. Second, we confirm the previously advanced but contentious notion that gas phase protein ions are readily compacted or stretched away from their crystal structure by capillary and Coulombic forces during the electrospraying process.

Key words: Protein, Surface tension, Rayleigh limit, Compaction, Shape transition, DMA, Mobility

Introduction

We have recently reported that the 0.8 MDa tetradecameric protein complex GroEL forms two different gas phase conformers when electrosprayed into air from aqueous ammonium acetate solutions [1]. The clearly observable differences between the two corresponding mobilities may be seen in Figure 1, where ion abundance is shown in a color scale versus inverse mobility (x axis) and m/z (y axis). The present paper is an effort to rationalize this observation. We also provide new evidence confirming the ability of electrospray ionization to alter native protein conformation.

already shown in the original Reference [1], the two conformers exhibit almost the same cross section when extrapolated to zero charge, and differ primarily in the magnitude of the slight dependence of their mobility on the charge state z . This important point is elaborated further in the Appendix. (2) The maximum charge state observed is $z=82$, substantially larger than the maximum theoretical Rayleigh charge [Equation (1)] expected for a water drop with the same volume as the GroEL ion. This is a highly unusual situation for proteins electrosprayed from neutral aqueous solutions [2]

$$z_{\max} = 0.0778 m^{1/2} \sim 71 \quad (1)$$

Discussion

Explanation for the Two Conformers Based on a Bimodal Charge Distribution

The following singular experimental observations provide the necessary insights for the present interpretation: (1) As

(3) The lowest z observed is close to $z=71$ for both conformers, but the highest charge state approaching $z=82$ is achieved only by the most mobile conformer. Point (1) suggested that the charges are distributed differently on the surfaces of both conformers, leading to different shapes [1]. But how could just two distinct ways of distributing so many charges come about, rather than

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many similar charge distributions sampling continuously a range of mobilities?

Why Two Distinct Charge Distributions

Note first that the two main mechanisms known to limit the charge on a drop are the Rayleigh limit and ion evaporation. The second is generally active at drop sizes considerably smaller than GroEL. This general trend is clearly verified for the GroEL data under discussion. For if the charge were limited by ion evaporation, it would be below the Rayleigh limit, not near or slightly above it. We therefore proceed under the assumption that ion evaporation is inactive.

Two Levels of Supercharging One possible mechanism leading to a dual mode of charging is suggested by observations (2) and (3). There is an unusual mechanism peculiar to GroEL enabling it to be charged substantially above $z_{\max}$ (2), while this mechanism is exploited almost twice as intensely by one conformer than by the other (3). For brevity we will use the term *supercharged* or *supercharging* to refer to ions holding more charge than the Rayleigh charge on a spherical solvent drop of the same volume.

The Importance of Being Nonspherical It is evident that a spherical ion originating from a charged solvent drop cannot be supercharged. Therefore, the observed supercharging mechanism must be associated to the nonspherical shape of native GroEL. It is well known that linear polymer chains and denatured proteins can take far more charge than globular proteins [2]. The probable reason for this phenomenon is that the non-spherical object enables (or even forces) a nonspherical shape in the drop carrying it, which in turn allows this drop to sustain a charge above the Rayleigh limit. In one picture put forth by Fenn and colleagues [3, 4] a PEG ion would spread on the drop surface and bind to a number of free surface charges. When the drop has shrunk sufficiently for all of its free charges to be tied to the polymer, as it reaches the Rayleigh limit, the drop can no longer eject any net charge, nor can it remain spherical, which leads to a supercharged non-spherical drop [2]. This mechanism leading to charge levels substantially above the Rayleigh limit would apply not only to PEG, but also to other ions capable of extending widely and of binding charge, including denatured proteins.

The Supercharging Sites are the Cylinder Bases PEG's extreme form of supercharging does clearly not apply to GroEL, which achieves a maximal charge of only 82/71 (=1.15) times the Rayleigh limit, and forms rather compact gas phase ions [1]. However, a more restrained supercharging mechanism is still available for an approximately

cylindrical but not freely extendible ion such as GroEL. This mechanism is most easily understood near the end of the drying process, when both bases of the cylindrical ion come in contact with the drop surface [sketch (a) in Figure 1]. Beyond this point in its evaporation history, the drop can no longer remain spherical. Therefore, its maximum charge is no longer the Rayleigh value for a sphere of the same volume, but greater. Several mechanisms contribute to this advantage. First, some charge is removed from the surface of the liquid drop by being tied directly to the dry corners of the protein protruding outside the drop [sketch (b) in Figure 1]. Second, given the enhancement of the field taking place at these highly curved corners, the dry area around them will sequester a disproportionate fraction of the charge. Third, the still wet polar regions of the drop also have relatively large curvature, which not only draws more than the average surface charge, but also stabilizes it better against the Rayleigh instability. Finally, although the equatorial region of the drop in sketch (b) in Figure 1 remains spherical and subject to the usual Rayleigh limit, since much of the charge has been moved away from it (into the corner regions and the polar caps), the deformed drop (sketch b) can hold more charge than a completely spherical drop. This higher value presumably coincides with the maximum charge observed, $z=82$. Evidently, the charge-holding capacity of the deformed drop in sketch (b) is only moderately increased because all the liquid-air interfaces are still subject to the Rayleigh instability. Once this limit is reached anywhere, one or several Taylor cones form [5], leading to substantial (13 %–50 %) charge loss.

Shape Evolution of GroEL After Drying The supercharging mechanism discussed requires that GroEL remains in its native form as long as it is captive in the drop, but does not imply that the dry ion will remain cylindrical. We have in fact argued that gas phase GroEL ions are substantially compacted into globular shapes [1]. Nor is it necessarily true that the full excess charge initially deposited on the cylinder bases is locked in that region of the protein. Some of it may

Figure 1. Mobility- m/z contour plot for GroEL tetradecamers in dry atmospheric pressure air (after Figure 1 of Reference 1), including sketches of the process leading to two different levels of supercharging

redistribute by proton transfer to other basic sites, though we believe some excess charge remains in the polar regions.

Objections One could object to the mechanism presented by arguing that a cylindrical macromolecule could intersect the drop on its sides as well as its base, hence supercharging could apparently take place laterally. Similarly, even a spherical ion could touch the drop surface and lead to supercharged globules, but because globules do not supercharge, this would apparently disprove our mechanism. Note, however, that neither the globule nor the side of the cylinder present edges where the field may be intensified and the charge concentrated. Edges are present only on the cylinder bases. Furthermore, the double intersection of sketch (a) is impossible on the cylinder sides. Even the single side intersection of sketch (c) is possible only in sufficiently large drops.

Our tentative explanation for the conformer exhibiting a lesser degree of supercharging is that the last Coulombic fission takes place when only one of the two bases touches the drop surface, hence only one base is involved in supercharging [sketch (c) in Figure 1].

Summary We therefore conclude that:

(1) GroEL is capable of holding an anomalous level of charge on either of its two bases; (2) one of the conformers is asymmetrically supercharged only on one base; (3) the other conformer is symmetrically supercharged on both bases; (4) two different conformers result from the different deformations associated to both charge distributions. In particular, the symmetrically supercharged ion is stretched along its axis.

Why the More Charged Conformer Is More Compact

GroEL enjoys the special feature of having an axial length approximately twice its radius. This geometry is singular in several respects [6]. First, for cylinders, it corresponds to a minimum surface area A for a given volume V . Second, it has an isotropic drag tensor (same resistance to motion in all possible orientations). Third, a small deformation away from a body shape having an isotropic drag tensor always increases its mobility/Area, whence this geometry is also associated to a minimum mobility for given surface area. Suppose now that GroEL is a cylinder with a length smaller than twice its radius. In this case, because protein matter is approximately incompressible (i.e., the protein volume is fixed) [7], a slight axial stretching will decrease A . Conversely, if GroEL's axial length is slightly larger than its radius, its area will increase upon axial stretching. In neither case could we assure that the mobility will either decrease or increase because ZA is also a maximum. Whether Z increases or decreases depends on the details of the curvature of ZA and $V^{2/3}/A$ versus elongation, as well as

on the initial departure of the crystal geometry from a geometry having an isotropic drag. These details are not known well enough to decide whether axial deformation will result in an increase or a decrease in mobility. The observed increase in mobility with axial stretching is therefore not unreasonable.

Protein Deformation Upon Electrospraying

We have argued [1] that the cross section of GroEL is drastically (~40 %) smaller than expected from the crystal structure. However, this quantitative conclusion depends on how the measured mobility is converted into a cross section. Reference 1 based this conversion on Millikan's drag law for spheres in free molecule flow. Although unusual in protein studies, this assumption agrees well with other calculation methods [1, 8, 9]. Further qualitative support on the general issue of protein deformation upon electrospraying follows directly from Figure 1. We should first note that earlier measurements of GroEL [10] had observed only one conformer with most charge states below $z=71$. The difference in charging level may be due to the fact that compared with nanospray, the electrospray source of Reference 1 uses similar liquid flow rates (~1.5 nl/s, computed based on Poiseuille flow for conditions reported in reference 1: a capillary i.d. of 40 μm , 25 cm long, driven by a pressure difference of 50 mbar), but larger capillary diameters (~40 μm). The lack of two structures in prior work could conceivably be attributed to the smaller charge states achieved, but this is unlikely given that we do see the two conformers at $z=71$ and 72, at which other studies have seen only one. The difference may be due to the fact that our instrument is unique in measuring mobility at atmospheric pressure, prior to any ion activation in the vacuum system of the mass spectrometer.

The existence of two conformers shows directly that proteins may be deformed by the process of entering into the gas phase. Additional evidence for this deformation is given by the observed dependence of the ratio z/Z (proportional to the cross section) on the charge state. This dependence is in part due to ion dipole interaction with the bath gas (the so called polarization effect). But there is more than that because otherwise Z/z plotted versus z^2 would exhibit the same slope for both conformers. In reality, the cross section of the most mobile conformer varies considerably less with z than that for the other, showing that the charge modifies the collision cross section in two different ways. First, indirectly through the polarization effect, without necessarily modifying the ion shape. Second, directly by deforming the ion through Coulombic stresses. These different roles are quantified in the Appendix.

Capillary Compaction

Because Coulombic stresses and capillary stresses are comparable at the Rayleigh limit, and the drops containing these ions

are charged close to this limit, the capillary stress exerted by the evaporating drop on the ion must also be capable of deforming it. This consideration reinforces our prior conclusion that the protein may be deformed both by Coulombic stresses and by capillary stresses. These two types of stresses have opposite effects, one tending to compact the ion, the other spreading it out. Capillary forces are expected to be dominant since the drop and the solute ion are typically charged below the Rayleigh limit.² However, capillary forces seem to dominate also for our GroEL complex, charged clearly above the Rayleigh limit. This is apparent from the fact that supercharged GroEL is more compact than most other proteins [1, 9], while a deformable ion charged above its Rayleigh limit would stretch considerably [11]. This simply means that the surface energy typically associated to protein matter in the gas phase is comparable to the surface tension of a water-air interface. We have recently provided independent evidence for this point based on new [12] as well as earlier [13] observation of a Rayleigh-like instability at a critical charge above which gas phase protein ions lose their compact conformation. This quantitative determination of the effective surface tension of protein matter is confirmed also by early measurements on the mass dependence of the maximal charge that can be held by clusters of dipeptides [14]. Therefore, the tendency for gas phase proteins to be compacted into spheroidal shapes already associated to capillary forces from the solvent should be interpreted more broadly as including also capillary forces associated to the bare protein itself. This notion has also been confirmed in measurements with polyethylene glycol (PEG) ions [11], and can be directly verified by computing the potential energy of a folded polymer molecule as a function of its size, and confirming that (at large enough sizes) there is an additive contribution proportional to ion surface.

Our mobility measurement [1] could be interpreted as implying that the crystal structure is approximately preserved in the gas phase, but only by assuming that the drag force exerted by the gas is the same as in the *ideal* case of a smooth sphere with elastic and specular reflections. This hypothesis, however, faces too many objections, including (1) Millikan's finding that the free molecule limit drag of oil drops is a factor $\xi=1.36$ times larger than the *ideal* drag. (2) We know from simulations that the atomic roughness of real ion surfaces leads to a similar drag enhancement factor ranging from 1.2 to 1.3 at ion masses going from 20 kD to relatively large sizes [15, 16]. (3) The *ideal* assumption $\xi=1$ leads to unrealistically small values ($\rho \sim 0.6 \text{ g cm}^{-3}$) for the gas phase protein density [9]. The solvent volume displaced by proteins is typically $0.75 \text{ cm}^3/\text{g}$ [7], while the void volume of proteins is rather low, typically 0.25 [17], leading to an expected protein ion density in the range of $\sim 1 \text{ g cm}^{-3}$. This is the range of densities obtained under the assumption $\xi=1.36$ [9]. (4) Recent mobility measurements of mass resolved ionic liquid nanodrops confirm a value of $\xi \sim 1.36$ down to drop diameters smaller than 2 nm [18], covering the full protein size range. In view of these many and serious difficulties, it would seem that the hypotheses that $\xi > 1.2$ for proteins and that electrospray

ionization leads to substantial compaction are far more reasonable than the alternative. This point is further confirmed by recent measurements on the relative mobilities of concanavalin A and its aggregates, showing without reference to any choice of ξ that the tetrahedral crystal structure of the tetramer is considerably spheroidized in the gas phase [12].

Now, if the electrospraying process is capable of spheroidizing large protein ions, how does it do so? In our view, this is achieved primarily by the capillary forces already alluded to. Take, for instance, the tetrahedral tetramer of concanavalin, roughly approximated by four symmetrically arranged touching spheres of radius R_1 [12]. Ignoring provisionally the area lost in the region of intersection of these spheres, their area of contact with the gas (or vacuum) is four times that of the monomer. However, if the same volume of four monomers is compacted into a single sphere, its area is only $4^{2/3}$ the monomer area. This level of compaction, therefore, reduces the surface area by a significant factor of $4^{1/3}=1.5874$. The surface energy reduction per unit mass associated to this process is

$$\frac{\Delta G_S}{m} = \frac{3\gamma}{\rho R_1} \left(1 - \frac{1}{4^{1/3}} \right). \quad (2)$$

In the case of concanavalin, with $R_1 \sim 2.2 \text{ nm}$, using the surface tension of water for γ , and $\rho=1 \text{ g/cm}^3$, we find a surface energy reduction of $3.5 \cdot 10^8 \text{ erg/g}$. A typical conformational energy for a protein with $m \sim 20 \text{ kDa}$ is $5\text{--}20 \text{ kcal/mol}$ [19], ($2.09 \cdot 10^7 \text{ erg/g}$ assuming a conformational energy of 10 kcal/mol). This is an order of magnitude below the surface energy reduction (Equation (2)), rationalizing the ease with which capillary forces are able to deform proteins.

The preceding calculation is of course tentative for many reasons, including the fact that the surface energy has been computed for a protein-air interface, while the conformational energy is in aqueous solution. This is, however, not a fatal flaw. Suppose that a protein originally in its native state in solution is removed from the solvent without shape change, and preserving all interior water. All interaction energies then remain unchanged except those taking place at the protein surface. One could then argue that the magnitude of the conformational energy is not drastically changed by removal of the surrounding solvent for two reasons. First, the conformational energy associated to surface interactions in solution cannot exceed that for bulk interactions, particularly so for large proteins, for which volume dominates over surface. Also, if surface contributions in solution were dominant, then proteins in solution would be spherical. Second, since these surface interactions can be quantified by the product of a surface energy times the interfacial area, and because this surface energy is considerably larger in air or vacuum than in water, if the surface

energy in air exceeds the full conformational energy in solution (as just found), it must exceed even more its bulk component (the only one surviving out of solution, and therefore the only one that would tend to preserve the native shape once in the gas phase).

Another limitation of Equation (2) is that because a true tetramer necessarily contains protein-protein interfaces, the calculated surface energy overpredicts the actual surface energy. We estimate the associated error by noting that most of the contact region between four tetrahedrally arranged identical spheres is contained within the tetrahedron whose vertices are at the centers of the four spheres. Excluding the surface of the spheres within this tetrahedron removes out of each the area associated to the solid angle of one vertex of the tetrahedron. This angle is $\arccos(23/27)$ steradians (i.e.: <http://en.wikipedia.org/wiki/Tetrahedron>), or 4.387 % of the full solid angle of 4π spanned by the sphere. Equation (2) should then substitute the factor $1-1/4^{1/3}$ by $1-0.04387-1/4^{1/3}$, leading to a reduction of 11.8 % in the calculated ΔG_s . The estimate based on Equation (2) would, therefore, be still acceptable even if one doubled the interaction area just considered.

We note finally that although the notion of protein surface tension is not widely used, the protein surface is undisputedly of the highest importance, and so should the associated Gibbs free energy [20].

Conclusion

In conclusion, the two conformers found for GroEL contribute further to the already considerable evidence available showing that the electrospraying phenomenon tends to compact the shape of protein ions, and that capillary forces play a leading role in this process. While the model proposed to explain GroEL's two conformers is tentative, several of its features are directly verifiable. In particular, other approximately cylindrical proteins should also exhibit anomalously high charge levels, and perhaps also exhibit two distinct charge distributions and two conformers.

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Appendix: Polarization Effect and Extrapolation of the Cross Section to Zero Charge

A comparison between the observed variation of Z/z with z^2 and that expected from polarization effects is instructive, and was included in the supporting material of reference 1. On the one hand, it helps quantify the role of Coulombic stresses in deforming the protein geometry. On the other, it provides a better basis for the linear extrapolation used in Reference 1 to conclude that both conformers have almost identical cross-sections in the limit of zero charge.

Figure 2 represents the data of Figure 3 from Reference 1 in a scale extending down to $z^2=0$, suitable to judge by eye the reliability of the extrapolation. The filled data points correspond to the most mobile ion, for which the ion intensity is higher and the number of charge states more extensive, so that the linear fit to the data shown can be used to determine with relatively high confidence the intercept with the Z/z axis at $z=0$ ($z/Z=85.502$ Vs/cm²). Almost the same intercept for the less mobile conformer ($z/Z=85.523$ Vs/cm²) is obtained, though with less confidence due to the smaller number of charge states, the weaker signal, and the broader peaks. The important statement that the two cross sections extrapolated to zero charge are essentially identical is therefore justified. Also, a general qualitative trend for the two cross-sections to approach each other at diminishing z is evident from the raw data irrespective of how one extrapolates them. What may be doubted is the basis for a linear extrapolation down to $z=0$, well below available data. These measurements do certainly not provide rigorous justification for such an extrapolation. However, for a globule of fixed geometry and variable charge state, theory supports a linear dependence of the cross section on z^2 (see below). Similarly, electrostatic stresses are quadratic in the electric field and, therefore, are also linear in z^2 . As long as these stresses produce linear deformations, a linear dependence of cross section on z^2 is equally justified. A linear response over the whole range of available data is also appropriate because the total change in cross section is relatively small (8 % reduction from the smallest cross section measured to the extrapolated $z=0$ value). The extrapolation of Figure 2 has, therefore, a substantial basis, and is undoubtedly the most reasonable approach available to compare the cross-sections of the two conformers corrected for Coulombic effects. For a hard sphere of fixed diameter d , the ratio between the actual mobility Z including polarization effects and the value Z_0 excluding them is a function of the parameter ξ

defined as the ratio between the polarization energy at contact and the thermal energy kT :

$$Z/Z_o = F(\xi) \quad (A1)$$

$$\xi = 2\alpha e^2 z^2 / [\pi \epsilon_o k T d^4], \quad (A2)$$

where α ($17.1 \cdot 10^{-31} \text{ cm}^3$ for air) is the polarizability of the gas, e the elementary charge, and ϵ_o the electrical permittivity of vacuum. Concrete predictions for $F(\xi)$ are available in the special case of a hard sphere of radius R with elastic and specular reflections at $r = R$ combined with a polarization potential for $r > R$ (see Tammet's review of prior literature [21]). This particular model is quantitatively unreliable (because real scattering on the atomically rough ion surface is not specular) as evident from its overprediction of Z_o by a factor 1.36. Still, the general result (A1) is model-independent, and a linear dependence for small ξ is reasonable in principle, and is confirmed by a variety of measurements [22] and models [23]:

$$Z/Z_o = 1 - b\xi; \xi \ll 1. \quad (A3)$$

An approximate value for the dimensionless parameter b in the range 0.4 was determined by Ude [24] for singly charged polyethylene glycol ions in air. Recent systematic studies based on mass and mobility measurements of highly charged nanodrops of ionic liquids confirm over a greatly expanded dataset the linear law (A3), and conclude with higher confidence than Ude that [22, 23]:

$$b = 0.45 \pm 0.1. \quad (A4)$$

The substantial ambiguity of this measurement is a result of the relatively small value of the $b\xi$ correction. In order to compare the b values associated to the two linear fits in Figure 2 with those expected from (A4), we proceed as follows. For Z_o we use Millikan's expression, which

Figure 2. Extrapolation to $z=0$ of the data from Figure 3 of Reference 1

(probably due to diffuse scattering from the atomically rough ion surface [15]) is a factor 1.36 smaller than the usual elastic hard sphere result [1]:

$$Z_o = \frac{z}{1.36} \frac{e}{p \pi d^2} \left(\frac{9 \pi k T}{8 m_g} \right)^{1/2} = 0.44 \frac{z e}{p d^2} \left(\frac{k T}{m_g} \right)^{1/2}, \quad (A5)$$

where p is the gas pressure and m_g is the effective mass for ion-gas collisions (which for the massive GroEL ion is indistinguishable from the molecular mass of the gas molecules). Interestingly, $\xi^{1/2}$ and Z_o have exactly the same dependence on z and d . Their ratio for a given ion-gas pair and given pressure and temperature is therefore the constant:

$$\frac{Z_o}{\xi^{1/2}} = 0.44 \frac{k T}{p} \frac{(\pi \epsilon_o)^{1/2}}{(2 \alpha m_g)^{1/2}} = 0.55 \frac{k T \epsilon_o^{1/2}}{p (\alpha m_g)^{1/2}}. \quad (A6)$$

For air at ambient conditions it takes the numerical value

$$Z_o / \xi^{1/2} = Z^* = 1.29 \text{ cm}^2/\text{V/s}, \quad (A7)$$

whence the polarization correction may be written

$$Z/Z_o = 1 - b(Z_o/Z^*)^2 = 1 - b z^2 (Z_1/Z^*)^2, \quad (A8)$$

where $Z_1 = Z_o/z$ is the polarization-corrected mobility of the singly charged GroEL ion.

Z/Z_o , therefore, varies linearly with z^2 with slope $b(Z_1/Z^*)^2$. As noted in the supplementary material to Reference 1, the experimental slopes b defined by (A8) and the GroEL data correspond to $b=0.23$ and 0.34 for the two conformers, respectively. These experimental slopes are therefore comparable to, but less, than would be expected from polarization effects alone in a Coulombically undeformed ion. There would accordingly be nothing surprising if the cross section varied linearly with z^2 down to $z=0$, with a slope even larger than those observed here. Earlier observations of GroEL cross sections in nitrogen that have no dependence on z , or even decrease with increasing z , would in this light appear as far more paradoxical than those of Reference 1.

The data of Figure 2 and the anomalously small slopes found demonstrate a second effect of the charge on cross section not due to polarization. Since this effect is different on both conformers, it must be a shape effect, and since it depends on charge, the conformation of the ions must be affected by Coulombic stresses.

Note that the polarization correction coefficient b derived from experiments corresponds to globules of relatively polar materials such as polyethylene glycol and ionic liquids. This situation approaches that of a uniformly charged sphere, since most of the net charge is shielded by dipoles and turned into relatively uniform polarization charge on the ion surface. In a globule made up of a relatively low dielectric constant substance (such as a protein), only about half of the charge would be delocalized, and the assumption of a uniform charge distribution would be more problematic.

Still, for an ion carrying 71–82 individual charges, most approaching gas molecules would feel primarily the global charge, while only the small fraction of those impacting directly on a protonated site would behave substantially different from the uniform charge model. We conclude that while the coefficient b may depend on the system and in particular on the dielectric constant of the ion, it is unlikely to take drastically different values for proteins than for PEG or ionic liquid nanodrops.

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