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High-energy collisions of multiply charged lysozyme ions in gases

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Abstract

An electrospray ion source has been mounted on an accelerator mass spectrometer of the isotope separator type. The multiply charged ions produced by the ion source are accelerated to a kinetic energy of 50*z* keV, where *z* is the charge state of the ion. The conformation of disulfide-bond intact and disulfide-bond reduced lysozyme ions with charge states between +7 and +17 has been studied by measurements of single collision destruction cross sections in collisions with He and Xe. For disulfide-bond intact lysozyme ions the cross sections are smaller and less influenced by charge state variation than for disulfide-bond reduced lysozyme. The destruction cross sections are modeled by semielastic collisions between individual molecular atoms and target atoms with an assumption of a charge-state dependent unfolding of the lysozyme structure. (Int J Mass Spectrom 207 (2001) 31–39) © 2001 Elsevier Science B.V.

Keywords: Large biomolecules; Gas-phase conformation; High-energy collisions; Destruction cross sections

1. Introduction

Recently there has been a growing interest in studies of shapes and sizes of gas-phase biomolecular ions [1]. The motivation for these studies is twofold. The mere existence of such ions, produced in either matrix-assisted laser desorption ionization [2] or electrospray ionization (ESI) [3] ion sources, calls for a better understanding of their gas-phase conformation. Second, the extent to which the solution-phase structure is preserved in the gas phase may shed some light on the influence of solvent on structure.

Methods for probing gas-phase structures of large biomolecular ions can be divided into two categories, namely studies of chemical reactivity and studies based on collisions with inert collision partners [4] or surfaces [5]. The former utilize differences in chemical reactivity among conformers and typically involve ion–molecule reactions such as proton transfer [6] or H/D exchange [7]. Ion mobility measurements, which have been used to determine collision cross sections of protein ions with different conformation [8], belong to the second category. In such measurements, low energy collisions with gas atoms reduce the speed of the ions in an electrical field but do not induce dissociation of the ions. We have measured total cross sections for destruction of gas-phase protein ions by high-energy collisions (up to 1 MeV kinetic energy). The dominating destruction mechanism at such collision energies is direct ejection of

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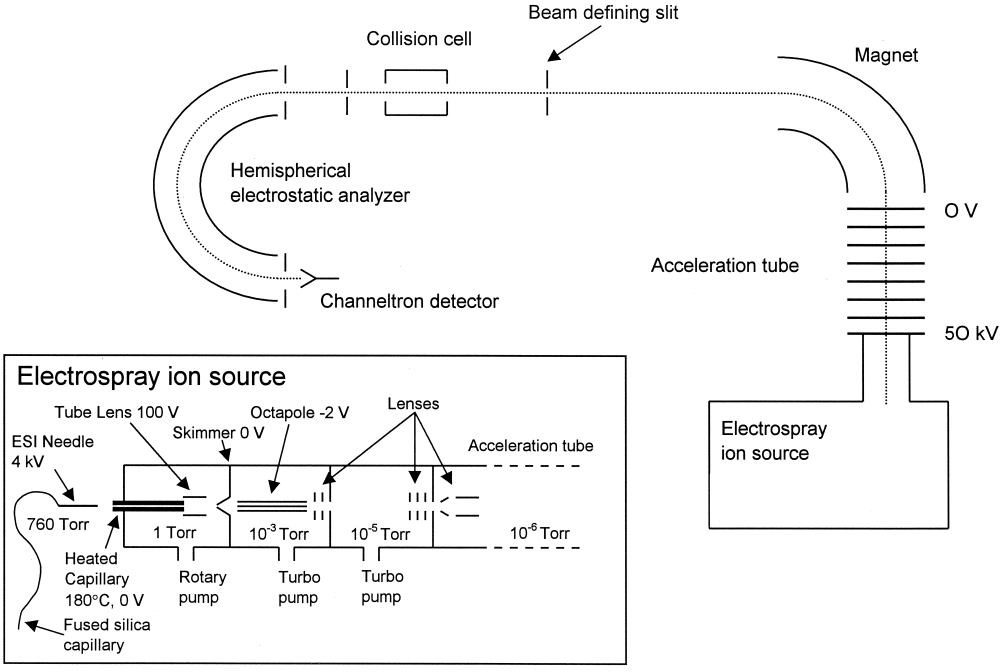

Fig. 1. Schematic diagram of the experimental apparatus; the inset shows the electrospray ion source.

atoms from the protein molecule due to a collision of an atom or a group of atoms in the protein ion with an atom in the gas. The destruction cross section provides more direct information about the geometry of a biomolecular ion than the results obtained from chemical reactivity studies. Further, the fact that the destruction cross section depends on the target gas may be used to obtain detailed shape information which cannot be derived from mobility measurements. At very low energies the van der Waals radii of atoms determine the collision cross section, and these radii are almost independent of the atomic numbers of the colliding atoms. We have measured destruction cross sections for both disulfide-bond intact and disulfide-bond reduced lysozyme ions. The cross sections for ions with low charge states, 7+ to 10+, were found to correspond to a shape near the native crystal conformation of lysozyme, whereas the larger cross sections found for high charge states indicate a more open conformation of these ions. It should be stressed here that the high charge states, 11+ to 17+, are only observed for disulfide-bond reduced lysozyme ions.

2. Experimental

A homemade electrospray ion source has been mounted on an existing electrostatic heavy ion accelerator of the isotope separator type. A schematic diagram of the experimental setup is shown in Fig. 1. The electrospray ion source (including rotary pumps and turbomolecular pumps) is mounted on the high-voltage platform of the accelerator. A stainless steel hypodermic needle is via a fused silica capillary connected to a syringe containing a solution of the analyte. A syringe pump (Harvard Apparatus) delivers a constant flow of the solution through the needle, which is biased at typically 4 kV relative to the heated capillary. The electrospray produces highly charged droplets at atmospheric pressure, and gaseous multiply charged analyte ions are formed from the droplets in the heated capillary. The capillary is a 10 cm long stainless steel rod with a 0.4 mm bore, normally heated to 180 °C. Due to pumping at the rear end of the capillary, ions emerge into the first vacuum zone where a rotary pump maintains a pressure of around 1

Torr. The ions are then focused by a tube lens through a skimmer into a second region, where the pressure is further reduced to 1 mTorr by a turbopump. The ions are steered through this section by an octopole beam guide, and enter the third vacuum region through lenses and another skimmer. This region is evacuated to 10^{-5} Torr by a second turbopump. An Einzel lens assembly guides the ions into the acceleration tube of the isotope separator, where the ions acquire a kinetic energy of 50*z* keV. After acceleration, an *m/z* analysis (*m* being the mass of the ion) is performed by a large, 2 m radius, 72° bending magnet capable of deflecting singly charged ions with mass up to 5000 Da. After magnetic analysis the mass-selected ions pass through a target gas contained in a 3 cm long differentially pumped cell with entrance and exit apertures of 1 and 3 mm in diameter, respectively. The ions exiting the target cell are separated according to their energy-to-charge ratio (*E*/*z*) by a computer-controlled 180° hemispherical electrostatic analyzer with a radius of 15 cm. The transmitted ions are detected by a channeltron, connected through amplifiers and discriminators to the PC and operated in the particle counting mode. In this way, mass-analyzed ion kinetic energy spectra could be obtained. Here we have used this analyzer to select ions that have not fragmented. The contribution from fragments with the same *m/z* ratio as the primary beam was negligible. The ESI *m/z* spectra illustrated in Fig. 2 were obtained from the intensity of the transmitted beam as a function of the magnetic sector field for zero pressure in the target cell. The total destruction cross sections for ions in a given charge state were derived from the exponential dependence of the beam intensity I on the thickness (μ atoms/cm^2) of the target gas, $I(\mu)=I(0)e^{-\sigma\mu}$. Gases of He and Xe were used as targets and lysozyme ions with charge states from 7+ to 17+ as projectiles.

Hen egg-white lysozyme (i.e. disulfide-bond intact lysozyme) was obtained from Sigma and used without further purification. Disulfide-bond reduced lysozyme was prepared by reduction of the disulfide bonds with dithiothreitol in 6 M guanidinium chloride and subsequent alkylation with 4-vinylpyridine. The reduced and alkylated lysozyme was purified by reversed-phase high performance liquid chromatography. For electrospraying the proteins were dissolved in 1:1 CH_3OH/H_2O (v/v) with 1.0% acetic acid (v/v) to a concentration of 10 μM.

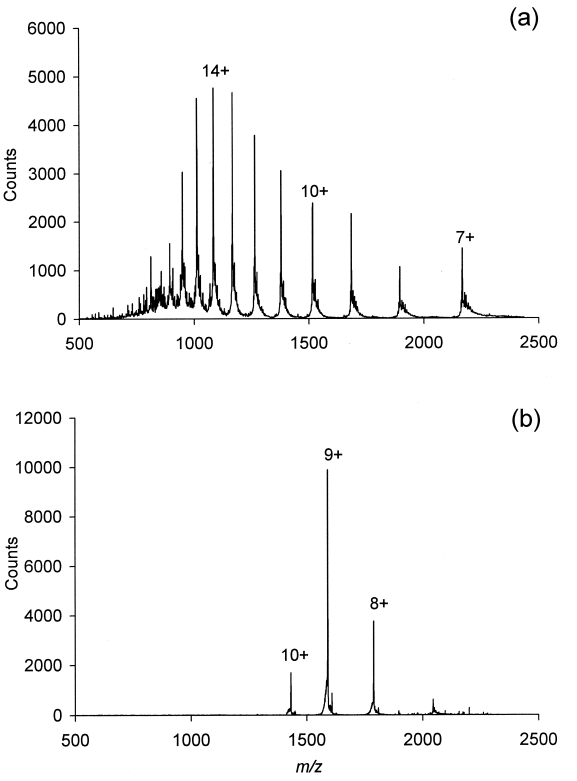

Fig. 2. Electrospray ion mass (*m/z*) spectra measured for (a) disulfide-bond reduced lysozyme and (b) disulfide-bond intact lysozyme.

3. Results and discussion

ESI mass spectra of disulfide-bond intact lysozyme (M_W 14305.1 Da) and disulfide-bond reduced lysozyme (M_W 15154.3 Da) are shown in Fig. 2(a) and (b). The intact lysozyme ions have charge states from 7+ to 10+, with 9+ as the most abundant. The distribution is broader for disulfide-bond reduced lysozyme, with charge states ranging from 7+ to 17+ and with 14+ as the most abundant. These results are in agreement with previously reported ESI spectra of lysozyme and other disulfide-bonded proteins [9,10].

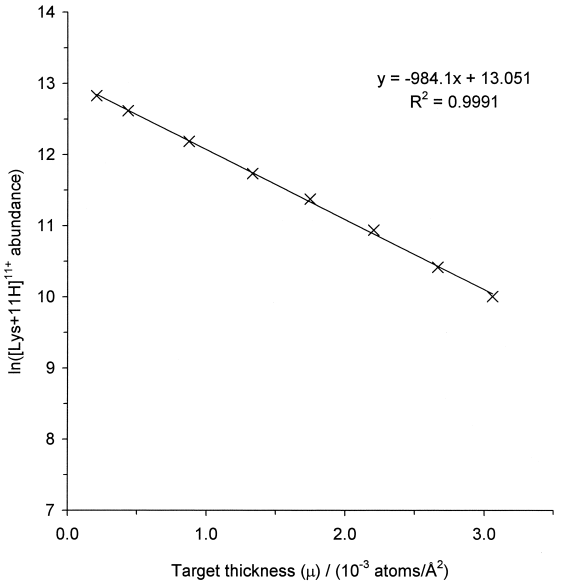

Fig. 3. Exponential fit to the measured beam intensity vs. target thickness for reduced and alkylated lysozyme 11+ colliding with a He target gas. The total destruction cross section is obtained from the slope of the fitted line.

The difference in charge state distribution is believed to reflect different gas-phase conformations. The reduction of the beam intensity with increasing target gas pressure is illustrated in Fig. 3 for lysozyme 11+ on He, and a cross section of σ=984.1 Å^2 was obtained in this case.

The measured destruction cross sections in He and Xe for the different charge states of disulfide-bond intact (native) and reduced lysozyme are shown in Figs. 4 and 5. In He the cross section for intact lysozyme ions is nearly independent of charge state (7+ to 10+) and the absolute value is about 750 Å^2. For reduced lysozyme the cross section increases with charge state, and the strongest increase is observed for low charge states, from 7+ to 10+. For the charge state 7+, the cross section is nearly the same for the two types of lysozyme. The measured cross sections in Xe show a different behaviour. The destruction cross section is about twice as large as in He and it increases with charge state, both for intact and for reduced lysozyme. For charge state 7+ the cross section is smaller for intact than for reduced lysozyme.

An analysis of the destruction of energetic molecular ions in collisions with atoms or molecules inevitably raises the question about how energy is trans-

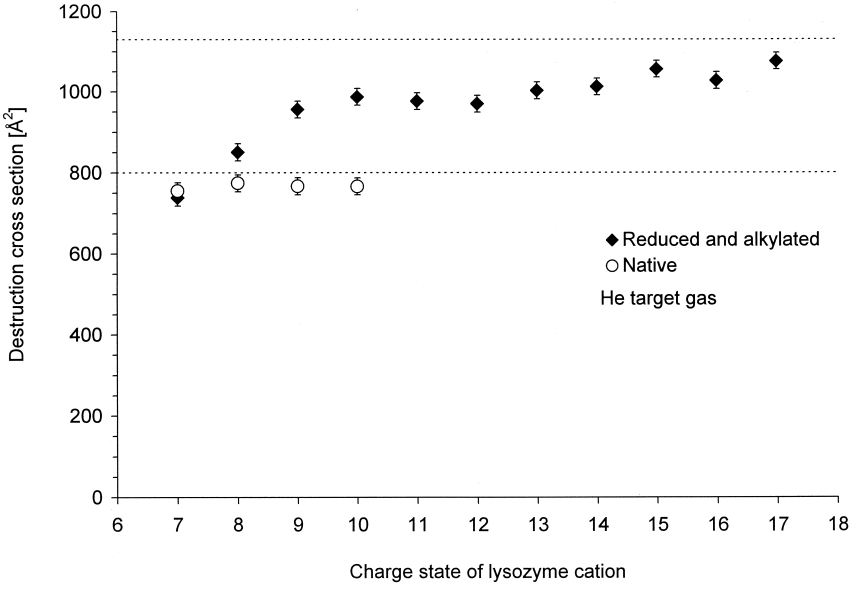

Fig. 4. Total destruction cross sections for disulfide-bond intact (native) and disulfide-bond reduced lysozyme in a He gas. The dotted lines indicate cross sections estimated for a native conformation (lower line) and an extended string (upper line), as described in the text.

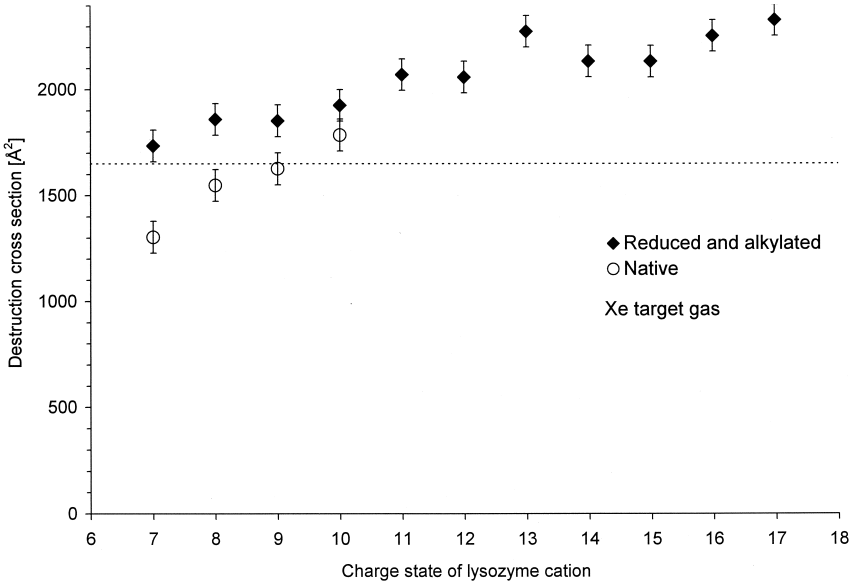

Fig. 5. Total destruction cross section in a Xe gas (as Fig. 4). The dotted line indicates the estimated cross section for the native conformation of lysozyme.

ferred to the molecular ion. It has been shown that at our collision velocities around 10^5 m/s the destruction cross section is dominated by ejection of an atom or a group of atoms due to binary elastic collisions with a target atom [11,12]. In the following we discuss a simple model that includes the essential features of this mechanism.

The disulfide-bond intact lysozyme molecule (formula: $C_{613}H_{951}N_{193}O_{185}S_{10}$) is approximated by a sphere with a radius of 20 Å, containing ∼1000 N atoms. The argument is that the heavy atoms are dominating the collisional energy transfer, and that the 1001 heavy atoms on the average have about the size of a nitrogen atom. The laboratory energy is 50z keV, corresponding to 350 and 850 keV for the charge states 7+ and 17+. Accordingly, the center-of-mass energy varies from 100 to 250 eV for collisions with He and from 3.3 to 8 keV for collisions with Xe. Let us now assume that an energy transfer of at least 5 eV to one or more of the 1000 N atoms results in destruction of the molecule. Our task is then to calculate the impact parameter corresponding to an energy transfer of 5 eV to a N atom in a collision with a He or Xe atom. Approximating the interaction potential by a screened Bohr potential [12], we find that the variation of this critical impact parameter with collision energy is from 0.69 to 0.57 Å for a He target and from 2.9 to 2.4 Å for Xe. The critical impact parameter depends only weakly on energy transfer [12]. Thus a change of the lower limit of the energy transfer from 5 to 10 eV changes the value of the critical impact parameter by only 25%.

A useful illustration of these collisions is obtained by plotting the lysozyme ion as shown in Fig. 6. Individual heavy atoms in the molecule are represented by dots and the collision partner, a gas atom, by a circle with radius equal to the impact parameter corresponding to a 5 eV energy transfer. In a collision with a gas atom, with relative velocity perpendicular to the plane of the figure, those atoms in the molecule will be ejected which are inside the circle centered at the point of impact. It is seen that the lysozyme molecule is to some extent transparent in collisions with He and typically very few atoms are ejected. In contrast, a collision with a Xe atom with any point of impact within the boundary of the lysozyme molecule will lead to ejection of many atoms.

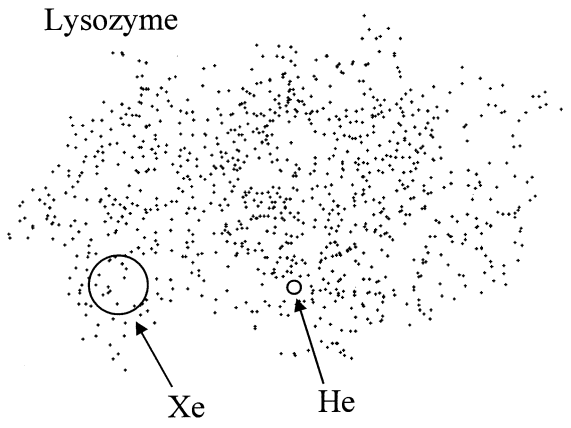

Fig. 6. Model of a lysozyme ion, based on the three-dimensional structure determined by x-ray crystallography. The dots indicate projected positions of heavy atoms. The size of the circles representing a He and a Xe atom are chosen such that atoms in the lysozyme molecule which are inside this circle will be ejected when the gas atom penetrates the molecule along a trajectory perpendicular to the plane of the figure.

As a first step, we now estimate the geometrical destruction cross section for the disulfide-bond intact lysozyme ions for the two target gases. Adding an average value of the critical impact parameter to the radius of the lysozyme sphere, we find the cross section for He as $\pi 20.6^2$ Å^2 = 1333 Å^2 and for Xe as $\pi 22.6^2$ = 1605 Å^2. These geometrical cross sections must be modified by a transparency factor accounting for the fact that for some points of impact the target atom passes through the lysozyme ion without causing fragmentation. The cross section for transfer of at least 5 eV to an atom in the lysozyme ion is $\sigma_a \simeq \pi 0.6^2 = 1.13$ Å^2 for He and $\sigma_a \simeq \pi 2.6^2 = 21$ Å^2 for Xe. The average projected density of heavy atoms in the lysozyme molecule is around $t = 0.8$ atoms/Å^2 and we estimate the transparency factor by its value for a random two-dimensional distribution, $T = e^{-t\sigma_a}$, which gives $T \simeq 0.4$ for He and a negligible value of T for Xe. Multiplying the geometrical cross section by $(1-T)$ we then arrive at cross sections of 800 Å^2 for a He target and 1605 Å^2 for a Xe target. These model cross sections are indicated by dashed lines in Figs. 4 and 5, and they are seen to agree roughly with the measured values for the lowest charge states. The extreme denatured conformation of lysozyme can be modeled by a string of pearls, with a distance between the pearls of 1–1.5 Å. In a collision with a He atom, there will then rarely be more than one lysozyme atom within the critical impact parameter for ejection, and the total destruction cross section can be estimated as a sum of atomic cross sections, $\sigma_a 1001 = 1130$ Å^2. As seen in Fig. 4, this value is not far from the cross sections measured for the highest charge states of reduced lysozyme. Due to the much larger value of the critical impact parameter for collisions with Xe atoms, the geometrical cross section in this case takes the form of the projection of a cylinder, which is about 1300 Å long and 5 Å in diameter. If we average over all orientations of this cylinder, we arrive at an average cross section of $\pi/4 \times 5 \times 1300 \simeq 5000$ Å^2. This value is much larger than the measured cross sections in Fig. 5.

Already the comparison with these simple estimates indicates that different information is contained in the measured cross sections for destruction in He and Xe gases. To extract this information we have applied the simple model illustrated in Fig. 7. The atoms in the lysozyme molecule are assumed to be randomly distributed within a sphere. Conformational changes are represented by a change in radius of the sphere, from the unrealistic limit where all atoms are at one point to the also unrealistic limit, where the distance between atoms in the molecule are several angstrom. The two inserts illustrate how the density of atoms decreases with increasing radius. The atoms in lysozyme are again indicated by dots, and the He and Xe target atoms are represented by small spheres with radius equal to the critical impact parameter, i.e. equal to 0.6 and to 2.6 Å. In a collision with a given point of impact of the target atom on the molecule, the lysozyme atoms within a small circle centered at this point will be ejected. The total destruction cross section equals the area covered by impact points for which at least one atom is ejected. The variations with the lysozyme radius of the cross sections in He and Xe are indicated by the dashed and solid curves in Fig. 7. It is seen that the sensitivity to a molecular expansion is about the same for the two cross sections at very high atomic densities, but the cross section in He saturates at densities corresponding to a lysozyme

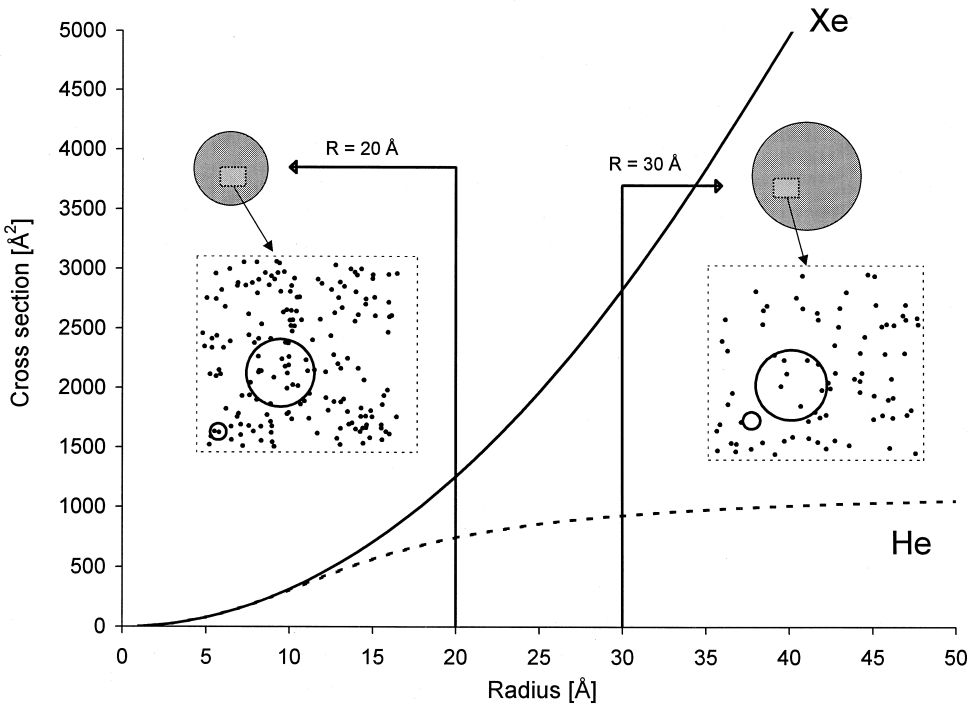

Fig. 7. Total destruction cross section of "lysozyme" as a function of the "radius" of the molecule. The variation of the radius simulates the change in atomic density in a lysozyme molecule due to a conformation change. The dots indicate randomly distributed molecular atoms with average densities corresponding to lysozyme radii of 20 and 30 Å. The inserts show circles representing the "size" of He or Xe atoms penetrating the structure. For a given point of impact, fragmentation happens if there is one ore more dots inside the circle.

radius of about 30 Å where it approaches the sum of atomic cross sections. The atomic cross section is larger by a factor of nearly 20 in Xe, and therefore the total destruction cross section saturates at a much larger value and at a larger lysozyme radius. This difference in sensitivity at low densities for measurements in He and Xe is used in the following qualitative interpretation of the results.

From a comparison between the experimental data with simple cross section estimates we can conclude the following. The conformation of the disulfide-bond intact lysozyme ions is well represented by the native compact form. For these ions the destruction cross section in collisions with He atoms is independent of the charge state whereas the destruction cross section increases with charge state for collisions with Xe. This observation indicates that the structure change of the molecule with increasing charge happens in regions with fairly high transparency in the He collisions.

For the disulfide-bond reduced lysozyme ions the destruction cross section increases with charge state in collisions with both He and Xe atoms. It should be noted that for the low charge states (from 7+ to 10+) the absolute change in cross section is nearly the same in He and Xe, indicating that in this case the structural changes take place in compact regions where the transparency is low even for He atoms. It is generally accepted [14] that the robustness towards coulomb repulsion is strongly reduced for these ions, and a gradual unfolding takes place with increasing charge state, from 7+ to 17+. The data for both He and Xe show that the conformation changes from a compact form to a more open form, and the Xe data indicate that the compact form is somewhat swollen as compared with disulfide-bond intact lysozyme ions with corresponding charge states. Again, the fact that there is no difference between the cross sections of intact and reduced lysozyme ions for low charge state ions

in He may, with reference to Fig. 7, be interpreted as evidence for a confinement of this expansion to low-density regions of the molecule. The Xe data also show that the ions with high charge states are not completely unfolded. This observation is in good agreement with molecular dynamics structure calculations by Reimann et al. [13], which show that the conformation changes as a function of charge state but also that the structure is still far from string like.

The present method of obtaining conformational information about gas phase protein ions can be compared with previously used techniques for obtaining similar information. Valentine et al. [14] used the so-called ion mobility mass spectroscopy technique, and Reimann et al. [5] utilized the technique of energetic surface imprinting. With the surface technique, structures, which have been dubbed "hillocks," are created when energetic protein ions hit a surface. The hillocks contain shape information about the objects impinging on the surface, but the resolution is as poor as 40 Å (full width at half maximum) [5]. The structure information obtained with the mobility technique is based on collisional slowing down of ions in a drift tube. The parameter determining the granularity of this method is the hard-sphere collision distance, which is around 2.5 Å in collisions between the atoms of interest in protein–He collisions [14]. The value of this collision distance corresponds in the present measurements to the critical impact parameter in Xe, and the absolute values of cross sections in low energy He collisions are similar to those for destruction in high energy collisions in Xe.

In the collision cross section measurements for multiply charged lysozyme ions Valentine et al. [14] could separate different conformations within individual charge states of lysozyme ions and were able to generate conformers with higher collision cross sections by increasing the energy used to inject ions into the drift tube (injection energy). With our method the presence of two or more conformers having different destruction cross sections will be revealed by a multiterm exponential dependence of the beam intensity on the target thickness. However, we have not attempted to resolve conformers within each individual charge state, and therefore each measured destruction cross section represents an average over the conformers present in the ion beam. For disulfide reduced lysozyme, the destruction cross sections in Xe are on average 20% lower than the corresponding collision cross sections determined by Valentine et al., but the variation with charge state is remarkably similar. The difference in absolute magnitude is not surprising since the methodologies and the definitions of cross sections are very different. Taking into account the 20% difference in magnitude, our results for disulfide-intact lysozyme in Xe are close to the larger of the cross sections measured in [14] for high injection energy, which was assigned to a partly unfolded conformer.

The strength of the new method we have introduced is that the critical atom–atom impact parameter can be changed dramatically, from about 0.6 Å for He to 2.6 Å for Xe. As explained above, this feature allows the extraction of additional information about the conformational changes taking place when protonated protein ions are brought into the gas phase. We have based our qualitative interpretation on simple model calculations, but it should be possible to derive quantitative information from a comparison of the measurements with simulations based on a more realistic molecular model of the protein.

4. Conclusion

Measurements of destruction cross sections for high-energy collisions between lysozyme ions in gases have shown that the conformation of these gas-phase protein ions depends on both the charge state and the presence of disulfide bonds. The destruction of the lysozyme ion takes place in a single collision with a gas atom as a result of semielastic binary collisions with one or several atoms in the lysozyme ion. By varying the atomic number of the target gas, we can change the granularity of the cross sectional area information. When probing the lysozyme structure with He impact, where the impact parameter with a lysozyme atom must be less than about 0.6 Å to induce ejection, we observe no conformational changes for disulfide-bond intact ions

when the charge state is varied from 7+ to 10+. When probing with Xe, on the other hand, the cross section is found to increase with charge state, indicating that some change of conformation does take place. The critical impact parameter in binary atom-atom collisions is much larger in this case, around 2.6 Å, and, in contrast to the destruction in He, the cross section is also sensitive to expansion in regions of the molecule which are not very compact. The disulfide-bond reduced ions are also highly folded for the lowest charge states, but probing with Xe indicates some swelling of the ion as a result of the breaking of the disulfide bonds. The fact that this swelling is not seen by the He probe, indicates that it is confined to fairly open regions of the molecule. The cross section increases with charge state (from 7+ to 17+) for both target gases. For high charge states He probing indicates an open structure of the ions, but probing with the "larger" Xe atoms reveals that some folding remains. This is in good accord with molecular dynamics calculations by Reimann et al. [13]. When compared with ion mobility measurements and energetic surface imprinting, it should be noted that the present technique combines the high resolution of the former with the time resolved properties of the latter. The time of passage through the target gas is less than 1 μs and the measurement gives a snapshot of the molecular size with this time resolution. Furthermore, the present technique is unique in providing structural information with variable granularity.

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