International Journal of Mass Spectrometry and Ion Processes, 111 (1991) 103–129
Elsevier Science Publishers B V , Amsterdam

Coulombic effects in the dissociation of large highly charged ions

Alan L Rockwood, Mark Busman and Richard D Smith[1]
Chemical Sciences Department, Pacific Northwest Laboratory, Richland, WA 99352 (USA)

(Received 2 April 1991)

ABSTRACT

Electrospray ionization has been shown to produce highly charged ions for classes of very large molecules These ions (at relatively low *m/z*) can be generally activated and dissociated with good efficiency The effect of coulombic interactions on the stability of large highly charged ions is discussed for a model based on evenly distributed charges for a linear polymer, i e a "charges on a string" model Both activation energy for dissociation and net thermodynamic stability are considered As a result of the long-range nature of the coulomb potential and the short-range nature of chemical bonding interactions, calculations predict a large coulombic effect on thermodynamic stability, and a somewhat smaller effect on activation energy for ion fragmentation The most important consequence of this analysis is that as the charge of an ion increases, it should become easier to dissociate Experimental support for the reduced stability of the higher charged ions is cited Another consequence of coulombic interactions is that we expect ions with high charge to give fragmentation patterns different from those of ions with low charge As charge increases, increased rates of dissociation are expected for sites towards the center of large molecules where the coulombic forces are greatest Limitations of the models and future directions for more rigorous calculations are discussed The present results are discussed within the context of preliminary experimental results

INTRODUCTION

Electrospray ionization has recently become very important in mass spectrometry, particularly for the analysis of biopolymers [1–3] One of the distinguishing characteristics of the technique is its ability to produce highly charged ions for important classes of molecules (e g proteins, oligonucleotides etc) This allows an analyzer of relatively modest mass-to-charge (*m/z*) range to be used to acquire information adequate for molecular weight determination [1–4] For example, apomyoglobin (myoglobin minus the non-covalently associated heme, M_r = 16 951) produces a series of ions with a charge distribution ranging from approximately 13+ to 27+ [4] Thus at least 15 peaks appear in the spectrum, even using an analyzer with a maximum *m/z* range of 1400 thomsons (the thomson is a recently suggested unit that is equivalent to one *m/z* unit) [5] Our laboratory has been investigating the

---

[1] Author to whom correspondence should be addressed

activation and dissociation of these large ions, and has shown that proteins at least as large as albumin may be dissociated with good efficiency, and that the most intense peaks in the dissociation spectra can be assigned on the basis of the known primary structure [1]

These developments present a great opportunity to study the chemistry of novel systems, specifically the chemistry of large highly charged ions Some of the most fundamental questions one might ask concern the effects of coulombic energy on the kinetic and thermodynamic stability of highly charged polyatomic ions A first response may be to assume that coulombic repulsion will tend to be destabilizing, but one must then wonder how strong the destabilizing effect is and whether it affects kinetic parameters (e g activation energies) to the same extent as thermodynamic parameters (e g net bond dissociation energies)

The present paper addresses these questions through model calculations By making a few simplifying assumptions about the structures of the ions and the nature of the interactions involved, one can easily perform a series of calculations relevant to ion stability Given the simplifications of the model, the calculations make no pretense at quantitative accuracy, but should be qualitatively useful in elucidating trends, and may even be semiquantitatively predictive

By framing the issues, elucidating trends, and estimating the magnitude of the expected effects, the present exploratory studies can serve as bases for discussions of coulombic effects and as a springboard to launch further theoretical and experimental studies In this spirit we discuss the general effects of superposing short-range (e g chemical bonding type) interactions with long-range (e g coulombic) interactions, make recommendations of how to perform more rigorous modeling calculations, predict experimental consequences of the theory, and briefly refer to early experimental results (to be published elsewhere) that indicate the existence of coulomb-induced stability differences for differing charge states of highly charged ions

MODEL CALCULATIONS

These calculations are based on a simplified "charge on a dissociating string" model Such a model would most accurately apply where the bond strengths between each monomer unit are equivalent in the absence of multiple charges For these exploratory studies we assume that the charges are equally spaced along a structureless string with the spacing between charges given by L/K, where L is the length of the molecule and K is the total number of charges The model specifically applies to backbone bond cleavages of a linear homopolymer, although a somewhat similar treatment could also be given to cleavages of charged side-chains The model does not treat fragmentation occurring through more complicated bond rearrangements

The dissociation of a backbone bond of an uncharged molecule is taken to be a Morse potential

$$U_{\mathrm{m}} = D_{\mathrm{e}}\{1 - \exp[-\beta(r - r_{\mathrm{e}})]\}^2 \qquad (1)$$

with a dissociation energy D_{e} of 5 20 × 10^{-19} J (3 25 eV) and a β parameter of 1 96 × $10^{10}\,\mathrm{m}^{-1}$ The bond stretch is $r - r_{\mathrm{e}}$ These parameters approximate typical covalent bonds such as C–N or C–C [6]

Assuming that the chemical part of the dissociation energy is not perturbed by electrostatic charging of the ion, the effect of charging is simply to add electrostatic repulsion terms to the Morse curve that characterizes bond dissociation In the model, the electrostatic contribution to the breaking of a particular bond is the coulomb energy given by

$$U_{\mathrm{c}} = \sum_{\substack{i\,(\mathrm{left}) \\ j\,(\mathrm{right})}} \frac{z_i z_j}{4\pi\varepsilon_0 r_{ij}} \qquad (2)$$

where z_i and z_j are the ith and jth charges, and r_{ij} is the distance between ith and jth charge with i and j limited to combinations taken from opposite sides of the dissociating bond We do not include the dielectric polarizability of the molecule in the model The total potential energy for bond dissociation is

$$U = U_{\mathrm{m}} + U_{\mathrm{c}} \qquad (3)$$

During dissociation it is assumed that the relative distances between charge sites to the left of the dissociating bond remain unaltered, and likewise for the charges on the right The two rigid portions of the molecule simply pull away from each other during dissociation

The calculations were performed using 64-bit floating point arithmetic in BASIC language on several computers running the IBM DOS (c) and MS DOS (c) operating systems

RESULTS

The basic information supplied by the calculations is the potential energy (for the dissociating bond) as a function of bond stretch Zero bond stretch is defined as the equilibrium bond distance of the uncharged molecule The ion is described by an ordered triplet notation as indicated in Fig 1 (charge left fragment, charge right fragment, "extended" molecule length in ångstroms) Our "baseline molecule" is a biopolymer of chain length 550 Å, a size comparable to myoglobin (153 amino acid residues) in an "extended" linear conformation Effects due to displacement of charge site from the polymer backbone, presumably localized on the side-groups of basic amino

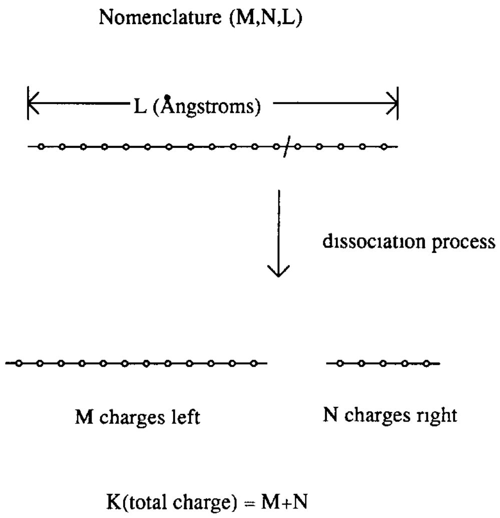

Fig 1 Notation for ordered triplets describing the dissociation of a multiply charged polymer

acid residues for positively charged (protonated) proteins, should be small and are ignored in the present treatment as are the small differences in bond dissociation energy due to the heterogeneous nature of proteins

Figure 2 presents potential energy curves for a series of charge states ranging from $K = 0$ to $K = 200$ for bond breaking in the center of the ion In this figure the energy zero is set at zero bond stretch

Figure 3 represents a 26-charge 550 Å molecule dissociating at various positions between the central and terminal positions The energy zero is taken to be at zero bond stretch The curve labeled (0, 26, 550) is identical to that of an uncharged molecule since in this case all the charge resides on one side of the dissociating bond and there are no coulomb repulsion terms operating across the dissociating bond

In Fig 4 we plot potential energy curves for the dissociation of a series of ions of increasing charge, but with the length (and therefore mass) scaled to keep m/z constant The presented calculations are for breaking the chain near the center of the ion

The plots described so far are limited to a bond stretch of about 5 Å and therefore do not adequately represent the asymptotic energy as the bond stretch approaches infinity In Fig 5 the potential energy function of a (13, 13, 550) dissociation is plotted on three scales of different length 5, 50 and 2000 Å For these plots, the energy zero point is chosen so that at a bond stretch of infinity the energy is 3 25 eV (i e the charged and uncharged systems asymptotically approach the same energy) From calculations such as those represented in Fig 2 one can calculate an energy barrier for bond dissociation The dissociation barrier is lowered by 0 31 eV for a (13, 13, 550) process and

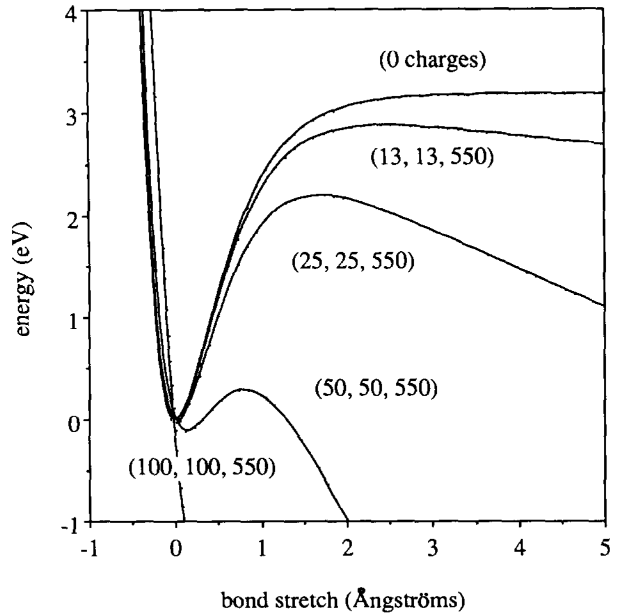

Fig 2 Potential energy curves vs bond stretch for the symmetrical dissociation of a polymer containing various numbers of equally spaced charges The curves correspond to a superposition of morse and coulombic potentials for an ion 550 Å long with between zero and 200 charges as indicated by the ordered triplet notation (Fig 1)

0 24 eV for a (10, 10, 550) process In Fig 6, the barrier lowering of a 550 Å ion is plotted as a function of charge for a dissociation into equal halves Also represented in this figure is a quadratic fit to the calculated values

As one can surmise from Fig 3, the energy barrier for breaking a bond near the end of the backbone is somewhat greater than the energy barrier for breaking a bond near the center of the ion This behavior is indicated in Fig 7 A few representative points are plotted along with the solid curve fit (a cubic spline interpolation of the calculated points is used to show the gross features of the curve that are of primary interest for this paper) Rigorously, the fit should consist of a series of stair steps with discontinuities occurring at each charge site

From curves such as those plotted in Fig 4, the scaling properties can be determined for barrier lowering for a set of ions of constant *m*/*z*, but varying *z* (or *m*) Figure 8 displays these in terms of barrier lowering vs the logarithm of the chain length A linear fit is also included in the plot

From Fig 5 we can see that a highly charged ion can become thermodynamically unstable This trend is summarized in Fig 9 as a plot of the net binding energy as a function of the number of charges for a 550 Å ion with cleavage at the center of the chain A quadratic fit to these data is given, as

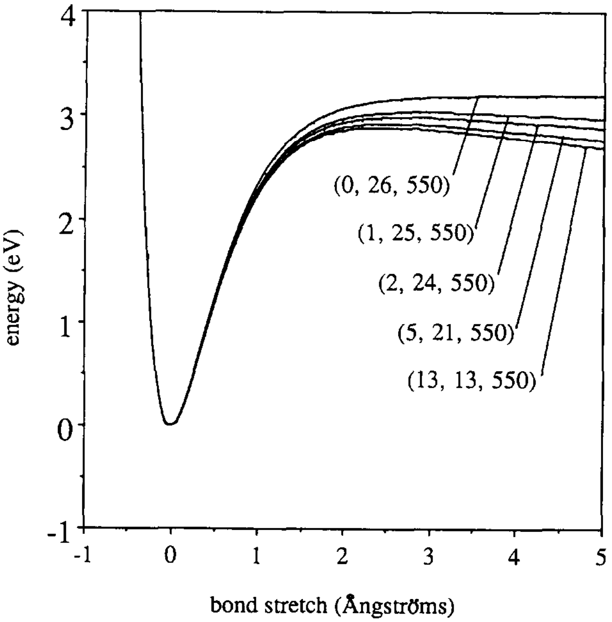

Fig 3 Potential energy curves vs bond stretch for fragmentation at various positions on the chain for an ion of length 550 Å containing 26 total charges (The curve labeled (0, 26, 550) is identical to the curve for an uncharged or singly charged ion)

well as a broken line indicating the limit of thermodynamic stability It is necessary to clarify the definitions of net bond energy and the limit of thermodynamic stability The net binding energy, also referred to as the net bond dissociation energy, is the difference between the potential energy curve at the local minimum that defines the "bound" system, and the potential energy of the fully separated fragments If the net binding energy is positive, the system is thermodynamically stable (at least if the ion is internally "cold") and will have no tendency to dissociate If the net binding energy is negative, the system is thermodynamically unstable, but may be inhibited from dissociation by an energy barrier, in which case the system is metastable A metastable system may have a long lifetime, even an infinite lifetime if it is cold enough and tunneling can be neglected Nevertheless the metastable state is thermodynamically unstable because the dissociated fragments are of lower energy than the bound system It is in the above context that we use the terms net binding energy and limit of thermodynamic stability in this paper However, consideration of "typical" levels of internal energy suggests that nearly all very large systems are energetically capable of dissociation For example, if we apply the equipartition condition to a system containing 1000 atoms, then even at room temperature the system would contain far more energy than needed to break any bond in the system Thus, all such systems would be

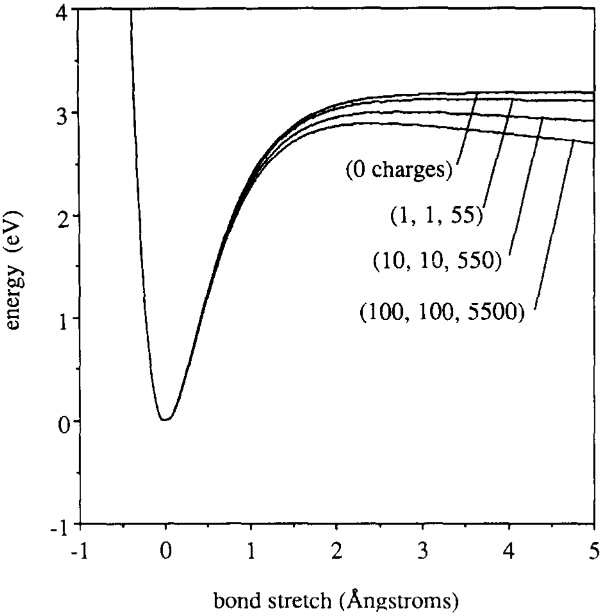

Fig 4 Potential energy curves vs bond stretch for a series of ions of increasing charge but with length scaled to maintain constant *m/z* The curve for an uncharged ion is also presented

"metastable", and the apparent stability would depend on the very small rate of fragmentation compared with the characteristic observation time of the experiment

Figure 10 also shows a plot of the thermodynamic stability, this time for cleavage of an ion of 26 charges and 550 Å length at different positions in the chain The broken line indicates the thermodynamic stability limit The solid line, a cubic spline interpolation, shows the gross trends Closely related to the thermodynamic stability is the energy released in dissociating fragments Since an ion must pass over the energy barrier to dissociate, we can reasonably approximate the energy release as the thermodynamic dissociation energy plus the barrier height, as illustrated in Fig 11

In the absence of coulombic effects, RRK theory predicts that breaking of an uncharged polymer chain is most favorable for residues near the chain ends [7] This can be thought of as basically a reduced mass effect resulting in lower vibrational frequencies for bonds towards the molecule center Using the expressions of Bunker and Wang [7], we can calculate the relative rates of chain breaking at various residue positions, as indicated in Fig 12 Here we have assumed a chain length given in terms of amino acid residues, 153 (The horizontal axis in this plot is the amino acid residue number, whereas the horizontal axes in Figs 7, 10 and 11 indicate relative positions of the charges

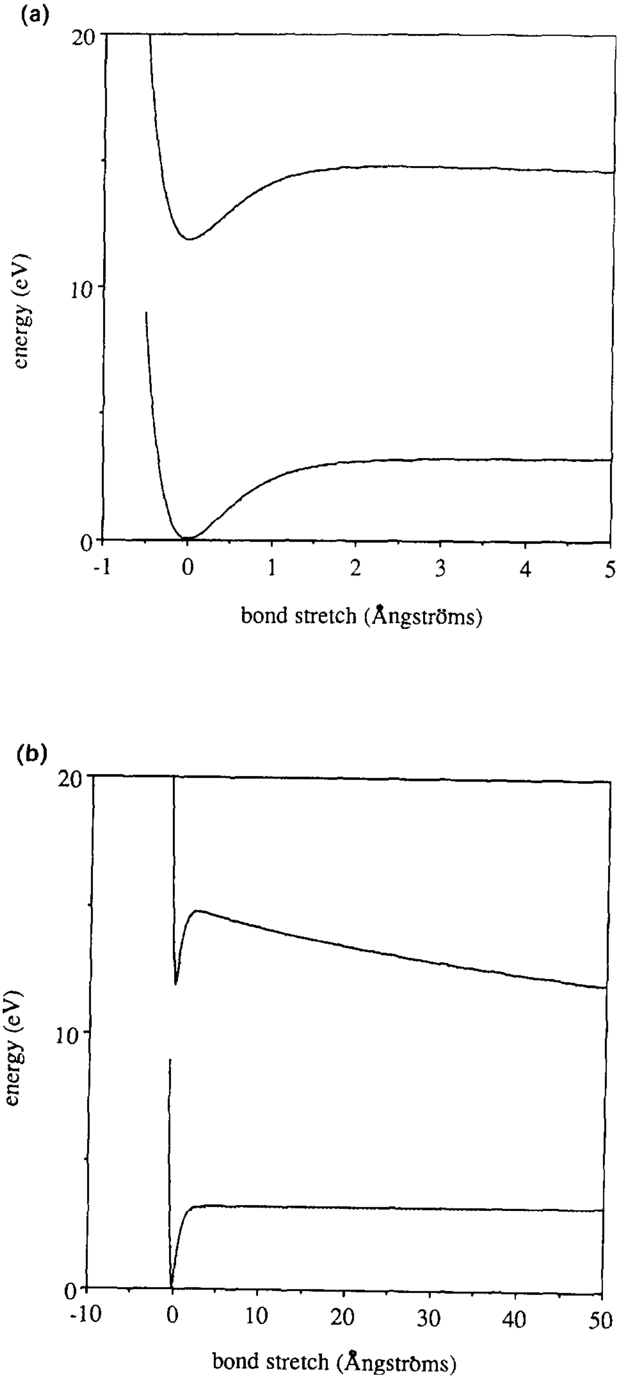
(a)
20
10
0
energy (eV)
-1
0
1
2
3
4
5
bond stretch (Ångströms)
(b)
20
10
0
energy (eV)
-10
0
10
20
30
40
50
bond stretch (Ångströms)

Fig 5

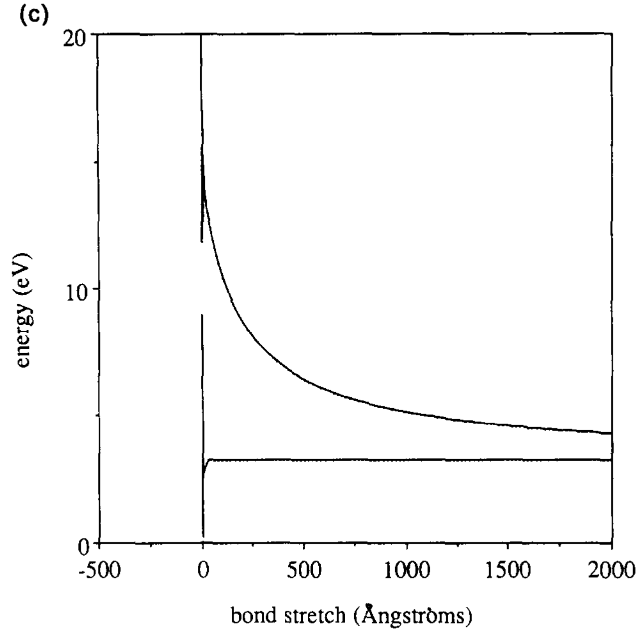

Fig 5 (continued) Potential energy curves vs bond stretch for the symmetrical cleavage of a 26-charge 550 Å ion plotted on three scales of different length (a) 5 Å, (b) 50 Å, (c) 2000 Å The lower curve is the morse potential alone The upper curve is the superposition of the morse and coulombic potentials The energy reference is chosen to give the same energy (3 25 eV) at a bond stretch of infinity This figure shows the importance of the long-range nature of the coulomb potential It also illustrates the presence of an energy barrier inhibiting the dissociation of a thermodynamically unstable ion

spaced along the polymer The horizontal axes on all these figures encompass the full length of the ions)

Coulombic energy will enhance the dissociation rates at various residues by lowering the barrier to dissociation The extent of rate enhancement depends on both the internal energy of the ion and the amount of barrier lowering Following Bunker and Wang, we parameterize the internal energy with an effective internal temperature T We do not know a priori reasonable choices for T, so we will arbitrarily choose 600 and 900 K (Although these temperatures do not necessarily correspond to any particular experimental results, they are sufficient to show the trends)

We assume a barrier lowering of ΔE will result in a dissociation rate altered by an Arrhenius factor $\exp(\Delta E/kT)$ (Here we use a sign convention that barrier lowering corresponds to positive ΔE) Enhancement factors due to coulombic dissociation rates for various charge states of a 550 Å ion are given in Fig 13 for cleavage near the chain center In Fig 14 are plotted the rate enhancement factors for a 26-charge 550 Å ion breaking at various positions

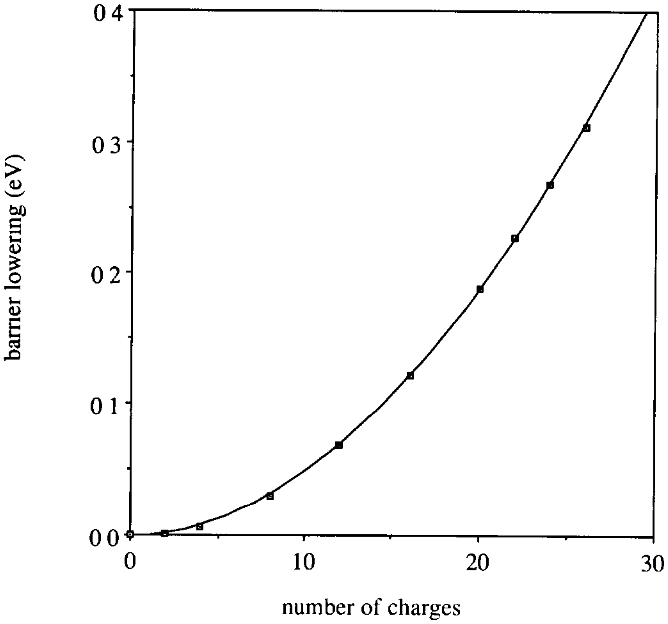

Fig 6 Barrier lowering (eV) for the symmetrical dissociation of 550 Å ions containing various numbers of equally spaced charges A quadratic fit is also included

in the chain It should be noted that in these two figures we have plotted rate enhancement factors, not absolute rates Thus the rate enhancement factors for 600 K are greater than those for 900 K, although the absolute rates of the 900 K case would be greater than for the 600 K case

When pre-exponential frequency factors are combined with coulomb rate factors, curves such as those given in Fig 15 result This figure shows cleavage rates for various chain-breaking locations for ions of small and large charge state, $K = 1$ and $K = 26$, for a chain length of 153 residues at a temperature of 900 K

DISCUSSION

Role of coulombic forces in large ion dissociations

There is little question that coulombic energy tends to destabilize a highly charged ion It influences both the thermodynamic stability and the dissociation rates of ions Our calculations indicate that this may be a significant effect for large highly charged ions such as those produced in electrospray ion sources In fact, as indicated in Fig 9, the model predicts that a "cold" myoglobin-sized ion with 26 evenly spaced charges should generally be

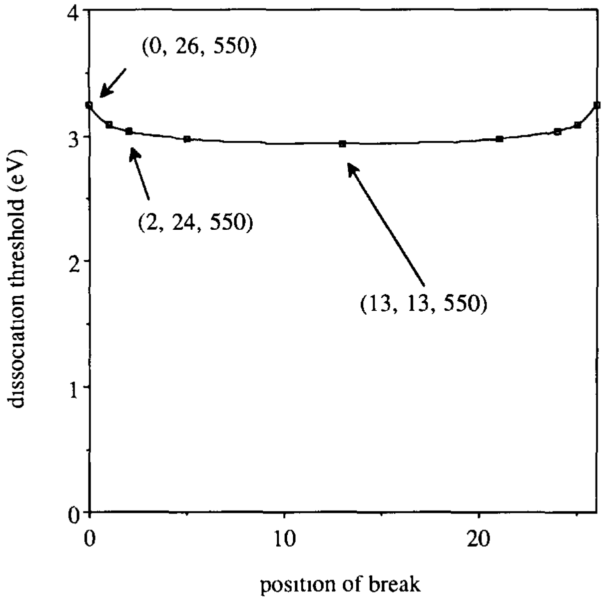

Fig 7 Total dissociation threshold energy for 26-charge 550 Å ions dissociating at a few selected positions The horizontal axis labeled "position of break" corresponds to the number of charges occurring to the left of the break (not to the total number of polymer repeating units to the left of the break) The smooth line is a cubic spline interpolation of the data showing the general trends in the data but not the detailed structure of the curve, which is actually a series of stair steps

thermodynamically unstable by about 9 eV, a substantial amount when compared with typical chemical bond energies of about 3–4 eV

Thermodynamic instability is only partially reflected in a dissociation barrier lowering The coulomb interaction, although quite strong, is of much longer range than typical "chemical bonding" interactions Over the length range that characterizes bonding interactions, the coulomb interaction is able to exert only a fraction of its total effect Therefore it contributes only modestly to lowering the dissociation barrier It is generally true that when a short-range attractive potential is combined with a long-range repulsive potential a barrier lowering will occur that will be less than the change in the net thermodynamic stability

In principle, if the coulombic contributions become large enough, it is possible to flatten the energy barrier substantially, or even to swamp it completely to produce a fully repulsive potential This behavior is illustrated by the curves labeled (50, 50, 550) and (100, 100, 550) in Fig 2 The m/z of these ions for our model protein corresponds to approximately 170 and 85 thomsons repectively It would be surprising to observe experimentally such extreme charging of large molecules by electrospray ionization For example,

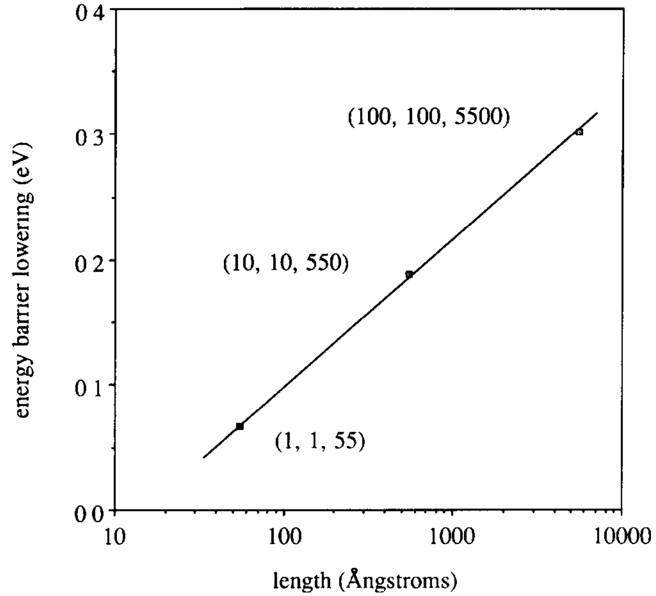

Fig 8 Energy barrier lowering vs $\log_{10}$ (chain length) for a series of polymer ions of constant *m/z* but of increasing charge Also included is a linear fit

in the electrospray ionization mass spectrometry of myoglobin, charge states only up to about 27+ are observed

If extreme barrier flattening is to be observed experimentally, it will probably be through the use of extremely large and highly "chargeable" polymer ions (However, see the discussion on hydrogen bonding and biopolymer conformation later in this paper) If both the chain length and charge are scaled up in such a manner as to keep *m/z* constant, the barrier lowering will grow approximately with the logarithm of the chain length This behavior can be concluded from our numerical calculations, as well as analytical expressions based on a continuum charge model not discussed in the present paper This functional dependence is indicated by the linear fit in Fig 8 Because of the logarithmic functional form, any amount of barrier lowering should be achievable However, truly enormous and unrealistic chain lengths would be required to suppress the dissociation barrier completely For example, extrapolating the line in Fig 8 (0 12 eV per decade) by about 24 decades would be required for flattening the barrier, making the ion too long to fit in the solar system without a considerable amount of folding

Raising the charge by electrospraying an ion that has many ionizable groups, producing a lower *m/z* ion, makes barrier flattening much more favorable From Fig 6 one concludes that the barrier lowering is approximately proportional to the square of the number of charges, so doubling the

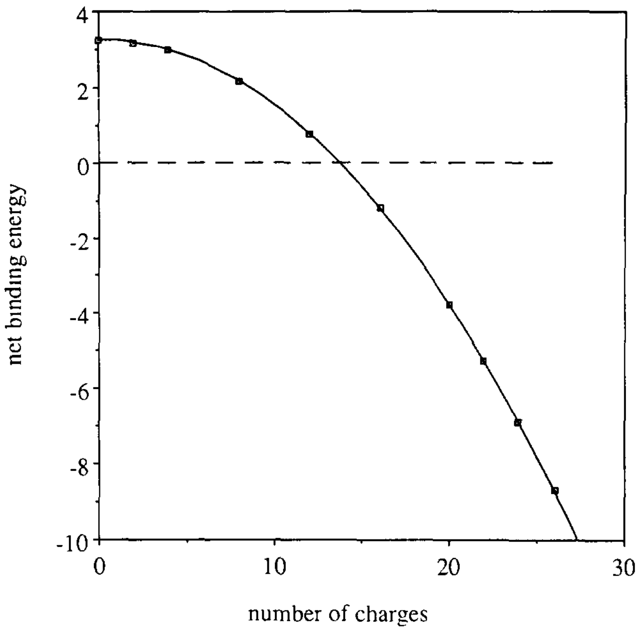

Fig 9 Net binding energy for the symmetrical dissociation of a 550 Å ion containing various numbers of charges The broken line indicates the limit of thermodynamic stability Also included is a quadratic fit to the data

number of charges should quadruple the slope of Fig 8, yielding barrier flattening in about 6 decades rather than 24 Quadrupling the number of charges should increase the slope 16 times Effects that place limitations on the maximum achievable charge, such as those described by Fenn and co-workers [2,8], might prevent the formation of such ultra highly charged ions, even for molecules containing many ionizable groups Thus, it is conceivable that ions could be produced that would spontaneously dissociate without an activation barrier We believe it is highly improbable that such species could actually be formed by the electrospray process

With the presence of thermal energy and partial barrier lowering, the dissociation becomes an activated process Therefore

$$\text{rate} \propto \exp(-D/kT) \tag{4}$$

where D is the dissociation barrier In terms of previously used symbols

$$\text{rate} \propto \exp\left(\frac{-D_e}{kT} + \frac{\Delta E}{kT}\right) \tag{5}$$

where D_e is the dissociation energy of the uncharged molecule, and ΔE is the amount of barrier lowering It should be noted that ΔE depends on the number of charges on the ion, the position of the break and the chain length

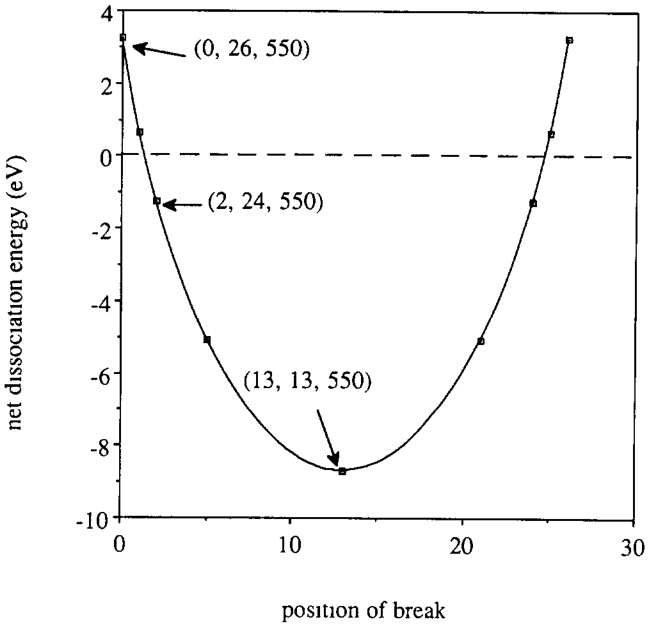

Fig 10 Net binding energy for the dissociation of selected 26-charge 550 Å ions at various positions The horizontal axis labeled "position of break" indicates the number of charges to the left of the break The broken line indicates the limit of thermodynamic stability Also included is a cubic spline interpolation of the data showing the general trends in the data

The dissociation rate also depends on a pre-exponential factor v (the simple pre-exponential frequency factor v of RRK theory) that we will assume is independent of charge and calculated from statistical mechanics as described by Bunker and Wang [7] This factor can, in turn, be split into a factor representing the dissociation rate of a terminal group and a "correction" factor that incorporates the frequency factor adjustment for breaks in the chain interior Our main interest is in relative dissociation rates, so the factors of interest are the relative RRK factor (as a function of chain breaking position) and the coulomb rate enhancement factor given by $\exp(\Delta E/kT)$ From Fig 13 we see that the coulomb rate enhancement is relatively small (with a value of about unity) for ions of low charge state, but increases dramatically beyond about 15 charges Thus it would appear that the dissociation rate for large highly charged ions may be dramatically increased by coulombic effects, but the dissociation rate for lower charge states is unlikely to be strongly affected by coulombic energy

From Fig 14 we see that coulombic effects favor dissociations near the center of the polymer backbone, but from Fig 12 we see that the RRK "reduced mass" factor favors chain breaking near the ends of the polymer

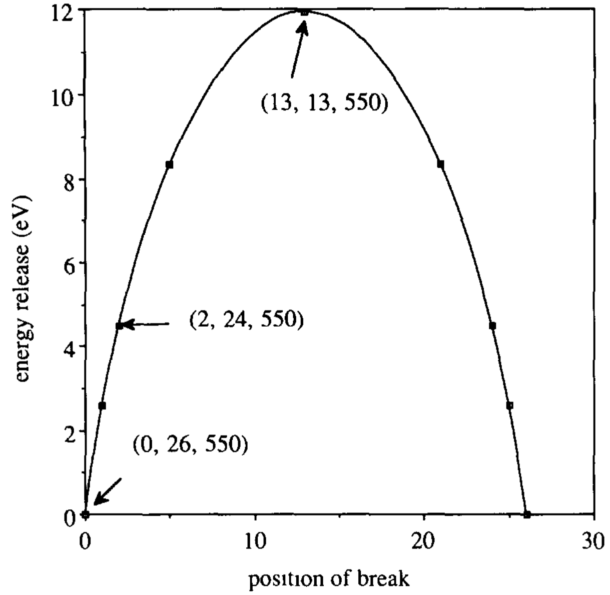

Fig 11 Energy release upon dissociation of a 26-charge 550 Å ion as a function of the position of break The axis labeled "position of break" is the number of charges to the left of the breaking bond The smooth curve is a cubic spline interpolation of selected data points indicating the general trends in the data

chain The competition between RRK and coulombic effects will favor central chain breaking for very highly charged ions and terminal chain breaking for low charged ions, as indicated in Fig 15 Thus, in the tandem mass spectrometry of electrosprayed ions, one might hope to be able to steer the preferred fragmentation pathways by selecting parent ion charge states Also, the relative importance of coulombic effects in guiding the dissociation pathways becomes less important for highly energetic ions, as indicated by the 600 K vs 900 K curves

Let us suppose we assume that once an ion passes the transition state it dissociates smoothly and without significant energy exchange between the reaction coordinate and the other degrees of freedom of the ion In this case the energy released in the dissociating fragments is approximately the difference between the energy at the transition state and the asymptotic energy of the dissociated fragments For example, Fig 11 shows that energy releases of as much as 12 eV may occur One practical result of this trend is that dissociation products of highly charged ions may be more strongly scattered than those from lightly charged ions and therefore possibly collected with poorer efficiency in tandem mass spectrometers Whether this last effect is actually

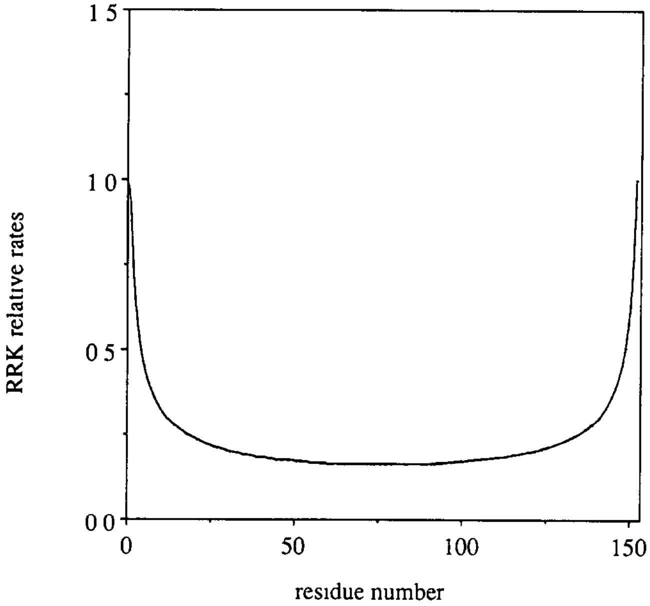

Fig. 12. Relative rates of bond cleavage at various positions of an uncharged polymer of 153 residues as predicted by RRK theory. Data are normalized to the rate of dissociation of the terminal residue.

large enough to worry about is a more complex question than can be answered here.

Comparison with preliminary experimental results

Can the effects of coulombic energy be observed experimentally? We have recently been experimenting with a technique we call "thermally induced dissociation" (TID), described in detail elsewhere [9], which may help to answer this question. Briefly, the experiments were performed in a heated metal capillary electrospray inlet somewhat similar to the one recently described by Chowdhury et al. [10]. In the previously reported studies, such capillaries have been heated in order to desolvate ions as they pass from atmospheric pressure into the mass spectrometer. Further desolvation is achieved by applying a voltage between the capillary and skimmer, so the ions undergo a series of accelerations and collisions to aid desolvation. Unfortunately in such studies, molecular ions of different charge state are collisionally excited to different extents, making it difficult to determine the coulombic contribution to dissociation. Our experiments differed chiefly in using no collisional heating (no field gradient) in the capillary/skimmer region and providing a substantially greater heating of the capillary. (Our capillaries were also shorter, about 10 cm long, but we do not yet know how important

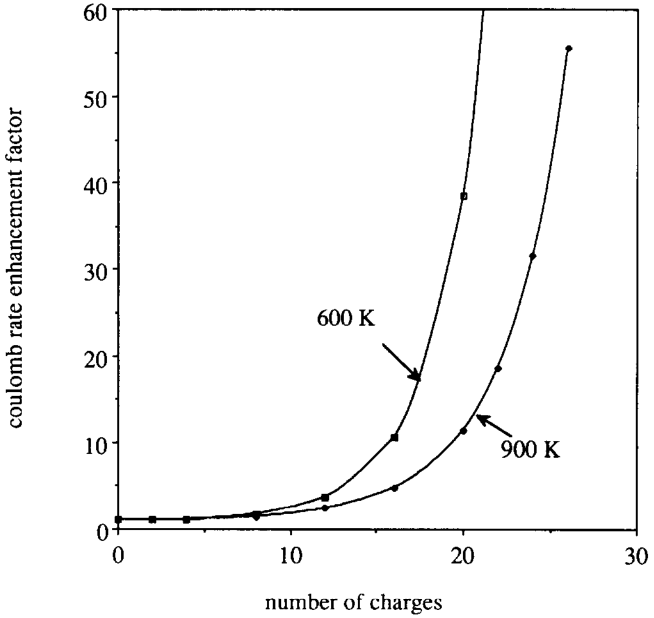

Fig 13 Effect of coulomb-induced barrier lowering on dissociation rates as a function of charge This is for a symmetrical dissociation of a 550 Å ion The data are normalized to the dissociation rate for an uncharged molecule and thus represent rate enhancement factors Two different temperatures were assumed, as indicated in the figure

capillary length is) We found that at somewhat higher temperatures than used previously, good desolvation can be attained by heat alone, without the need for collisional heating in the interface Furthermore, we found that by increasing the capillary temperature above that needed for desolvation, fragmentation could be induced by "thermal activation " (Here we define thermal activation as occurring in a high pressure region in the absence of an external electric field used for collisional activation in the capillary-skimmer region) In typical sets of experiments, involving electrospray ionization of the protein myoglobin and melittin (M_r = 2845), we found that as capillary heating was increased, the intensity of parent ions decreased, and the intensity of fragment ions increased Significantly, we observed that the more highly charged parent ions were the first to disappear

This is illustated in Fig 16, which shows the positive ion spectrum of melittin at two different levels of capillary heating At low heat, we see a typical [1,4,11] melittin spectrum, composed of charge states from 6+ through 3+ due to protonation The similarity of this spectrum to published electrospray spectra of melittin is evidence that processes involving partial discharge on the capillary wall are not very important At high heat the higher charge states have disappeared leaving only the 3+ parent ion A series of

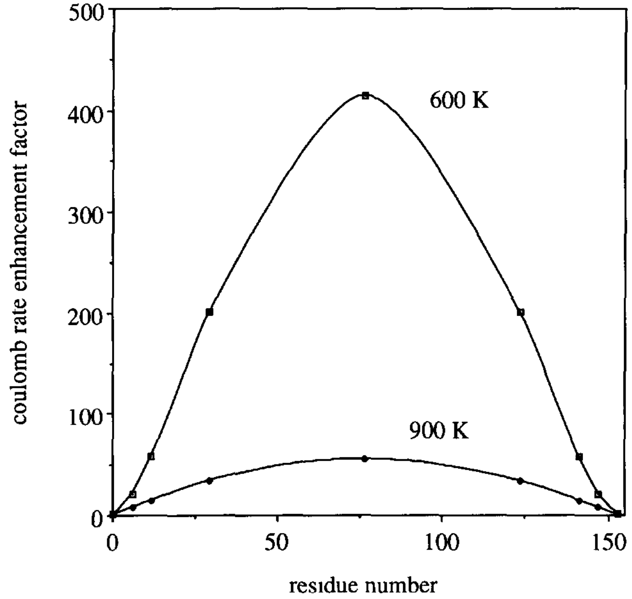

Fig 14 Coulomb-induced rate enhancement factors for the dissociation of a 26-charge 550 Å ion as a function of the position of break at 600 and 900 K For this figure the position of break is given in terms of residue number rather than the number of charges to the left of the break

known melittin fragment ion peaks [4,11] also appeared in the thermally activated spectrum We have observed that the disappearance of the charge states is progressive, i e the more highly charged ions disappear at lower heating levels than the lower charged ions The simplest explanation of these experiments is that ions absorb heat during their transit through the heated bath gas (air) in the hot capillary, become activated, and undergo dissociation This is consistent with the presence of known fragment ions in the high heat spectrum The fragments correspond to cleavages of the peptide backbone Since the more highly charged ions are more easily dissociated, the obvious conclusion is that they are less stable than the lower charged ions and that coulombic repulsion is responsible for this reduced stability

Coulombic effects may also show up in the product distributions found in collision dissociation studies We have observed that products evident in the tandem mass spectrometry spectra of the $(M + 49H)^{49+}$ ion of sheep albumin differ from the products of the $(M + 68H)^{68+}$ ion [12] This is a possible manifestation of coulombic effects, with the more highly charged ion showing an increased likelihood of dissociating near the center of the chain [12] Similar effects may show up in the tandem mass spectrometry of multiply protonated melittin When the various protonation states (3+ to 6+) of melittin are dissociated under approximately similar conditions of ion kinetic

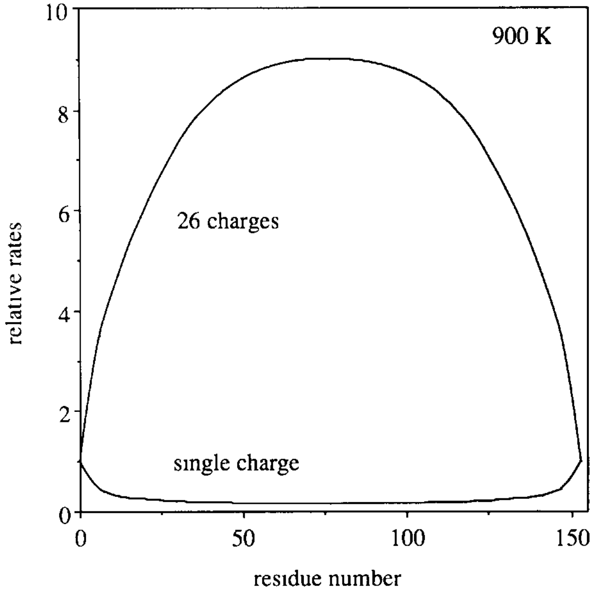

Fig 15 Relative cleavage rates vs cleavage position based on the combination of coulombic (e g Fig 14) and reduced mass (e g Fig 12) effects for a 550 Å, 153 residue ion as a function of the position of break Two curves are shown, one for a singly charged ion and one for a highly charged ion Increasing the charge produces an increasing tendency to dissociation near the center of the polymer chain

energy and collision gas thickness [11], there appears to be a progressive shift in the relative abundance of fragments Dissociation of the 3+ ion produces fragmentation mainly near the chain termini (such as a_2, b_3, y_{20}^2, y_{24}^3 etc fragments) whereas dissociation of the 6+ ion produces fragmentation mainly from the chain interior (such as y_{13}^4, y_{13}^3, y_{12}^2, y_{11}^2 etc) This is consistent with the notion that for highly charged ions coulombic energy tends to favor breaking of interior rather than terminal bonds

Another place in which one might observe effects related to barrier lowering is in the conformation of highly charged ions For solution-phase biopolymers, the tertiary structure is determined largely by relatively weak non-covalent forces such as hydrogen bonding [13] Gas phase ions can probably also show a degree of "tertiary structure " For example, the structure of protonated non-ionic surfactants in the gas phase seems to be cyclic rather than linear as a result of a strong intramolecular hydrogen bond [14] Such effects could extend to highly charged biopolymer ions For example, the electrospray mass spectra of cytochrome *c* [15] and ubiquitin [16] have been interpreted in terms of tertiary structure of the solution-phase species (and hence the charge state distribution ultimately observed for the desolvated

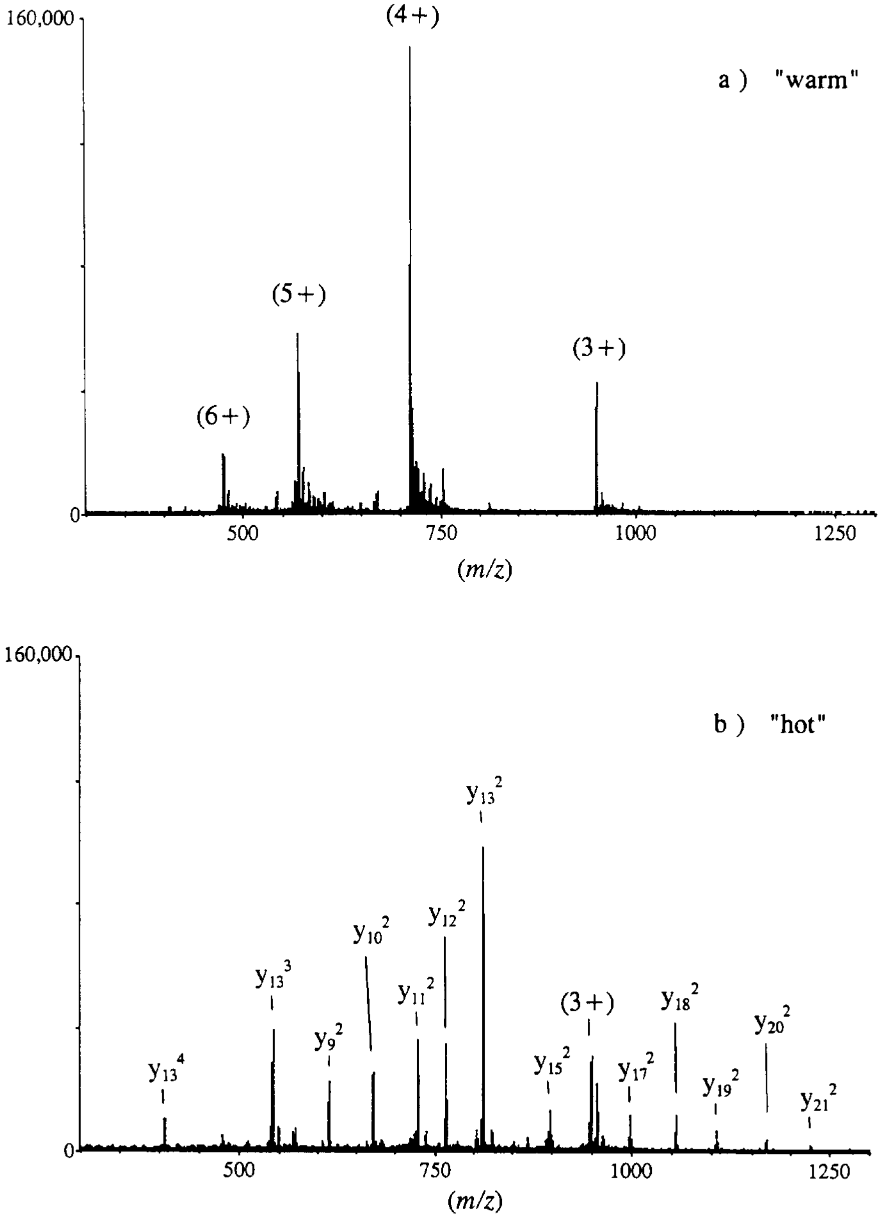

Fig 16 Thermally induced dissociation mass spectrum of electrosprayed melittin in a heated metal capillary inlet [9] (a) capillary heated with 25 W, (b) capillary heated with 49 W Labels such as (3+), (4+) etc indicate undissociated parent ions of various degrees of protonation Selected fragments are labeled using conventional nomenclature (i e y_{13}^4, y_{13}^3, y_{15}^2 etc), with the superscripts indicating the degree of protonation of the fragment

molecular ions), but it is interesting to speculate that gas-phase conformation may also be an important consideration For a highly charged ion, coulombic forces will tend to favor a linear conformation However, just as for covalent bond dissociation, the short-range (hydrogen bonding) and long-range (coulombic) forces may combine in such a way as to produce an energy barrier against the unfolding process Thus it is conceivable that a gas-phase multiply charged biopolymer may tend to remain in a metastable "globular" form on the time scale of the mass spectrometric experiment, even though the energetically preferred form may be linear In this case, coulomb-induced denaturation will be an activated process At a high enough charge state, the barrier will be completely flattened by coulomb-induced barrier lowering, and the ion will have no tendency to remain in a metastable globular form Similar considerations may apply to the dissociation of highly charged dimers that are non-covalently bound, i e held together by the same forces responsible for the tertiary structure of globular proteins Such dimers have apparently been observed experimentally [1], lending weight to the notion that non-covalent forces may be important in determining the structure of some highly charged gas phase ions, although the general observation is that non-covalently bonded protein subunits usually separate during electrospray ionization, losing their quaternary structure [17]

Highly charged gas phase ions are generally assumed to be linear [2,3,17], but there are some experimental results that can be interpreted in terms of more complex or less extended structures For example, myoglobin is usually electrosprayed from slightly acidic solutions, and the positive ion spectrum corresponds to myoglobin minus the non-covalently attached heme group (apomyoglobin) However, Smith and co-workers [18,19] and Katta and Chait [20] have observed that if myoglobin is electrosprayed from higher pH solutions, an additional series of peaks appears that corresponds to heme-attached myoglobin One consistent explanation is that gas-phase myoglobin ions electrosprayed from low pH solutions may be lost in solution or may exist in a stretched conformation that does not retain the non-covalently attached heme, but at higher pH a fraction of the gas-phase myoglobin ions may retain a folded structure that is able to retain the heme group This could relate to such coulomb effects on tertiary structure as just discussed More work needs to be done to answer questions in this area

General remarks on combining of coulombic and short-range forces

As mentioned earlier, a result of the combination of a long-range potential, such as the coulomb potential, and a short-range potential, such as chemical bonding interactions, is an energy barrier that separates reactants and products This is a rather general result and shows up in many places in the natural world For example, in nuclear physics the combination of a short-range (nuclear) interaction and long-range (coulombic) interaction dominates the dynamics of nuclear reactions [21] Thus, for example, in many nuclei, such as ^{238}U, the coulombic repulsion is strong enough to make the system thermodynamically unstable, and the only reason the nucleus does not instantly explode is that there is an energy barrier against dissociation because of the short- vs long-range nature of the interactions involved Consequently, the ^{238}U nucleus has a lifetime of thousands of centuries, and the only reason it decomposes at normal temperatures is because of tunneling of α particles through the barrier The thermionic or photoelectric emission of electrons from a charged metal is another example It is possible for a metal to be so highly charged that electron emission is thermodynamically favorable, yet rates are low in the absence of thermal or photon-induced electron emission Again, the short-range (chemical-like) interaction of the electron with the lattice combines with the long-range (coulombic) interaction of the electron with the metal's net charge imbalance, resulting in an energy barrier against electron emission If the electrostatic field at the metal surface is strong enough, it can reduce the emission threshold through the "Schottky effect" [22], a nearly exact analogy to the barrier-lowering mechanism discussed in the present paper

Similar considerations apply to the properties of ions in highly charged droplets However, in this case the situation is complicated by various "flavors" of short-range forces including liquid cohesive forces and ion solvation interactions The result is that one can engage in endless discussions over the relative importance of Rayleigh explosion vs field-assisted ion evaporation for the production of ions in thermospray and electrospray ion sources [23,24]

Model limitations and future refinements

As mentioned earlier, the calculations in this paper are based on a simplified model and are therefore important mainly for qualitative purposes, or for semi-quantitative predictions Some of the limitations of the model are now discussed, together with measures one might take to perform more rigorous calculations

The first limitation of the model is that it ignores quantum mechanics The most likely refinement of a quantum calculation would be to add a slight coupling between the "coulombic" contributions to bond dissociation and the "chemical" contributions Thus the net bond dissociation potential would depart somewhat from a simple superposition of these terms For example, bonding electrons in the presence of a large electric field may be strongly polarized, leading perhaps to field-induced bond weakening (or strengthening) This should be a weak effect for remote-site fragmentations, but could be very significant for bond rearrangements in close proximity to charge sites Many peptides (such as melittin) apparently fragment at positions quite remote from likely protonation sites, and for these cases our assumption of the simple superposition of "coulombic" and "chemical" contributions should be quite good

The second limitation of the model is that it ignores the presence of electrically polarizable groups within the ion, instead treating the molecule as an infinitely thin non-polarizable string Inclusion of dielectric polarizability into the electrostatic calculation will alter the calculated electrostatic energy This will affect both the net thermodynamic stability and the barrier lowering If we include dielectric polarization effects, the potential curves may be significantly altered The general trend will be to weaken coulombic interactions and therefore to stabilize the ion It is difficult to predict the magnitude of this effect, but we suspect that in some cases it could be as high as a few tens of percent relative change in the barrier lowering, with a slightly smaller effect on the net thermodynamic stability Including the effect of dielectric polarizability would increase the computational difficulty some orders of magnitude because one would then have to include the effects of an irregularly shaped dielectric of non-uniform polarizability, a rather difficult electrostatic problem The relative importance of the effect will decrease as the system becomes increasingly "string-like" or, in other words, as the polymer chain becomes longer

The third limitation is that the model considers only evenly spaced charges placed on the axis of the string This will affect the thermodynamic stability and the barrier lowering There are two issues involved here One is the spacing of charges along the string axis, and the other is displacement of charges off the axis If the charges are unevenly spaced on the axis, the general effect is to destabilize the ion at positions between closely spaced charges Thus, allowing uneven spacing is likely to increase the importance of coulombic effects for specific bond cleavages This can be easily incorporated into more refined versions of the model Next, allowing the charge group to be placed off the axis of the string will generally decrease the effect of coulombic repulsions, particularly if some twisting of the backbone occurs to stagger the charges However, displacing the charges off the axis affects the coulombic energy only in the second-order (and higher) terms Therefore, if the spacing along the string axis between ionizable groups is large compared with the displacement of the ionizable groups from the axis, we can ignore displacement of charge from the string axis In any case, this is an effect that could be included in the model without much difficulty

The fourth limitation concerns the quality of the model assumed for the chemical bonding interaction Here, mainly for convenience, we have used a morse potential and have parameterized it to give a dissociation energy of $3.25\,\mathrm{eV}$ ($5.2 \times 10^{-19}\,\mathrm{J}$) and a force constant of $4\,\mathrm{mdyn}\,\text{Å}^{-1}$. These are roughly what one might expect for a generic C–N bond [6], but we have not optimized these parameters for the amide linkage of the peptide backbone or the subtle variations due to differences in amino acid sequence. We have also not considered other bonding models. To a good approximation, the thermodynamic stability will be affected only by the dissociation energy, but the barrier lowering will be affected by the choice of the functional form of the model potential, the bond dissociation energy, and the force constant as well as perhaps other parameters in the potential model.

Closely related to the model potential is the effect of molecular geometry, a fifth limitation of our model. We have assumed that the molecule is string-like and that the tension on the string pulls in-line with the bond axis, corresponding to a simple bond stretch of the dissociating bond. For molecules with sp^2 and sp^3 bonding, a more realistic model would have a "zigzag" geometry. Bond dissociation then corresponds to a combination of scissor opening and bond stretching. If the scissors motion has a force constant much lower than that of the simple in-line bond stretch, then the effective force constant along the reaction coordinate will be reduced compared with that of a simple in-line bond stretch, and this will increase the coulomb-induced barrier-lowering effect. (In general, anything that makes the polymer easier to stretch makes coulomb-induced barrier lowering more important.) It has little effect on the thermodynamic stability. Except for the scissors motion, the uncertainty introduced by these geometry and bonding potential dependent factors could be as much as a few tens of percent for the barrier-lowering part of the problem, with a somewhat lower effect on thermodynamic stability. The scissors motion could alter the calculated barrier lowering by a factor of two or so. These effects would be relatively easy to include in the calculation, provided one has the improved model parameters.

Any residual higher order structure, i.e. secondary and tertiary structure in proteins, will obviously give rise to major deviations from coulombic forces calculated on the basis of a linear configuration. Although higher order structure has been related to the charge state distribution produced by electrospray ionization, it is at present unknown to what extent, if any, that higher order structure is retained in the gas phase. In particular, there is at present no evidence regarding the retention of structural differences for the conditions relevant to dissociation studies (i.e. collisional activation to levels sufficient to cause dissociation on a microsecond time scale).

Overall, the most reliable part of these calculations is thermodynamic stability, which depends almost entirely on the bond dissociation energy and dielectric screening effects. The barrier-lowering calculation depends on these factors, but also on other model parameters such as force constant, geometry and the functional form of the chemical bonding interactions Therefore, except for the scissors motion, the calculations are probably reliable to a few tens of a percent When the effect of scissors motion is included one might expect reliability to within perhaps a factor of 2 for the barrier lowering calculation

Finally, it is most difficult to provide an accurate estimate of the rate enhancement factors, which depend on all the previously mentioned parameters as well as the temperature Furthermore, the dependence is exponential Our calculations suggest that in the experimentally accessible range of molecular charging, the effect of coulomb energy could range from slight to very important, depending on the specific details of the system Therefore the present results should be considered as very informative preliminary results A more definitive assessment of the importance of coulombic effects must rely on more rigorous model calculations combined with experimental confirmation However, on the basis of our preliminary modeling calculations and experiments, we believe that at least in certain cases coulombic effects make a significant contribution to the dynamics of dissociation

Obviously, this discussion of the reliability of the model calculations is based on an underlying assumption that dissociation occurs by simple bond rupture, and not by subtle electronic or nuclear rearrangements Also excluded from consideration (as mentioned earlier) are more indirect effects in which the electronic structure of the ion is strongly perturbed by charging These and other related effects are very interesting and could be important in specific cases, but their consideration is well outside the scope of this paper Such issues might be more fruitfully addressed by semiempirical and/or ab initio quantum calculations However, it is to be generally expected that if there is a significant geometry change as the system goes through a transition state, simple coulomb repulsion effects such as those considered here, will affect the energy of the transition state

It is worth noting that coulombic effects have also been discussed by Fenn and co-workers [2,8] and by Mann [3] in a somewhat different context In that case, predictions of the degree of charging expected for a highly protonatable polymer were based on the thermodynamic energy required to place additional charges on an (already) highly charged ion (They were not concerned with fragmentation processes) When the cost of adding a proton to the ion exceeded the energy of protonation, the species was considered to be fully protonated The two approaches treat different problems and would overlap slightly only in the case where one might want to use the methods presented here to estimate the energy barrier and net reaction energy of removing a proton from a highly charged ion In that case the net reaction energy of removing the proton would also be the relevant energy one would use to estimate the maximum achievable charge

SUMMARY AND CONCLUSIONS

This paper discusses the effect of coulombic interactions on the stability of highly charged ions Both the activation energy for dissociation and the net thermodynamic stability are considered As a result of the long-range nature of the coulomb potential and the short-range nature of chemical bonding interactions, model calculations predict a large coulombic effect on the thermodynamic stability and a somewhat smaller effect on the activation energy Even a small change in the activation energy (much less than 1 eV) will produce a large change in the dissociation rate The most important consequence of this analysis is that as the charge of an ion increases, it should become easier to dissociate

For a model based on evenly distributed "charged on a string", dissociation barriers are lowered to a greater extent near the center of the string However, reduced mass considerations arising from statistical mechanics (i e RRKM theory) somewhat favor dissociation near the ends of the string, independent of the charge on the string [7] The combined result is competition between breaking near the middle of the polymer chain and breaking near the end, and we therefore expect that relative fragmentation rates will depend on the number of charges residing on the ion, even for the same molecular species Coulombic effects, similar to those discussed for covalent bond breaking, may also be important for intramolecular conformation changes and tertiary structure of gas-phase ions, and will be a subject of future experimental and theoretical studies

Finally, we have cited experimental support for the reduced stability of the higher charged ions based on thermally induced dissociation of ions Such experiments show that highly charged ions are more easily dissociated than ions with low charge Other tentative evidence exists suggesting that in the case of albumin molecular ions, dissociation of higher charge states (i e about 70) results in greater differences in fragmentation patterns than does dissociation of lower charge states (about 50) [12], and that the differences between fragmentation patterns of highly protonated (6+) melittin and less protonated (3+) melittin may correlate with coulombic effects Of particular importance will be quantitative experimental verification of shifts in relative fragmentation rates for molecular ions of different charge state

ACKNOWLEDGMENTS

Discussions with Professor Jean H Futrell are gratefully acknowledged This work was supported by the U S Department of Energy and the Molecular Science Research Center of Pacific Northwest Laboratory Pacific Northwest Laboratory is operated by Battelle Memorial Institute for the U S Department of Energy under Contract DE-ACO6-76RLO 1830

REFERENCES

1 R D Smith, J A Loo, C G Edmonds, C J Barinaga and H R Udseth, Anal Chem , 62 (1990) 882

2 J B Fenn, M Mann, C K Meng, S F Wong and C M Whitehouse, Mass Spectrom Rev , 9 (1990) 37

3 M Mann, Org Mass Spectrom , 25 (1990) 575

4 R D Smith, J A Loo, C J Barinaga, C G Edmonds and H R Udseth, J Am Soc Mass Spectrom , 1 (1990) 53

5 R G Cooks and A L Rockwood, Rapid Commun Mass Spectrom , 5 (1991) 93

6 G C Pimentel and R D Spratley, Chemical Bonding Clarified Through Quantum Mechanics, Holden-Day, San Francisco, CA, 1969, p 192

7 D L Bunker and F -M Wang, J Am Chem Soc , 99 (1977) 7457

8 S F Wong, C K Meng and J B Fenn, J Phys Chem , 92 (1988) 546

9 A L Rockwood, M Busman and R D Smith, Rapid Commun Mass Spectrom , in press

10 S K Chowdhury, V Katta and B T Chait, Rapid Commun Mass Spectrom , 4 (1990) 81

11 C J Barinaga, C G Edmonds, H R Udseth and R D Smith, Rapid Commun Mass Spectrom , 3 (1989) 160

12 J A Loo, C G Edmonds and R D Smith, Anal Chem , 63 (1991) in press

13 C R Cantor and P R Schimmel, Biophysical Chemistry Part 1 The Conformation of Biological Macromolecules, W H Freeman, San Francisco, CA, 1980, pp 110–113

14 H Y Lin, A L Rockwood and D P Ridge, Proc 38th ASMS Conf Mass Spectrometry and Allied Topics, Tucson, AZ, 1990, p 471

15 S K Chowdhury, V Katta and B T Chait, J Am Chem Soc , 112 (1990) 9012

16 J A Loo, R R Ogorzalek Loo, H R Udseth, C G Edmonds and R D Smith, Rapid Commun Mass Spectrom , 5 (1991) 101

17 J A Loo, H R Udseth and R D Smith, Anal Biochem , 176 (1989) 404

18 C G Edmonds, J A Loo, C J Barinaga, H R Udseth and R D Smith, J Chromatogr , 474 (1989) 21

19 A L Rockwood and R D Smith, unpublished results

20 V Katta and B T Chait, Proc 39th ASMS Conf Mass Spectrometry and Allied Topics, Nashville, TN, 1991, in press

21 R T Weidner and R L Sells, Elementary Modern Physics, Allyn and Bacon, Boston, MA, 1968, pp 403–405

22 R M Rose, L A Shepard and J Wulff, The Structure and Properties of Materials, Vol IV, Wiley, New York, 1966, p 41

23 V Katta, A L Rockwood and M L Vestal, Int J Mass Spectrom Ion Processes, 103 (1991) 129

24 G Schmelzeisen-Redeker, L Butfering and F W Rollgen, Int J Mass Spectrom Ion Processes, 90 (1989) 139