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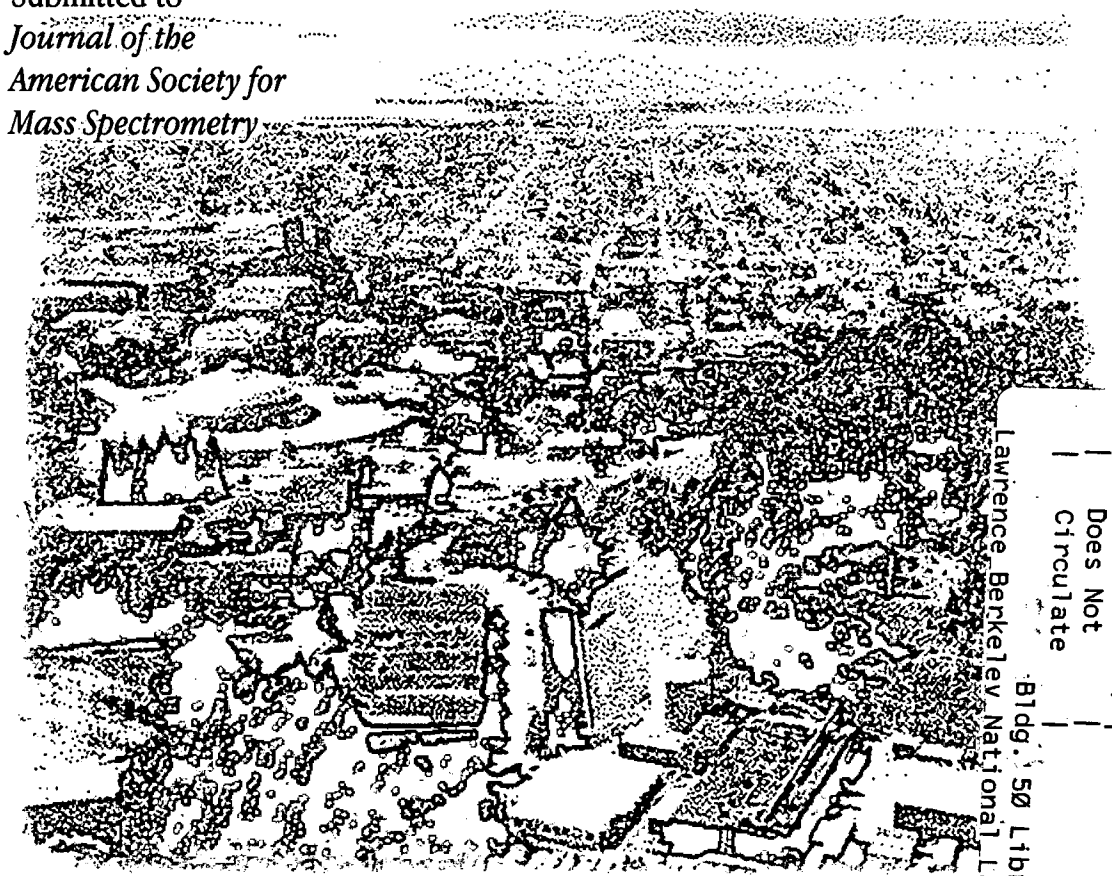

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Abstract

Charge detection mass spectrometry has been used to determine the mass of double-stranded, circular DNA and single-stranded, circular DNA in the range of 2500 to 8000 base pairs (1.5-5.0 MDa). Simultaneous measurement of the charge and velocity of an electrostatically accelerated ion allows a mass determination of the ion. Positive ion mass spectra of electrosprayed commercial DNA samples supplied in Tris - EDTA buffer, diluted in 50 vol. % acetonitrile, were obtained without cleanup of the sample.

Introduction

We recently reported the development of a novel mass spectrometer capable of determining the mass of highly charged electrospray ions generated from samples of megadalton-sized molecules.[1] Electrospray ionization can generate intact, highly-charged, gas-phase ions from megadalton-sized molecules. The mass spectrometric technique used in that work, charge detection mass spectrometry (CD-MS), quantifies the charge on an individual ion and, from a velocity measurement of each electrostatically accelerated ion, also determines its mass-to-charge ratio. Together these measurements allow a calculation of the mass for a highly-charged ion.

We demonstrated in that initial report that mass spectra for megadalton-sized DNA could be obtained. In this work we apply the same mass spectrometric technique to a series of double-stranded (ds), circular DNA and single-stranded (ss), circular DNA in the range of 2500-8000 base pairs (1.5-5.0 MDa). Obtaining several mass spectra for each sample and applying statistical analysis to the mass values, we have begun the process of evaluating the ability of CD-MS to provide a more automatable alternative to sizing of DNA by gel electrophoresis.

Molecular weight determination of megadalton-sized molecules has also been achieved by Smith and co-workers using electrospray ionization with Fourier transform ion cyclotron resonance (FTICR) mass spectrometry.[2-4] In particular they have obtained the molecular mass of a double-stranded, circular, megadalton-sized DNA sample (pGEM).[2] The technique used involves manipulating individual highly-charged electrospray ions in the FTICR cell by reaction with reagents to induce charge state shifts. A molecular mass for an ion was calculated from measurements of the mass-to-charge ratio of different charge states of that ion. An average molecular mass for the sample was obtained from measurements of several individual ions. This technique has a higher resolution than the CD-MS technique demonstrated here, although it is not

as yet as amenable to routine analysis of samples as is CD-MS. Smith and co-workers have also applied an alternative FTICR approach where a direct charge determination of very large individual DNA ions (Coliphage T4 with a molecular weight of 10^8 Da)[5] was made. This FTICR technique offers lower precision than the one Smith and co-workers applied to megadalton-sized molecules.

Experimental

Instrumentation

CD mass spectra were acquired on an instrument with an electrospray ion source and vacuum interface from Analytica of Branford (Brandford, CT). Sample solutions, at flow rates typically between 0.5 - 1.0 µl/min maintained by pressurizing the sample reservoir with helium, enter the electrospray chamber through a 0.1 µm ID stainless steel capillary tube (at ground voltage). The other elements of this interface are (with representative applied voltages for obtaining positive ion spectra in parenthesis): a cylindrical electrode around the spray needle (-1000 to -2000V), an end cap (-4100V) that directs the nitrogen drying gas over the capillary inlet, inlet and exit ends of the glass capillary that passes the ions into the first stage of the vacuum system (-4600V and 415V, respectively), the skimmer between the first and second vacuum stages (350V), the RF-only hexapole ion guide (230V bias voltage, 700V peak-to-peak RF) and hexapole exit lens (226V). The ion guide produces a narrowly confined ion beam with a small energy spread. The hexapole voltage was chosen to accelerate the ions to a velocity with a convenient flight time (typically 8-12 µs). The exit end of the glass capillary and the skimmer between the first and second vacuum stages are +185V and +120V, respectively, relative to the hexapole voltage. As described in a later section, they were optimized to provide maximum desolvation of the DNA ions. To obtain negative ion CD mass spectra, voltages of similar magnitude but opposite sign are applied, and SF_6 is added to the drying gas to prevent discharges. Nitrogen drying gas, used to enhance electrospray droplet desolvation, flows at 1.8 L/min through a heater typically set at 300C. In negative ion mode, SF_6 at a flow of roughly 10 cc/min is added to the nitrogen drying gas to suppress corona discharge. A resistive wire heater, wound on the outside of the middle section of the capillary, was added in-house. Under typical operating conditions, we estimate that a section of the inside of the capillary reaches 180C with the heater on. To obtain positive ion CD mass spectra, the end of the stainless steel spray needle is positioned approximately 2.2 cm from the entrance to the capillary and pointed slightly off to the side so that the edge of the spray is sampled.

The first and second pumping stages are at 1 torr and 80 mtorr, respectively. The ion guide extends through the second pumping stage and into the third pumping stage, which is at 0.3 mtorr. The inlet to the fourth vacuum stage, a 3.0 mm hole in a 1.0 mm thick plate, is 15 mm from the hexapole exit lens. The fourth vacuum stage contains the analyzer and is at 2×10^{-8} torr. The details of the charge detector analyzer are given elsewhere.[1] Briefly, the charge detector consists of a thin-walled brass tube 3.61 cm in length with a 6.35 mm bore. The bore of the tube is aligned with the ion beam axis. The entrance to the tube, which is 75 mm from the entrance to the fourth pumping stage, is restricted by a 2 mm diameter hole, so that only near-axis ions are allowed to enter. The signal induced on the tube is amplified by a low-noise, charge-sensitive preamplifier and then shaped and differentiated by a second amplifier. The resulting output for each ion is a double pulse signal, where the leading positive amplitude pulse corresponds to the charge impressed on the cylinder by a positive ion as it enters the tube and the trailing negative amplitude pulse results as the ion exits. The time between the two pulses corresponds approximately to the flight time required for the ion to traverse the length of the tube. The peak-to-peak amplitude is proportional to the charge on the ion.

A LeCroy 9350 digital oscilloscope records the entire waveform for each ion passing through the detector tube and also calculates the time between the leading and trailing pulses and the amplitudes of the two pulses. The data are transferred to a desk-top computer where they are used to compute the charge and mass of each ion. The present signal processing and data transfer rates allow the acquisition of up to 5 events per second. This determines the rate of spectra acquisition, since ion count rates for these samples are typically higher than 5 per second. Mass spectra can be obtained for single component samples in about 10 minutes.

Some experiments were carried out under nanoelectrospray conditions.[6] The gold coated silica tips (from The Protein Analysis Company, Odense, Denmark) were mounted on an in-house constructed manipulator, with electrical contact between the tip and the manipulator made with silver conductive paint. The end of the tip was positioned ~1 mm from the capillary inlet, inside the end cap that normally directs the drying gas to the front of the capillary inlet. No nitrogen drying gas was used in these experiments, since it enhanced evaporation of the sample directly from the tip. However, in the case of negative ion experiments, roughly 10 cc/min SF_6 was flowed through the drying gas heater set at 150C to suppress corona discharge. Typical interface voltages for negative ion production were: emitter tip (-700 V), end cap (0 V), and capillary end (500 V). The cylindrical electrode was removed from the interface when operating under nanospray conditions.

Mass Calculations and Instrument Calibration

The ion's total kinetic energy ($1/2\ m\ v_m^2$, where v_m is the measured velocity in the tube detector) in our system is the sum of the initial kinetic energy imparted to it by the free jet expansion of the gas as it leaves the capillary plus the kinetic energy due to its subsequent acceleration in the electric field. By equating the electrostatic potential energy defined by the bias voltage on the ion guide (since the detector tube is at ground) to the kinetic energy imparted to the ion by this electric field and solving for the mass we obtain:

$$m = 2\ q\ V / (v_m^2 - v_g^2)$$

where m is the mass of the ion, q is the ion charge, V is the bias voltage on the ion guide, and v_g the velocity due to the gas expansion.

There are several aspects to calibrating the instrument. A test capacitor in the tube detector's circuitry allows a known amount of charge to be pulsed onto the tube for the purpose of calibrating the tube detector's amplifier. Test pulses are created with a shaping pulse generator to mimic the ion signals in terms of rise and fall time. A linear fit to the test data yields a relationship between the peak-to-peak amplitude of the double pulse signal recorded by the oscilloscope and the charge on the ion. An ion with 450 charges generates an approximate 1V peak-to-peak pulse.

To determine what exact physical length of the detector tube should be associated with the time between the two peaks in the pulse, so as to calculate the ion's velocity, a dual tube detector was installed temporarily into the system. The time between entering the first detector and entering the second detector was compared to that between entering and exiting the first detector. The first event should be directly proportional to the physical distance between the two tube entrances, since the full ionic charge would be impressed at the same position relative to the edge of the tubes, and the second event should be proportional to the apparent length of the tube to be used in the velocity calculations. By comparing the time ratios and the physical length ratios, it was determined that the ion velocities should be calculated from the peak-to-peak separation and an apparent length of the tube 1.7% longer than its physical length.

The ion velocity due to the gas expansion was estimated by grounding all electrostatic elements in the interface and timing the passage of ions through the detector. Under these conditions, only residue particles make their way to the detector, the molecular ion beam apparently becoming greatly defocused without the operation of the ion guide. Residue particles have a higher mass but lower charge than typical molecular ions; presumably they are formed when a droplet dries to a solid residue. A velocity of 552 m/sec was measured for the residue particle beam created using a standard pBR322 sample. For ions with m/z ~3600 (typical for ds DNA used in these experiments) and a hexapole bias voltage of 230V, v_g^2 is 2.6% of v_m^2, and therefore a small correction to the calculated mass.

The threshold limit of charge detection is set by the trigger level of the oscilloscope, and is typically equivalent to a charge of 330 electrons in these experiments. Detecting ions with a significantly lower charge is difficult with the present configuration because the system exhibits RMS noise which is equivalent to 50 electrons. This threshold of charge detection also results in a lower limit to mass detection. For an m/z of ~ 3600, a threshold limit of 330 electrons corresponds to a threshold limit on the mass of ~1.2 MDa.

DNA Samples

All DNA samples were bought commercially: ΦX174 and M13mp18 samples from New England BioLabs, M13mp8 samples from Sigma, pUC18 samples from Pharmacia and pBR322 samples from Boehringer Mannheim. They are supplied in 10 mM Tris-HCl and 1 mM EDTA (pH 8) (TE buffer). A 1 mM EDTA standard solution is expected to include ~ 3 mM Na^+.[7] A standard electrospray sample was made by diluting the DNA to 0.0125 μg/μl in 1:1 water:acetonitrile. For experiments carried out with the nanospray inlet option, the DNA was diluted in 1:3 water:acetonitrile. Acetonitrile presumably enhances the electrospray process by reducing liquid surface tension and possibly enhancing solvent evaporation. The Tris-EDTA concentration was also adjusted to 0.5 mM Tris-HCl and 0.05 mM EDTA, except in the case of double-stranded, circular M13mp18 where the buffer concentration was already higher after diluting the DNA concentration to the standard value.

For some experiments we wanted to remove the buffers supplied with the DNA and replace them with ammonium acetate (NH_4OAc). This was done with the pBR322 sample by ultrafiltration using an Amicon 100 Micron filter. Four sequential washings were done using the same filter, following Amicon's procedure for DNA. 50 μl pBR322 (250 ng/μl) was washed three times with 400 μl 20 mM NH_4OAc, then once with 400 μl water. The DNA solution recovered was diluted with water to a total volume of 50 μl, containing an estimated 0.2-0.4 mM NH_4OAc. Sample recovery was 100%, determined using UV absorbance at 260 nm.

Results and Discussion

Positive ion mass and charge data

A representative positive ion CD mass spectrum of ds, circular pBR322, diluted in our standard manner, is depicted in Figure 1(a). This histogram plot of masses represents the measurement of about 3000 individual ions sampled over 10 minutes. The resolution demonstrated in this spectrum is $m/\Delta m = 6$ (Δm = FWHM of the mass peak). The low resolution of CD-MS in the present configuration is largely due to noise in the determination of the charge. The CD-MS technique can be compared to gel electrophoresis (resolution ~30) which takes 3 to 5 hours to run and requires

calibration standards. The matching CD-MS scatter plot of charge vs. mass for this data set is shown in Figure 1(b), and the matching m/z histogram plot for this data set is shown in Figure 1(c). The m/z data is generated by dividing the mass (in Da) calculated for each individual ion sampled by the number of unit charges calculated for that ion. The maximum in the histogram of the masses from one data set (assigned by graphing with a somewhat finer bin size than shown in Figure 1(a)) is assigned to the measured mass for the DNA sample.

The spectrum presented for pBR322 is fairly typical of those observed for the ds, circular DNA samples, although signal below the mass of the unfragmented DNA can often be somewhat more intense. In most cases no distinct peak that could be assigned to the ss fragment, at half the mass of the intact ds DNA, was observed in spectra of ds DNA samples. The spectra of the ss, circular DNA are usually more complex than the spectra of the ds, circular DNA, with significant signal below and sometimes above the mass of the intact DNA. As an example, the mass spectrum of ss, circular M13mp8 is shown in Figure 2(a). Peaks assigned to the unfragmented ss M13mp8 and the dimer of ss M13mp8 are present in Figure 2(a) We assigned the peak at 4.5 MDa to a dimer of ss M13mp8, rather than to ds M13mp8 since agarose gel analysis of commercial ss DNA samples done early on in this study showed no presence of the related ds DNA. The band between these two peaks is presumably due to clusters of two DNA pieces, at least one being a fragment of ss M13mp8. The band below the peak assigned to ss M13mp8 is presumably due to fragmented DNA. The corresponding scatter plot of charge vs. mass is shown in Figure 2(b). The m/z histogram plot for only those data points whose mass is consistent with unfragmented DNA (between 2.0 and 2.8 MDa) from this data set is shown in Figure 2(c). The positive ion mass values obtained from a series of ds, circular and ss, circular DNA samples are collected in Table 1. In cases where the mass spectrum is not dominated by one peak (for example ss DNA), the expected DNA mass is used to identify the mass peak associated with unfragmented DNA and a mass value is assigned to that peak. Each value is the average of several determinations, including values obtained at least a month apart from each other. The scatter in the measurements is reflected in the standard deviation listed. Also listed in Table 1 are the calculated bare DNA masses, computed using 615.9 Da/base pair for ds DNA and 307.9 Da/base for ss DNA and the number of base pairs or bases quoted in the supplier's catalogue for each DNA sample. In solution the phosphate groups on the sugar backbone will be deprotonated, resulting in a negatively charged DNA molecule. The masses of the individual nucleotides, without counterions, are: T 303.2 Da, A 312.2 Da, C 288.2 Da, and G 328.2 Da. For the complimentary base pairs the masses are GC 616.4 Da and AT 615.4 Da. Thus for the double stranded DNA samples, regardless of the sequence, the average value of 615.9 Da per base pair is a good approximation to use in calculating the mass of the negatively charged DNA molecule without any counterions, the "bare" mass. For single stranded DNA samples the average value of 307.9 Da/base is a good approximation if the base composition is roughly evenly distributed among the four bases. The last column in Table 1 lists the percentage difference between the CD-MS measured mass and the calculated bare DNA mass.
In Figure 3 the mass values listed in Table 1 for the DNA positive ions measured by CD mass spectrometry are plotted against the calculated bare DNA masses. The CD-MS determined masses are close to or higher than the respective bare DNA masses.

In Figure 1(b) the charge vs. mass distribution for unfragmented, ds, pBR322 electrospray ions forms an oval of densely spaced data points centered at a charge of 750 and positioned about the line characterized by m/z = 3650, as indicated by the m/z histogram plot in Figure 1(c). Such a single mode charge distribution is typical of that manifested by positive electrospray ions of ds, circular DNA. Under our experimental conditions, the peak in the m/z distribution for unfragmented, positive, electrospray ions from a ds, circular, megadalton-sized DNA sample was in the range of 3200 to 4100. Note that Figures 2(b) and 2(c) indicate that there appear to be two different charge states for ions with a mass corresponding to unfragmented, ss, circular M13mp8, one centered at 940 (m/z = 2450) and the other at 660 (m/z = 3500). We have observed both single and bimodal charge distributions have been observed for positive electrospray ions from ss,

circular DNA. Under our experimental conditions, maxima in the m/z distribution for unfragmented, positive, electrospray ions from a ss, circular, megadalton-sized DNA sample were in the range of 2200 to 3600. If a single mode charge distribution was manifested, it was usually centered at the lower range of m/z values (2500-2800).

Evaluating the CD-MS mass determinations

In order to obtain the molecular mass of the sample, it is necessary to remove efficiently any solvent molecules attached to the electrospray ions. Methods others have used to achieve this include a strong countercurrent flow of heated gas for warming ions before they enter the vacuum of the mass spectrometer,[8,9] a heated capillary extending between the atmospheric pressure electrospray chamber and the first differentially pumped stage,[10] and collision-induced desolvation in the differentially pumped interface regions.[9-11] Our optimal experimental conditions included a heated capillary, a weak counterflow (1.8 L/min) of heated nitrogen gas and collision-induced desolvation.

The heated capillary, first introduced by Chait,[10] not only lowers the measured mass but improves the reproducibility of the measured CD-MS mass. Experiments were performed with ds, supercoiled pBR322, our benchmark sample, comparing the mass obtained with the heater on with the mass obtained with the heater off. A lower mass value was always obtained with the heater on. Sometimes only a small difference, less than 0.05 MDa, was observed. At other times, the difference was as large as 0.4 Mda. This is consistent with the capillary heater helping to remove solvent/neutral molecules from the DNA ions. (As a point of reference, two water molecules per base pair adds 0.16 Mda, or 5.8%, to the bare weight of pBR322). With the heater off, the degree of solvation appears to be quite variable. However, even with the heater on, some samples display more variation in the measured mass than others. For three of the samples (ss M13mp8, ss M13mp18, and ϕX174 RF II) the standard deviation of the mass is 0.02 MDa. Three of the four samples which display the largest standard deviation (pUC18, pBR322 and ϕX174 RF I) are the smallest ds, supercoiled molecules. Perhaps the desolvation process is more variable because these are tightly configured. M13mp18 RF II, the sample which displays the largest standard deviation, does not follow this pattern.

In the absence of a heated capillary, others [8,9] have found it necessary to use a strong countercurrent flow (6-9 L/min) of a heated gas to achieve desolvation. In agreement with Chait and co-workers [11] experience with relatively small molecules, we have found that a strong countercurrent flow of heated gas was not necessary in combination with a heated capillary to optimize desolvation for these large DNA molecules.

By the application of electric fields in the differentially pumped interface regions, ions can be collisionally "heated." This process has been used for both desolvation [9-11] of electrospray ions and, at higher voltages, for collision-induced dissociation.[9,11,12] Using our benchmark sample, ds, supercoiled pBR322, and with the bias voltage on the ion guide set at 230V, the voltages on the exit end of the glass capillary (L1) and on the skimmer between the first and second vacuum stages (L2) were adjusted in order to minimize the CD-MS measured mass (maximizing desolvation) while avoiding the appearance of fragment ions in the spectrum. Increasing either L1 or L2 independently while maintaining L2≤L1, sometimes decreased the measured mass indicating that collisional "heating" can occur in both of the first two differentially pumped regions. After the optimal voltages where determined (L1=415V, L2=350V), spectra of pBR322 were taken with both L1 and L2 changed by the same proportion. Increasing both voltages to 130% of the optimal voltage did not cause a change in the measured mass value of pBR322 (within the standard deviation observed for this sample), but did result in a decrease in unfragmented ion signal and an

increase, compared to the unfragmented ion signal, in signal in the 1-2 MDa region from fragment ions. This is consistent with efficient desolvation occurring at and above the optimal voltages, and with increasing collision-induced dissociation occurring at higher voltages. The maximum in the charge distribution also shifts to lower charge values with higher voltages, consistent with the more highly charged ions experiencing more energetic collisions and these ions fragmenting or being knocked out of the beam. Decreasing both voltages to 80% of the optimal voltages increased the mass by ~0.4 MDa, consistent with less efficient desolvation at lower voltages.

To further evaluate the CD-MS measured mass values, the mass of counterions must be considered. A positively-charged DNA molecule is formed by neutralizing the phosphate backbone and then adding additional cautions equal to the measured ionic charge. Using an m/z value of 3660 (the average value obtained from many pBR322 spectra) to predict the number of counterions, a 0.4% high mass value would be expected if all the counterions were H+. The DNA phosphate backbone is known to have a high affinity for sodium ions and our standard electrospray sample is expected to contain 0.15 mM Na^+. A 8.1% high mass value would be expected if the positive DNA ion were composed of all Na+ counterions. (Note that a 7.5% high value would be expected for DNA neutralized by Na+, with no net positive charges, so the exact m/z value used to estimate the mass difference has only a small effect.) The observed range of percentage differences between the CD measured mass and the bare DNA mass (+0.4% to +8.2%) are nearly bracketed by the +0.4% and +8.1% values for all protons or all Na+, respectively. Furthermore, we have experimental evidence that sodium may in fact be playing a role in positive ion formation for the DNA samples in TE buffer. Experiments with pBR322, replacing the TE buffer with ~0.2-0.4 mM NH_4OAc by ultrafiltration then adding 25 mM piperidine, yielded a positive ion mass of 2.68±0.03 MDa (n=3). This is to be compared to the calculated protonated mass of 2.70 MDa for pBR322. Piperidine has been shown to suppress sodium adduction.[13] The fact that the CD mass obtained in the absence of Na+ is very close to the calculated protonated mass provides an indication of the accuracy of the instrumental calibration (at least in the 3 MDa region, for an ion with m/z ~3600).

The difference between the CD-MS determined DNA ion mass and the bare mass is larger in both absolute value and percentage at the low masses than at the high masses. The presence of sodium ions would be expected to increase all the masses above the 0.4% high value by the same percentage. The presence of trapped solvents might be expected to scale with the molecular volume of these molecules. With the present, low-resolution instrument, only peak position information is available since the peak width and shape remained relatively constant with changes in experimental conditions and sample composition. The next generation instrument[14] will provide much higher resolution spectra due to its repetitious measurements of each detected ion. We expect that these higher resolution spectra will provide a much more detailed picture of the complex ion chemistry. Applying sample cleanup techniques and then obtaining higher resolution spectra should allow us to better assign masses for ions of similar character (i.e. fully protonated and desolvated).

Evaluating charge state distributions

While we have focused on the molecular ion mass as a means to characterize the DNA sample, the charge state may also provide qualitative information. To compare charge states of molecules with different masses, we compare m/z values. Solution-phase conformation has been shown to influence the charge-state distributions of biomolecular ions produced by electrospray ionization.[15-19]

A higher-charge-state distribution has been associated with a more extended molecular conformation. Several explanations have been offered for these observations: (1) a more open conformation allows "buried" sites to be more readily protonated [15,16,19]; (2) a more extended molecule can encompass more charges on the surface of a charged droplet and thus can desorb with a higher number of charges

[20]; and (3) Coulomb repulsion between charges in one molecule contributes to limiting the maximum charge state observed, so that a more extended molecule allows for more charges because they can reside further apart.[21] Regardless of the mechanism that determines the maximum charge state observed in the electrospray process, it might be expected that differences in DNA topology could be reflected in the m/z values exhibited. Nicked ϕX174 has a looser conformation than supercoiled ϕ X174,[22] and might be expected to display a higher charge state. However, under our present experimental conditions, these two DNA samples can not be distinguished by their positive ion charge distributions. Either the topological differences are not large enough to be reflected in the charge state distributions, or under our conditions most supercoiled samples are nicked during the electrospray or vacuum transfer process. Single-stranded circular DNA on average has a higher charge state (lower m/z) than ds, circular DNA, which would be expected from a more open, less dense molecule. However, it can display a bimodal distribution with one charge distribution typical of a ds, circular DNA and one of a higher charge state. One explanation to consider for this observation is that during the electrospray droplet formation process a bond is broken in some of the ss DNA. In this case the droplets would contain two conformations of DNA (ss, circular and ss, linear) which would be expected to have different charge states, with the lower charge state being assigned to the ss, circular DNA. Preliminary experiments with ds, linear DNA indicate that it fragments easily under the experimental conditions used here, but under softer conditions a mass spectrum can be obtained of unfragmented electrospray ions in a charge state (m/z ~ 2000) higher than that displayed by the ds, circular DNA. It is unlikely, therefore, that a significant amount of ss, linear DNA, which would be expected to be even more fragile than ds, linear DNA, could survive the electrospray interface conditions without fragmenting further. We are left with assuming that the ss, circular DNA remains intact but that either two different conformations or two different charging mechanisms give rise to the bimodal charge distribution.

Negative ion data

Negative ion CD mass spectra of our benchmark sample, ds, supercoiled pBR322, have been obtained. The spray conditions under negative ion mode using the standard pressurized electrospray inlet were much more difficult to reproduce and maintain than those under positive ion mode. The mass values obtained are similar, although only two spectra were obtained under instrumental conditions comparable to those used for the positive ion spectra tabulated here, yielding mass peaks at 2.78 and 2.71 MDa. The m/z values are noticeably lower (2500 and 2900) and thus the charge states higher than those obtained in positive ion mode. Several negative ion spectra have been obtained using the nanospray inlet option. A mass spectrum and charge vs. mass scatter plot for pBR322 obtained using negative ion nanospray are shown in Figures 4(a) and 4(b), respectively. Figure 4(c) presents the histogram of m/z values for ions in this data set assigned to unfragmented pBR322 (2.6-3.6 MDa). Negative ion nanospray of pBR322 often yielded two populations of unfragmented DNA ions, as shown in Figures 4(b) and 4(c), one centered at m/z ~ 3100-3300 and the other with m/z ~ 1200-1500. (While few positive ion nanospray spectra of pBR322 have been obtained, those that have do not look qualitatively different than positive ion spectra obtained using the standard pressurized electrospray inlet.) The mass peak derived only from the higher charged population (lower m/z) is very narrow (FWHM ~0.15 MDa), consistent with the higher instrumental resolution expected as the value of z increases (and the ratio of the signal to noise increases). The more highly charged ion population has a lower mass (2.78 MDa) than the lower charged ion population (3.05 MDa). The two ion populations could reflect two different modes of formation for negative nanospray pBR322 ions. The more highly charged population is either formed with fewer adducts (most likely neutral adducts since the mass difference between the two populations is so large) or more easily loses these adducts in the interface before detection. The latter appears to be the case since when the capillary heater is turned down, the mass of the higher charged ions increases by 0.4 MDa whereas the mass of the lower charged ions increases by only 0.1 MDa.

Instrumental improvements

The next generation of this instrument was recently described.[14] By trapping an ion between sets of plates at the entrance and exit of the charge detection tube, the same ion can be remeasured hundreds of times, greatly improving the signal-to-noise of the charge measurement and thus improving the mass resolution. The anticipated instrumental resolution will be 10 to 20 times better than that of the single pass instrument. We expect this will greatly enhance our ability to explore questions of electrospray ion character for large electrospray ions.

Summary

Charge detection mass spectrometry has been used routinely and rapidly to obtain positive ion mass spectra of DNA samples in the 1.5 - 5.0 MDa range without extensive cleanup of the sample. The instrument calibration is determined independently of samples. A CD mass spectrum constructed from 3000 ion measurements takes 10 minutes to acquire and yields the plasmid mass directly (resolution = 6). The data collected represents progress toward a more automatable alternative to sizing of DNA by gel electrophoresis.

In addition to the mass spectra, CD-MS generates charge vs. mass plots which provide another means to investigate the creation and fate of large electrospray ions.

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Disclaimer

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Figure Captions

Figure 1. (a) A positive ion CD-MS histogram mass spectrum of electrosprayed ds, circular pBR322. The associated (b) plot of charge vs. mass, with the short line representing m/z = 3650, and (c) the m/z histogram plot.

Figure 2. (a) A positive ion CD-MS histogram mass spectrum of electrosprayed ss, circular M13mp8 showing evidence for both unfragmented ss M13mp8 and the dimer of ss M13mp8. Both the associated (b) plot of charge vs. mass, with the short lines representing m/z = 2450 and m/z = 3500, and the (c) m/z histogram plot (for only those data points whose mass is consistent with unfragmented DNA) show that there are two charge states associated with unfragmented ss M13mp8 electrospray ions.

Figure 3. Data from Table 1 is plotted. The mass values for the DNA positive ions measured by CD-MS are plotted against the calculated bare DNA masses (without counterions). The linear fit through the measured mass values is described by $y = 0.973x + 0.157$, with correlation coefficient $r^2 = 0.99967$. A 0.4% higher mass value compared to the bare DNA mass would be expected if all the counterions were protons. The dotted line for the expected mass for full sodium ion adduction is 8.1% higher than the bare mass.

Figure 4. (a) A negative ion CD-MS histogram mass spectrum of nanosprayed ds, circular pBR322 showing evidence for both unfragmented ds pBR322 and the dimer of ds pBR322. Both the associated (b) plot of charge vs. mass, with the short lines representing m/z = 1250 and m/z = 3100, and the (c) m/z histogram plot (for only those data points whose mass is consistent with unfragmented DNA) show that there are two charge states associated with unfragmented negative nanospray pBR322 ions.

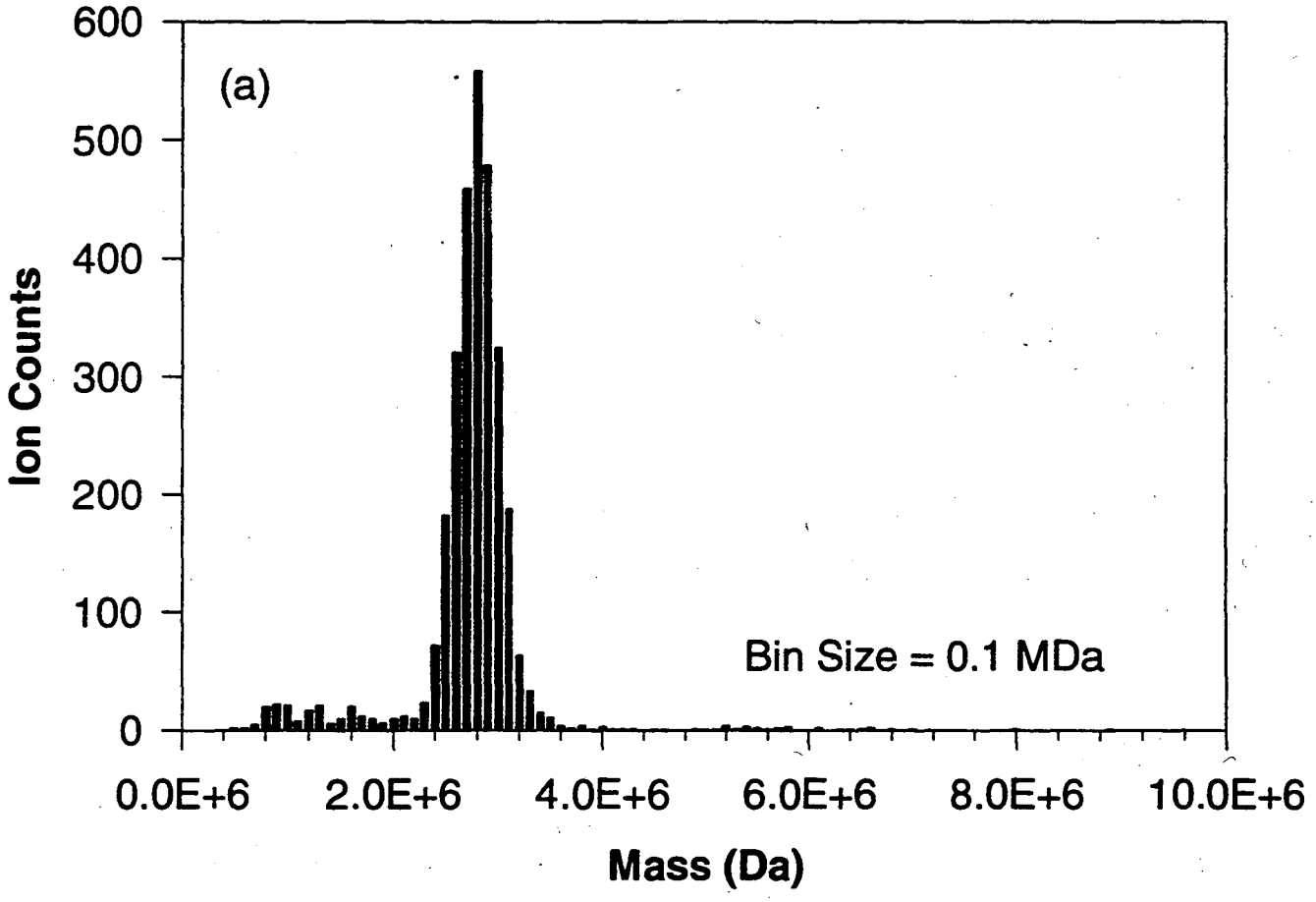
(a)
600
500
400
300
200
100
0
Ion Counts
Bin Size = 0.1 MDa
0.0E+6
2.0E+6
4.0E+6
6.0E+6
8.0E+6
10.0E+6
Mass (Da)

Figure 1(a)

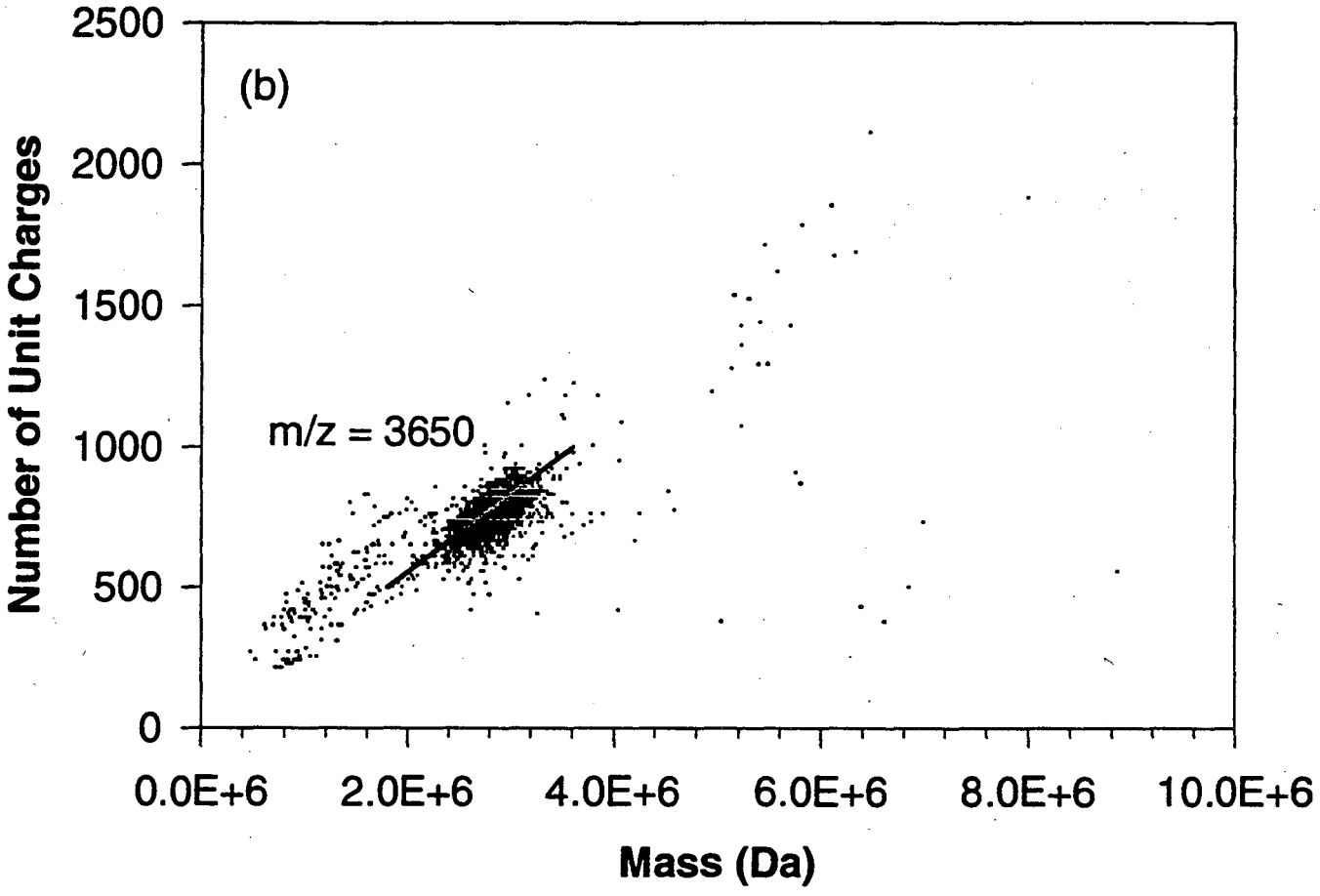
(b)
m/z = 3650
Number of Unit Charges
2500
2000
1500
1000
500
0
0.0E+6
2.0E+6
4.0E+6
6.0E+6
8.0E+6
10.0E+6
Mass (Da)

Figure 1(b)

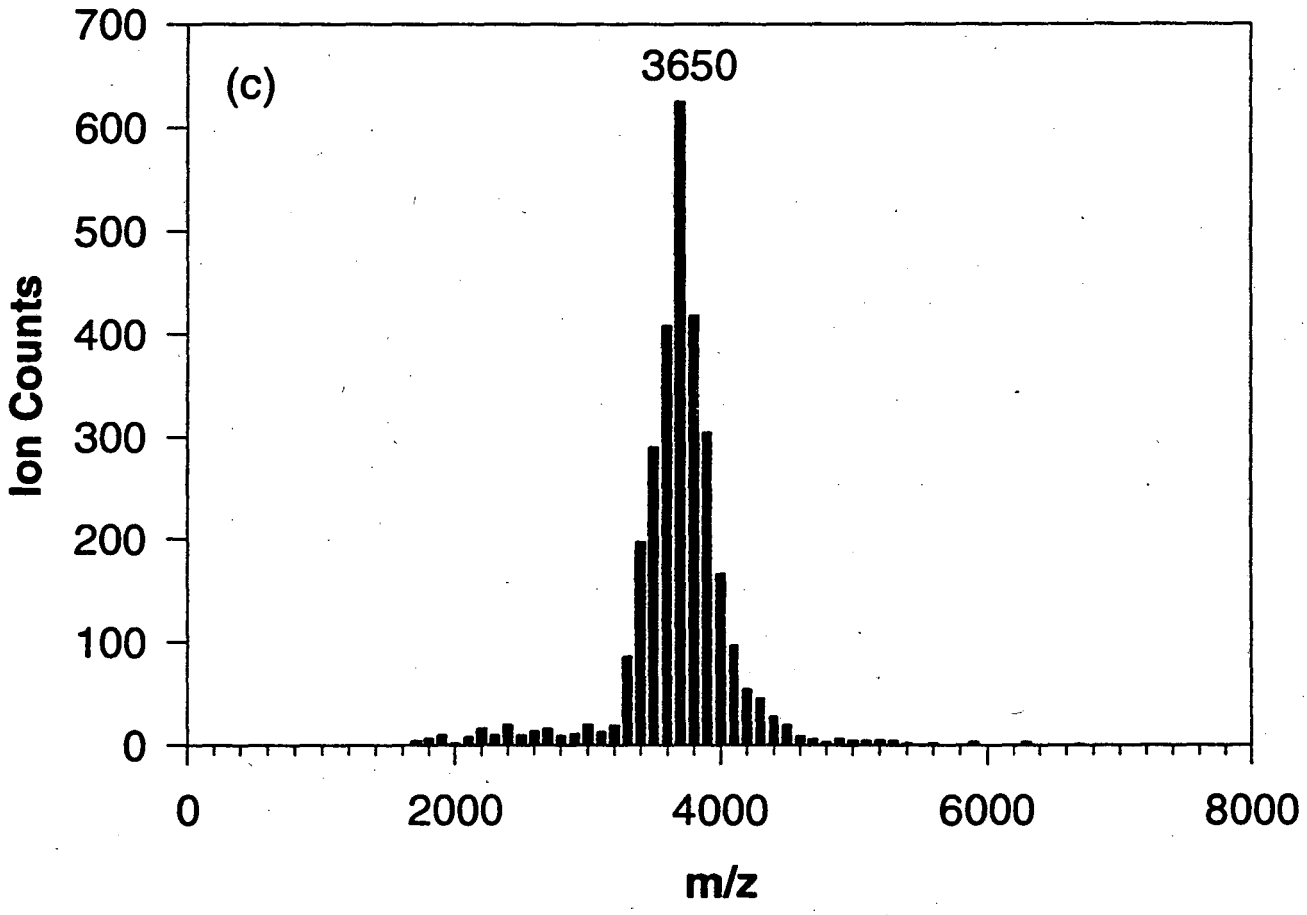
(c)
3650
Ion Counts
700
600
500
400
300
200
100
0
0
2000
4000
6000
8000
m/z

Figure 1(c)

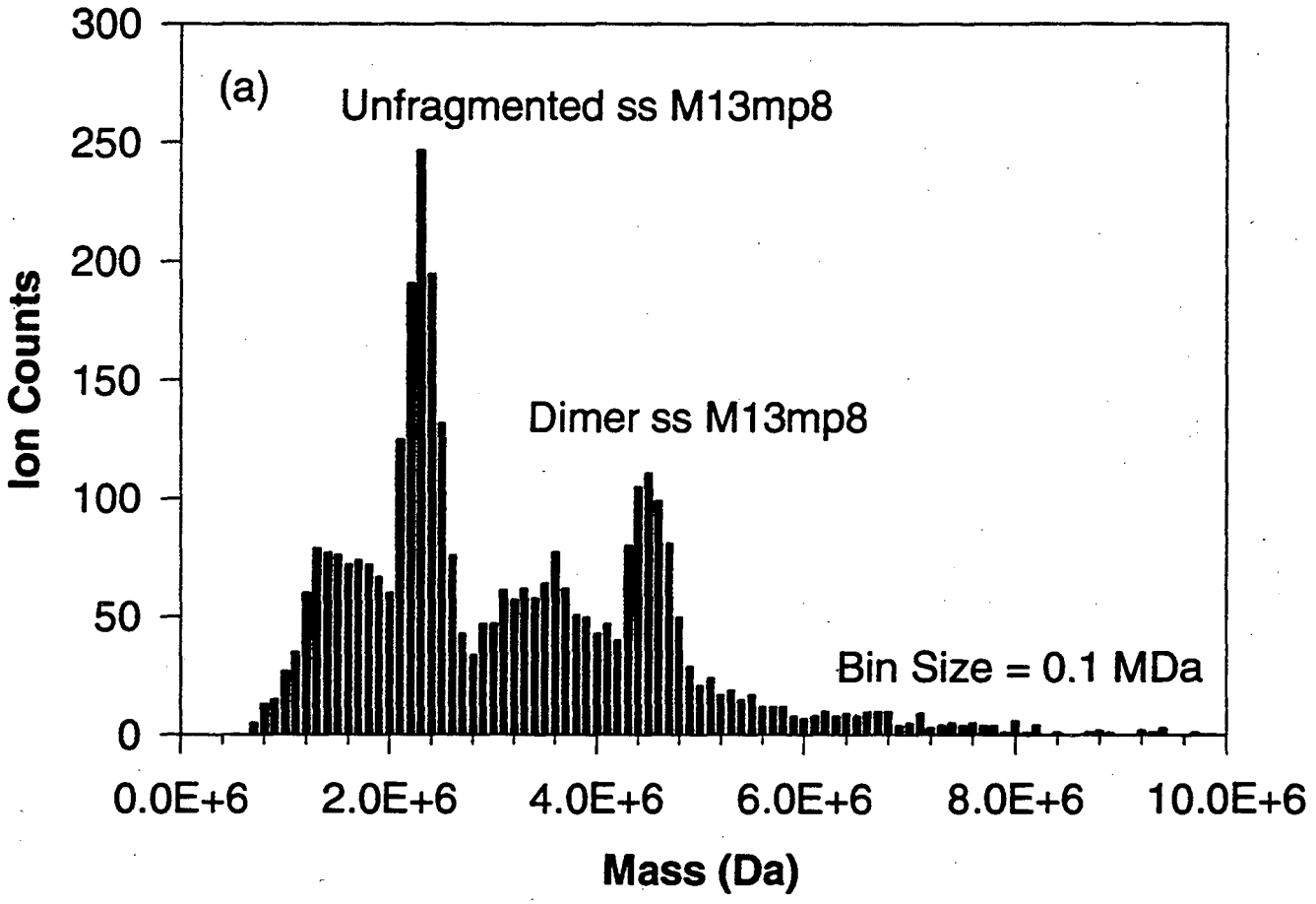
(a)
Unfragmented ss M13mp8
Dimer ss M13mp8
Bin Size = 0.1 MDa
Ion Counts
300
250
200
150
100
50
0
0.0E+6
2.0E+6
4.0E+6
6.0E+6
8.0E+6
10.0E+6
Mass (Da)

Figure 2(a)

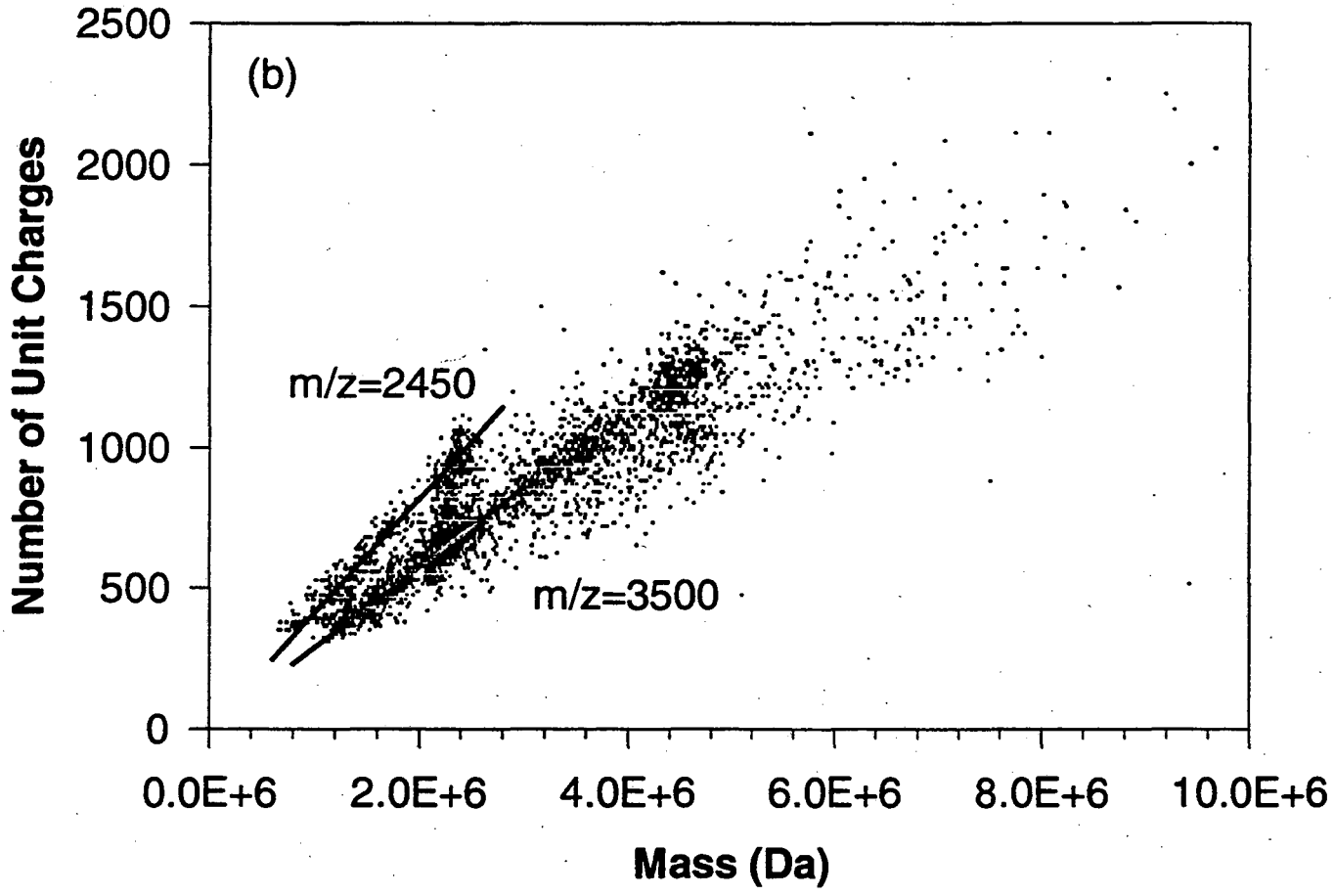
(b)
Number of Unit Charges
2500
2000
1500
1000
500
0
m/z=2450
m/z=3500
0.0E+6
2.0E+6
4.0E+6
6.0E+6
8.0E+6
10.0E+6
Mass (Da)

Figure 2(b)

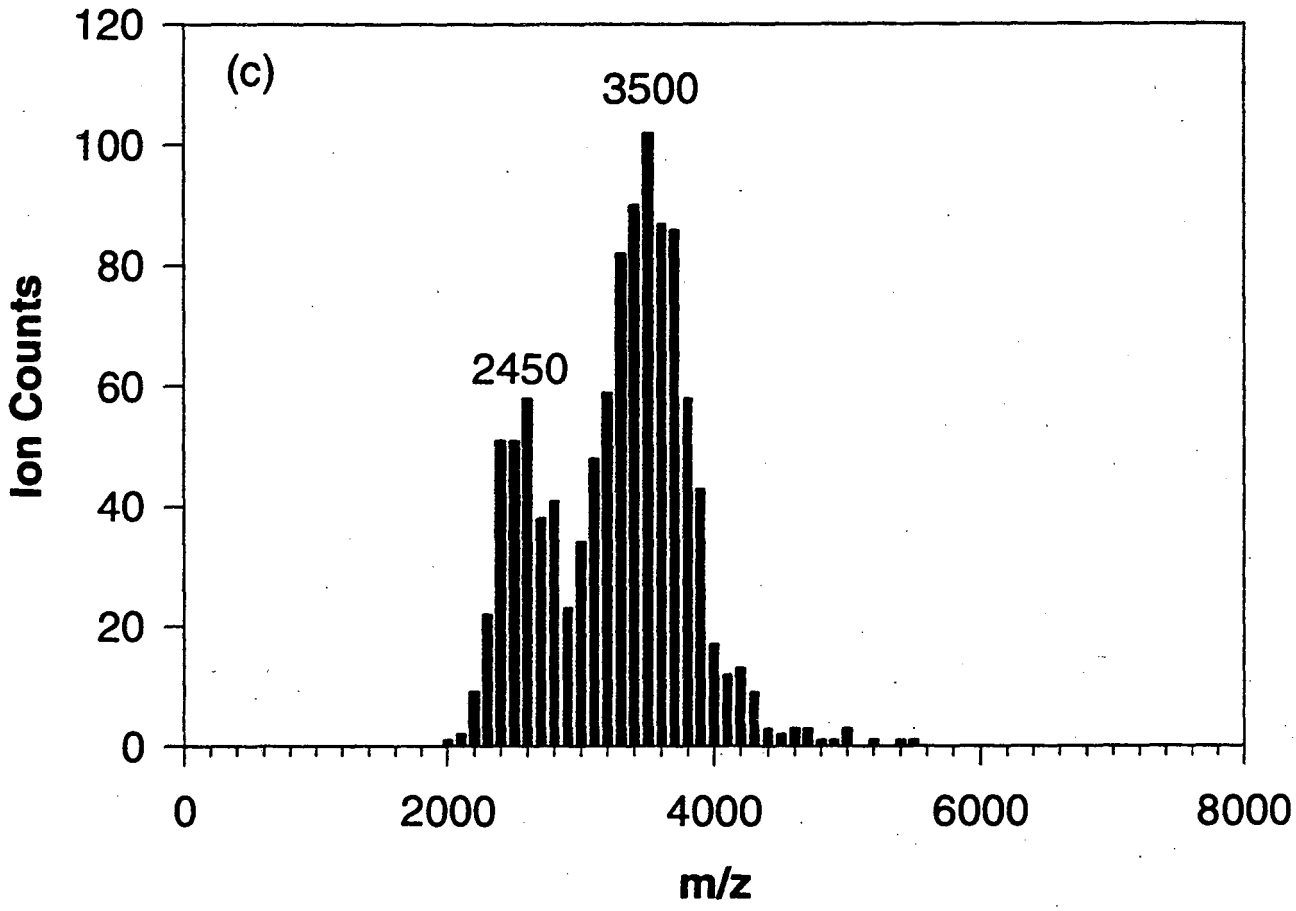
(c)
3500
2450
120
100
80
60
40
20
0
Ion Counts
0
2000
4000
6000
8000
m/z

Figure 2(c)

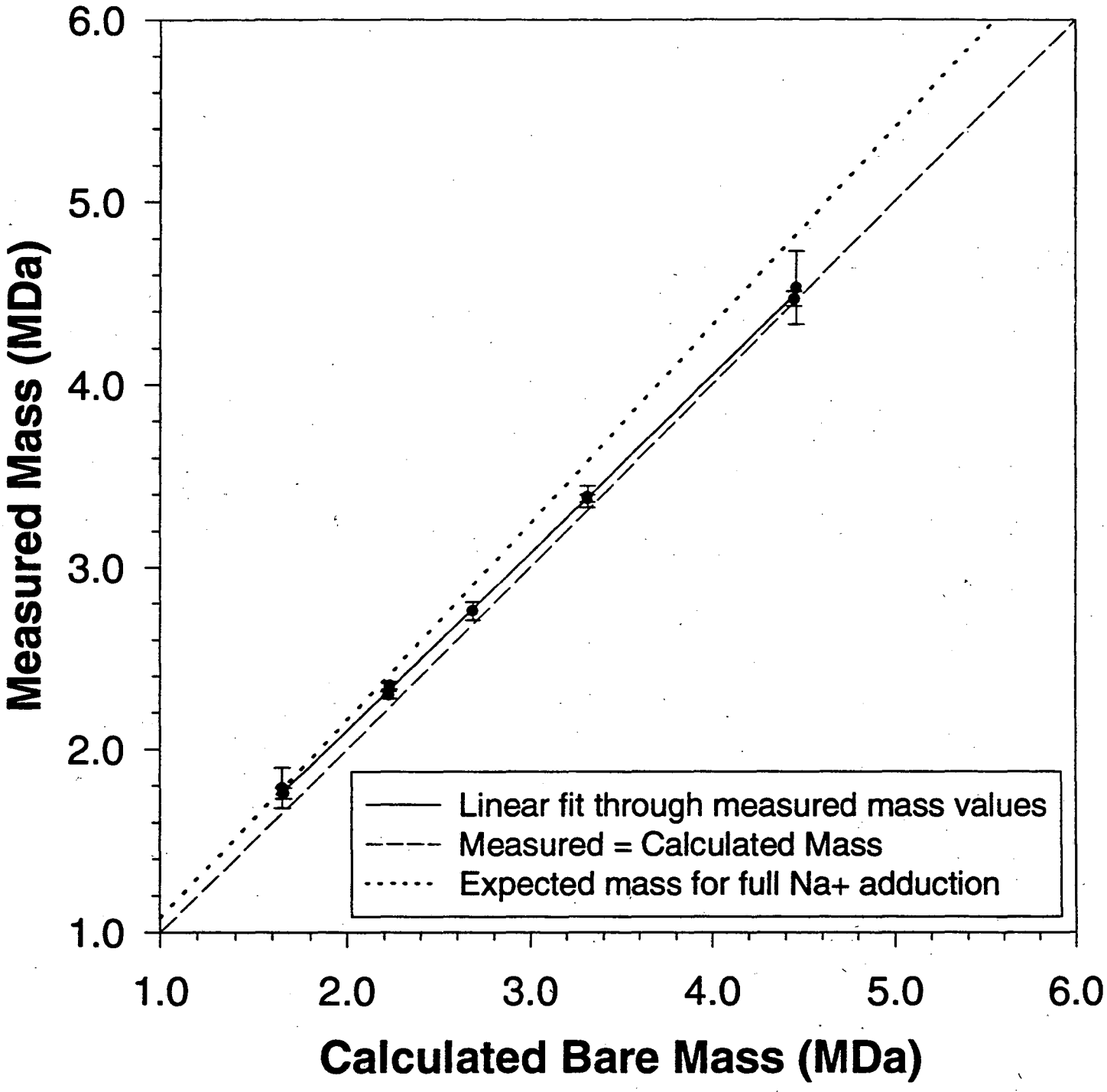
6.0
5.0
4.0
3.0
2.0
1.0
Measured Mass (MDa)
1.0
2.0
3.0
4.0
5.0
6.0
Calculated Bare Mass (MDa)
Linear fit through measured mass values
Measured = Calculated Mass
Expected mass for full Na+ adduction

Figure 3

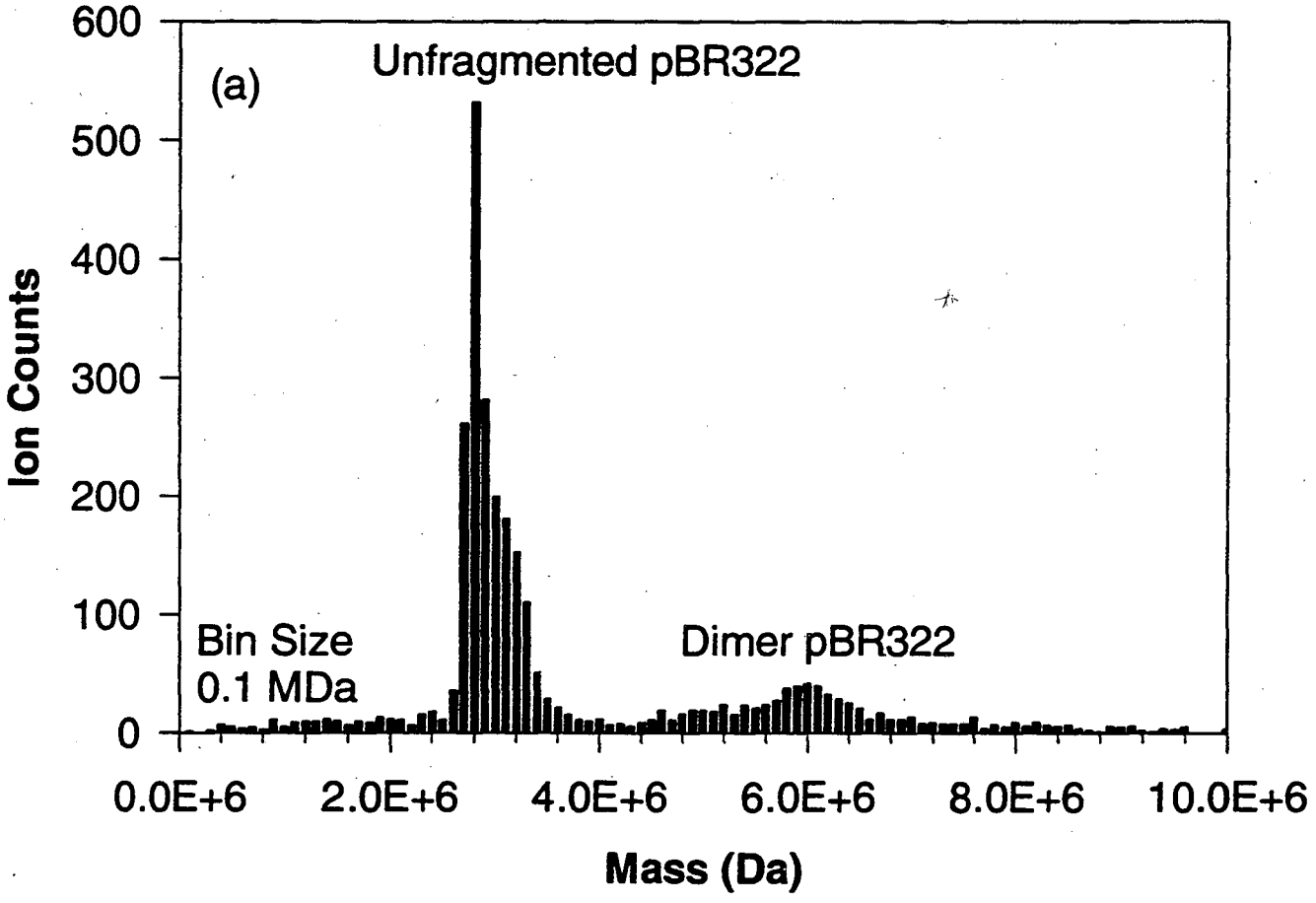
600
500
400
300
200
100
0
Ion Counts
(a)
Unfragmented pBR322
Bin Size
0.1 MDa
Dimer pBR322
0.0E+6
2.0E+6
4.0E+6
6.0E+6
8.0E+6
10.0E+6
Mass (Da)

Figure 4(a)

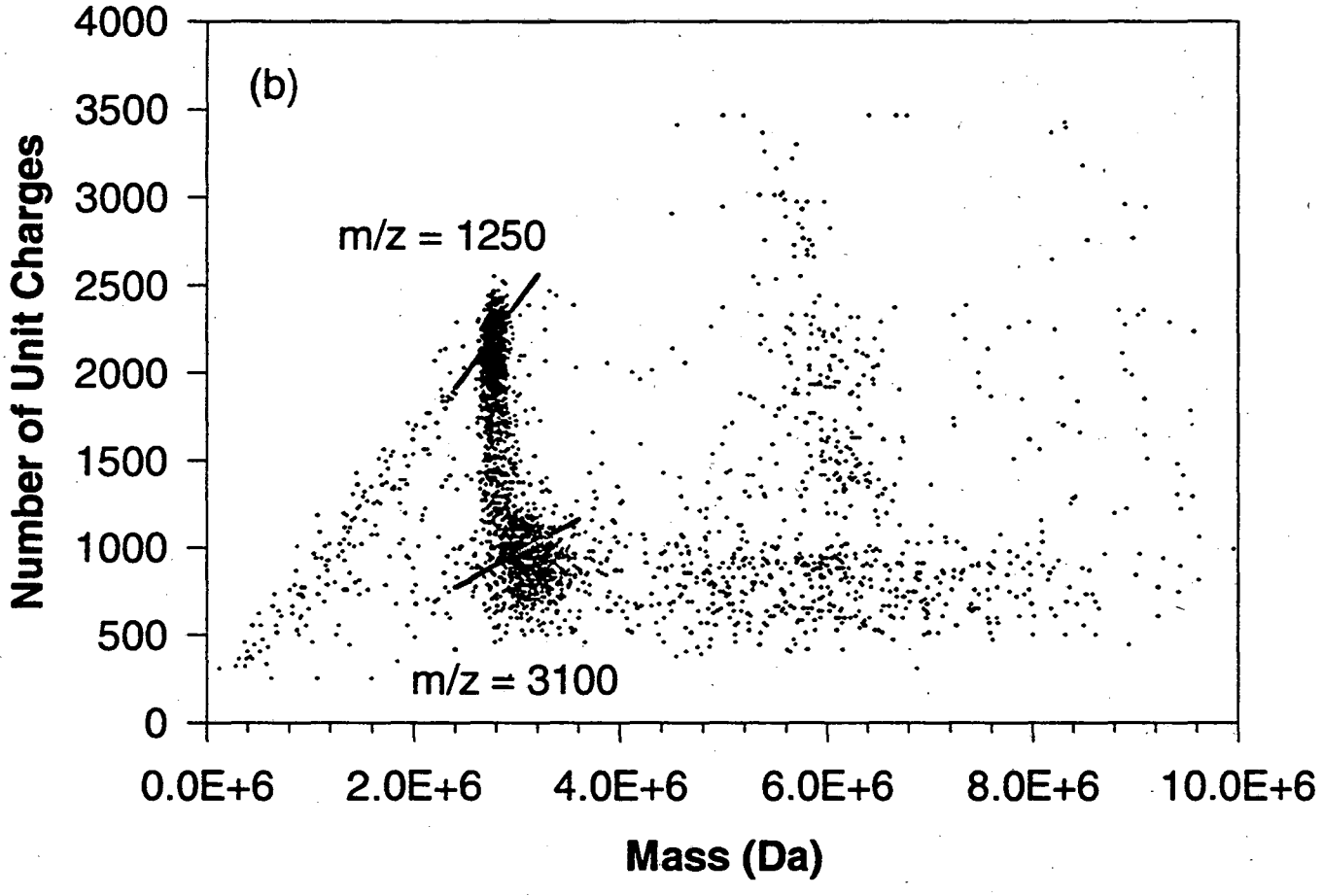
(b)
m/z = 1250
m/z = 3100
Number of Unit Charges
4000
3500
3000
2500
2000
1500
1000
500
0
0.0E+6
2.0E+6
4.0E+6
6.0E+6
8.0E+6
10.0E+6
Mass (Da)

Figure 4(b)

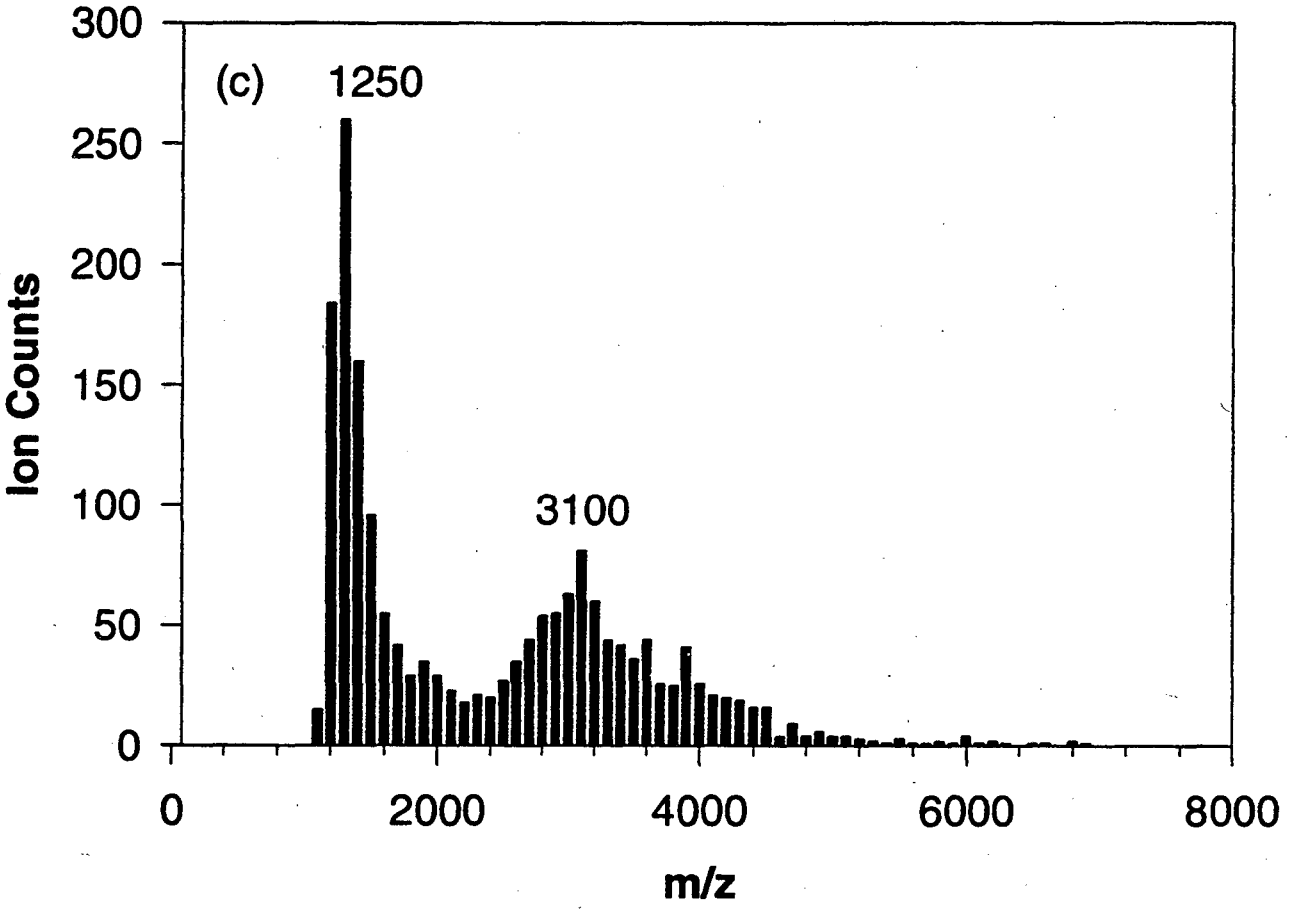
(c)
1250
3100
Ion Counts
m/z
300
250
200
150
100
50
0
0
2000
4000
6000
8000

Figure 4(c)

Table 1. Charge Detection Mass Spectrometric Data

DNA Sample	Characteristics[a]	Bare Mass[b] (MDa)	CD Mass[c] (MDa±s.d.(n))	% Difference CD vs Bare Mass
ϕX174 Virion	ss,circular,5386b	1.659	1.76±0.03 (4)	+6.1
SS M13mp8	ss,circular,7229b	2.226	2.30±0.02 (5)	+3.3
SS M13mp18	ss,circular,7249b	2.232	2.35±0.02 (3)	+5.2
pUC18	ds,supercoil,2682bp	1.654	1.79±0.11 (3)	+8.2
pBR322	ds,supercoil,4361bp	2.687	2.76±0.05 (29)	+2.7
ϕX174 RF I	ds,supercoil,5386bp	3.317	3.39±0.06 (4)	+2.2
ϕX174 RF II	ds,nicked circular,5386bp	3.317	3.38±0.02 (3)	+1.9
M13mp8 RF I	ds,supercoil,7229bp	4.452	4.47±0.04 (5)	+0.4
M13mp18 RF II	ds,supercoil,7249bp	4.465	4.53±0.2 (5)	+1.5

[a]bp=base pair, b=bases

[b]Bare mass calculated from average value of 615.9 Da per base pair for paired nucleotides without counterions or 307.9 Da per base without counterion for ss DNA

[c]s.d.=standard deviation, n=number of measurements

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