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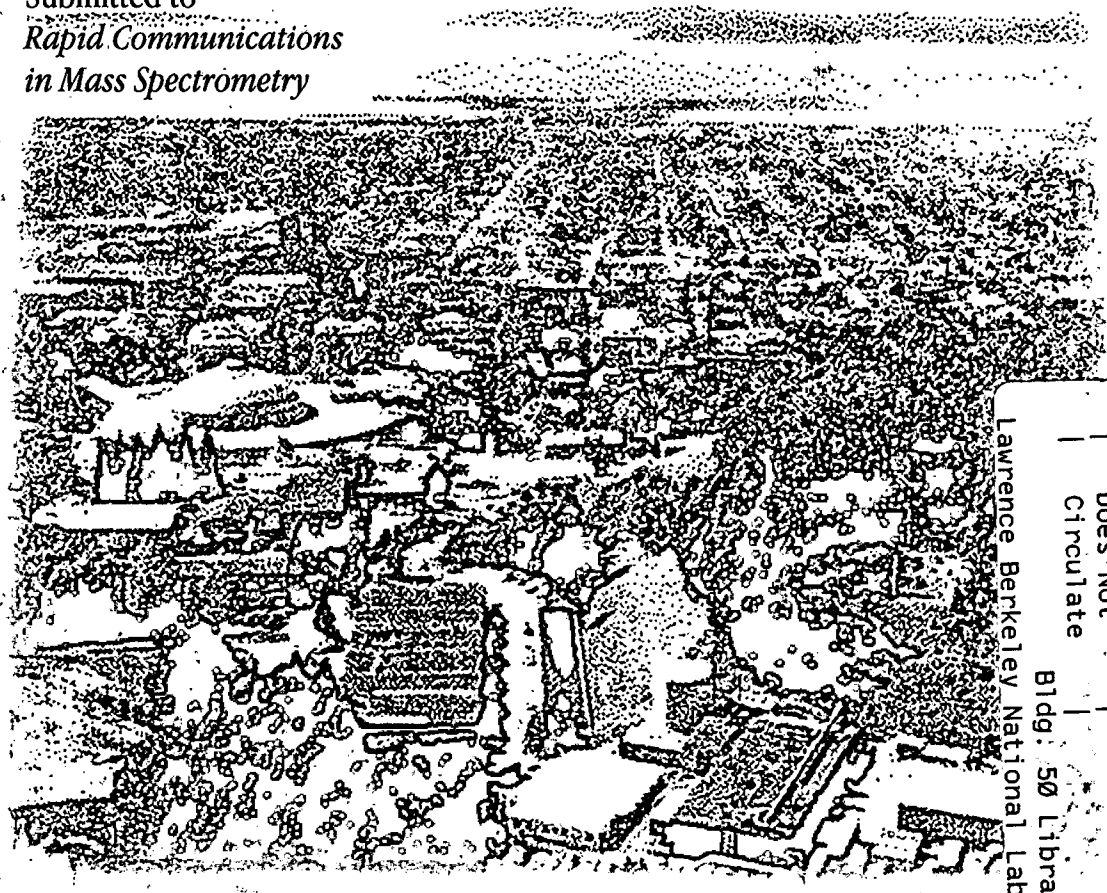

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Polymerase Chain Reaction Products Analyzed by Charge Detection Mass Spectrometry

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ABSTRACT

Charge detection mass spectrometry (CDMS) has been used to rapidly analyze polymerase chain reaction (PCR) products with a minimum of sample cleanup after the PCR reaction. PCR products of 1525 base pairs (bp), 1982 bp and 2677 bp were detected by CDMS, where simultaneous measurement of the charge and velocity of an electrostatically accelerated ion allows a mass determination of the ion. We extended the investigation to a longer double-stranded, linear DNA sample by also analyzing linearized pBR322 (4361 bp). Positive ion mass spectra and ion charge-state distributions were obtained for these electrosprayed DNA samples. Instrumental conditions were studied using a commercially available, linearized pUC18 sample (2682 bp).

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INTRODUCTION

In previous work[1,2] we demonstrated that charge detection mass spectrometry (CDMS) can be used to determine the mass of highly-charged electrospray ions generated from samples of double- and single-stranded circular DNA in the range of 2500 to 8000 base pairs (1.5-5.0 MDa). In the work presented here we applied the same mass spectrometric technique to polymerase chain reaction (PCR) products of 1525 base pairs (bp), 1982 bp, and 2677 bp. By the nature of the PCR reaction, PCR products are double-stranded (ds), linear DNA. We also applied CDMS to other ds, linear DNA samples: linearized pUC18 (2682bp) and linearized pBR322 (4361 bp). Double-stranded, linear DNA samples much smaller than 1525 bp can not be analyzed by CDMS in its present configuration due to a lower limit in the amount of charge that an ion can carry and be individually detected by the charge detection tube.

CDMS 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. The work presented here was undertaken to make further progress toward developing CDMS as a faster and more automatable alternative to sizing of PCR products by gel electrophoresis. Since an ion charge state distribution is obtained in addition to a mass spectrum, CDMS can be used to explore questions of electrospray ion character for large electrospray ions.

By the careful choice of primers, the PCR reaction leads to the amplification of a target DNA sequence. In diagnostic assays based on PCR, primers are designed to attach to a specific DNA sequence. The presence of a PCR reaction product scores the test as positive in some types of tests, while in others the specific length of the product is definitive. The diagnostic information is usually obtained by gel electrophoresis. If it is necessary to distinguish between different sizes of possible amplified DNA at a higher resolution than that afforded by the method used for size analysis, a restriction enzyme can be used to cleave the PCR product. By choosing an enzyme that cleaves at a location that varies between possible PCR-amplified products, the resulting ds, linear DNA fragments can have very different sizes depending on the identity of the PCR product. The analysis of PCR products and the application of restriction fragment sizing of DNA for sequencing and sequence checking could all take advantage of more rapid sizing of large, ds, linear DNA. While CDMS in its present configuration is not a high resolution technique, it yields mass directly, it does not require extensive cleanup of samples, it has the potential of being automatable, and upgraded versions of the instrument are expected to extend the range of detection to somewhat smaller total charges and have been shown to have improved resolution.[3]

Other mass spectrometric studies using electrospray ionization (ESI) of PCR-amplified DNA have focused on high-resolution analysis of products in the 50 to 115-bp size range.[4,5,6,7,8,9] Single base substitutions have been identified by mass analysis of the DNA single strands (ss) of 89 and 114-bp PCR products using ESI with Fourier transform ion cyclotron resonance MS[5] and of a 44-bp product using ESI with double-focusing tandem MS.[9]

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) has been used to detect the hepatitus B virus by verifying the presence of a 67 bp DNA fragment after nested PCR of blood samples.[10] Due to the low resolution for high mass molecules and in order to maximize the diagnostic information obtained, many studies using MALDI-TOFMS have included restriction enzyme digestion of PCR products in order to obtain smaller pieces for mass analysis or distinct fragmentation patterns due to the presence of mutations.[11,12,13] A recent report of PCR product analysis, that employed an affinity capture purification resulting in single stranded DNA and delayed extraction MALDI-TOFMS, demonstrated sufficient resolution and mass accuracy for a single base substitution in a 69 bp PCR product to be identified by direct molecular weight determination.[14] Using MALDI-TOFMS, large ds, linear DNA over 500 bp, detected as single-stranded DNA, have been mass analyzed.[12,15] However, in a recent report using IR-MALDI-TOFMS and frozen glycerol as matrix, ds, linear DNA up to 750 bp long has been detected (apparent resolution m/Δm~50) as the intact double-stranded DNA.[16]

EXPERIMENTAL

DNA Samples

DNA is characterized not only by the number of bases it contains but also by its structure. Double-stranded DNA can be linear – where the DNA has two free ends – or circular – where the ends covalently join forming a circle. PCR products are double-stranded (ds), linear DNA.

A 1525-bp segment of pUC19 was PCR-amplified using the following primers:

Forward: 5'-ATC AGC TCA CTC AAA GGC GG-3'
Reverse: 5'-GCT ATC TGG CGC GGT ATT AT-3'

A 1982-bp segment of pUC19 was PCR-amplified using the following primers:

Forward: 5'-GGC CTC TTC GCT ATT ACG C-3'
Reverse: 5'-GCT ATG TGG CGC GGT ATT AT-3'

A 2677-bp segment of pBR322 was PCR-amplified using the following primers:

Forward: 5'-TGT CCA GGC AGG TAG ATG ACG AC-3'
Reverse: 5'-GAG GAC CGA AGG AGC TAA CCG-3'

PCR primers were synthesized on a PerSeptive Biosystems (Framingham, MA, USA) model 8909 synthesizer. For PCR templates, pBR322 (purchased from Boehringer Mannheim, Indianapolis, IN, USA) and pUC19 (grown and prepared "in-house") were used. The 1525-bp and 1982-bp amplifications, following standard PCR protocol, were produced in a 100 μl reaction volume which included 10 ng of plasmid pUC19 DNA template, 50 pmol of each primer, and 2.5 units of Taq Polymerase along with reaction buffer and deoxynucleotidetriphosphate (dNTP). Thermocycling conditions were as follows: an initial denaturing step of 2 minutes at 94°C was followed by thirty cycles of 94°C denaturation for 15 seconds, 55°C annealing for 15 seconds, and 72°C extension for 60 seconds. The reaction was held for 5 minutes at 72°C at the completion of the run to assure no incomplete extensions. The

2677-bp amplification, following "long PCR" protocol,[17] was produced in a 100 μl reaction volume which included 10 ng of plasmid pBR322 DNA template, 50 pmol of each primer, and 2.5 units of Taq Polymerase along with reaction buffer and dNTP. Prior to the addition of the enzyme, the reaction mixture was heated to 78°C for 90 seconds to prevent false priming and extension. Following this, the reaction underwent an initial denature at 94°C for 60 seconds and then 30 cycles of 94°C denaturation for 15 seconds, 63°C annealing for 30 seconds, and 72°C extension for a variable amount of time. The extension step was 4 minutes long in the first cycle, and 15 seconds were added to the extension step in each subsequent cycle. PCR reactions were carried out in a Perkin-Elmer (Norwalk, CT, USA) model 9600 thermocycler.

Gel electrophoresis confirmed the success of the PCR reactions, after which the products were purified by ethanol precipitation. We assumed that some cleanup of the PCR reaction to remove nucleotides, primers, and buffers would benefit the electrospray CDMS analysis. It has been shown[18] that the presence of the much smaller dNTPs and oligonucleotide primers can cause severe electrospray-ion-signal suppression of the larger PCR product. To purify the PCR products by ethanol precipitation, ammonium acetate was added to the products to a concentration of 1.05 M, after which 2.5 volumes of pure ethanol were added. The solution was incubated at –80°C for 30 minutes and then centrifuged at 4°C for 30 minutes, leaving a DNA pellet. The supernatant was removed, and the pellet was rinsed with 70% ethanol. The solution was centrifuged again at 22°C for 10 minutes, and the supernatant was removed. The pellet was resuspended in water or 10mM tris(hydroxymethyl)aminomethane pH 8 with HCl (Tris-HCl), and the DNA concentration was determined by UV absorbance at 260 nm. Gel electrophoresis confirmed the presence of a unique product of approximately the expected size.

PCR product masses quoted are based on the number of base pairs of the targeted sequence defined by the PCR primers. Actually, the PCR products most likely have two more nucleotides containing an adenine base, but the additional mass (614 Da) is inconsequential. The additional nucleotides are due to the *Taq* polymerase used in the PCR reactions adding a non-templated deoxyadenosine phosphate to the 3'-end of each strand of the PCR products.[19]

Linearized pUC18, produced by using the enzyme *Bam*H I to cleave open pUC18 (ds, circular DNA of 2682 bp), was purchased from Pharmacia Biotech (Piscataway, NJ, USA) and used as is. We generated linearized pBR322 using the restriction enzyme *Eco*R I to cleave open pBR322 (ds, circular DNA of 4361 bp). *Eco*R I, its corresponding reaction buffer, and bovine serum albumin (BSA) were purchased from New England Biolabs (Beverly, MA, USA). 25 μg of pBR322, purchased from Boehringer Mannheim (Indianapolis, IN, USA), was incubated with 80 units of *Eco*R I and 100 ng/μL of BSA in a 100 μL reaction volume for one hour at 37°C. Gel electrophoresis confirmed that the digestion was complete and formed a unique product of approximately 4300 bp. The digestion product was purified by means of ethanol precipitation as described above.

An electrospray sample was made by diluting the DNA to 12-25 ng/μL in 1:1 water:acetonitrile. The standard electrospray sample also contained 0.5 to 0.75 mM Tris-HCl. We have found that the presence of Tris-HCl buffer enhances the positive ion electrospray process for DNA. Some electrospray samples also had 0.05 to 0.075 mM ethylenediaminetetraacetic acid (EDTA).

Mass Spectrometry

CD mass spectra were acquired on an in-house built instrument described in detail elsewhere.[2] The ion beam was generated with an electrospray ion source and vacuum interface from Analytica of Branford (Branford, CT, USA). 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 mm ID stainless steel capillary tube (at ground voltage). The other elements of the electrospray ion source are (with representative applied voltages for obtaining positive ion spectra in parenthesis): a cylindrical electrode around the spray needle (-1000 to –2500 V), the inlet end of the heated glass transfer capillary that passes the ions from the electrospray chamber to the first stage of the vacuum system (-4400 V), and an end cap that directs nitrogen drying gas (1.8 L/min flowing through a heater set at 300°C) over the glass capillary inlet (-4000 V). A skimmer separates the first and second vacuum stages. The ions then pass through a RF-only hexapole ion guide, which produces a narrowly confined ion beam, with a small energy spread. The ion beam is electrostatically accelerated between the ion guide and the charge detection tube, which is located in the third vacuum stage. The exit end of the glass capillary and the skimmer between the first and second vacuum stages are +95 to +185 V and +20 to +120 V, respectively, relative to the hexapole ion guide voltage, with the exit end of the glass capillary always at a higher voltage than the skimmer.

The ion detector is a thin-walled, brass tube 3.61 cm long, with a 6.35 mm bore. It is aligned so that the ion beam is directed along the axis of the tube. The charge induced on the tube is amplified by a low-noise, charge-sensitive preamplifier and then shaped and differentiated by a second amplifier. More details of the charge detector analyzer are given elsewhere.[1,2] The signal output for each ion passing through the charge detection tube 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; thus a velocity for the ion can be calculated. Since the ion has undergone a known electrostatic acceleration, the ion's mass-to-charge ratio can be calculated. The peak-to-peak amplitude is proportional to the charge on the ion. Simple multiplication then allows the calculation of the mass from the signal pulse for an individual ion passing through the charge detector tube. Calibration of the instrument is determined independently of samples. The details of the calibration procedure are discussed elsewhere.[2]

The threshold limit of charge detection is set by the trigger level of the oscilloscope, and is typically equivalent to a charge of ~300 unit charges in these experiments. Detecting individual 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.

RESULTS AND DISCUSSION

A positive ion CD mass spectrum of the 1982 bp PCR product is depicted in Figure 1(a). We obtained positive ion mass spectra of the large DNA samples because we have found positive ion electrospray conditions are easier to maintain and reproduce than negative ion conditions with a standard electrospray ion source. The mass spectrum shown in Figure 1(a) is a histogram plot of the masses determined for several hundred individual ions as the PCR sample is electrosprayed into the mass spectrometer. The main peak is assigned to the unfragmented 1982 bp PCR product. The band below this peak is presumably due to fragmented DNA. In some of the mass spectra of the ds, linear DNA samples the intensity of the band of fragment DNA was of similar intensity to that of the peak assigned to the unfragmented DNA. The band above the main peak is presumably due to clusters of DNA pieces. In some mass spectra we observe a distinctive dimer peak.

Most CDMS mass spectra of PCR products, generated by measuring ~1000 individual ions, were obtained in 15 minutes. This was a consequence of the fairly low (~1 cps) ion count rates observed with these samples. The instrument in its present configuration is capable of processing at a rate of 5 ions per second. The typical resolution demonstrated in the CDMS mass spectra of the PCR products is calculated to be $m/\Delta m = 5$ (Δm = FWHM of the mass peak). Its low value is largely due to noise in the determination of the charge by the instrument in its present configuration. A higher resolution version of the charge detection mass spectrometer, displaying at least a five-fold improvement in resolution over the configuration used here, has been described.[3]

The matching scatter plot of charge versus mass for this data set is shown in Figure 1(b). The electrospray ions assigned to the unfragmented 1982 bp PCR product roughly form an oval of data points centered approximately at 620 unit charges in the charge vs. mass scatter plot. The maximum in the m/z distribution for these ions is 2250. A ray of data points extending to lower masses than the unfragmented 1982 bp PCR product, also with m/z ~2250, are present in Figure 1(b). These are presumably fragment ions generated either during the electrospray process or in the vacuum interface by collision-induced dissociation. No ions were detected with less than ~300 unit charges, which is the threshold limit of charge detection set by the trigger level of the oscilloscope. We must detect all the ions in an oval of data points in the charge vs. mass plot, that are associated with a mass peak in the mass spectrum, in order to accurately determine the mass. Therefore a 1525 bp PCR product, with characteristic m/z ~ 2100 and thus most probable number of unit charges ~500, is close to the smallest ds, linear DNA amenable to mass analysis by CDMS in its present configuration.

For the ds, linear DNA samples analyzed, the maximum in the m/z distribution for the unfragmented ions ranged from 1800 to 2600, with the average ~2100. Typically a single-mode charge distribution was observed, i.e. a single oval of data points dominated the charge vs. mass plot at the unfragmented DNA mass, as seen in Figure 1(b). However, in a few cases bimodal charge distributions were observed. Figures 2(a) and 2(b) display the histogram mass spectrum and the charge vs. mass plot, respectively, of one case where a bimodal charge-state distribution was observed. The peak dominating the mass spectrum is assigned to the unfragmented 2677 bp PCR product. In the charge vs. mass plot two different charge states are seen for ions with a mass corresponding to the unfragmented 2677 bp PCR product; one characterized by m/z = 1850 and the other by m/z = 2650. It is important to note that a more complex charge distribution in no

way hinders our ability to generate a mass spectrum, since for each individual ion detected a mass is calculated. It would appear that there can be two different electrospray mechanisms leading to two different charge states for the unfragmented ion, or that the ion is present in solution in two different conformations, each leading to a different charge state. Solution-phase conformation has been shown to influence the charge-state distributions of biomolecular ions produced by electrospray ionization.[20,21,22] However, the first case seems most likely since we have observed that changing voltages in the electrospray ion source region can have an effect on the charge-state distribution of the detected ions. We have observed bimodal charge distributions before for electrospray megadalton DNA samples.[2] Again extending from each oval of data points at the unfragmented PCR product mass in Figure 1(b) are rays of data points at lower masses but of the same m/z; these are presumably DNA fragment ions. There is also a ray at m/z = 1250 with no unfragmented DNA oval of ions associated with it. Perhaps there were unfragmented DNA ions of this m/z formed in the electrospray process, but due to the high charge state, underwent more energetic collisions and thus either fragmented or were knocked out of the ion beam before reaching the charge detection tube.

While positive electrospray ions of ds, linear DNA display a m/z of ~2100, positive ions of ds, circular DNA are characterized by (using the same electrospray source and electrospray conditions) a m/z ~3600.[2] The higher charge state for linear DNA is consistent with a higher charge-state distribution for electrospray ions being associated with a more extended molecular conformation, which one would expect for the linear compared to the circular configuration. Others have also shown that solution-phase conformation influences the charge-state distributions of biomolecular ions produced by electrospray ionization.[20,21,22]

The measured CDMS masses are tabulated in Table 1. The maximum in the histogram mass spectrum of the peak assigned to the unfragmented ds, linear DNA sample is assigned to the measured mass for the DNA sample. Each value in Table 1 is the average of more than one determination except in the case of ds, linear pBR322. 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[2] for paired nucleotides without counterions and the number of expected base pairs for the target DNA (excluding the additional two bases due to the *Taq* polymerase). The calculated bare DNA mass is for the negatively charged DNA molecule without counterions, whereas the measured DNA mass is that for a positively charged ion and thus must include the presence of counterions. The last column in Table 1 lists the percentage difference between the CDMS measured mass and the calculated bare DNA mass. The DNA phosphate backbone is known to have a high affinity for sodium ions. Using a typical m/z ~2100 for a ds, linear DNA, a 8.6% high mass value would be expected if all the positive charges were supplied by sodium ions. (Note that a 7.5% high value would be expected for DNA neutralized by sodium ions, with no net positive charges, so the exact mass-to-charge value used to estimate the mass difference has only a small effect with respect to this low resolution measurement.)

Incomplete desolvation may also contribute to a mass higher than the bare mass. In order to obtain the molecular mass of the sample, it is necessary to efficiently remove any solvent molecules attached to the electrospray ions. Our optimal experimental conditions include a weak counterflow of heated nitrogen gas warming the ions before they enter the vacuum of the mass spectrometer, a heated glass capillary that transfers the ions between the electrospray chamber

and the first differentially pumped stage, and collision-induced desolvation in the differentially pumped interface regions. The data listed in Table 1 were collected under vacuum interface conditions that were a compromise between maximizing the collisional "heating" while optimizing the ion signal.

Figure 3 shows the effect of the vacuum interface conditions on the CDMS measured mass of a ds, linear DNA sample, linearized pUC18. The electric fields present in the differentially pumped interface region are related to the voltage difference between the exit of the glass transfer capillary and the ion guide voltage. This voltage difference is plotted on the x-axis. (The skimmer, located between these two elements, is held at an intermediate voltage.) As the voltage difference increases, the measured mass decreases, as shown in Figure 3. This is consistent with increasing desolvation of ions at higher electric fields. A similar effect was observed for ds, circular pBR322.[2] In this case, however, the voltage difference could be increased further without significant loss in ion signal and a leveling off of the CDMS determined mass was observed (at 2.7% above the bare mass). The lowest mass, the one closest to the bare mass of 1.654 MDa for linear pUC18, is obtained at the highest voltage difference applied as shown in Figure 3. Under these conditions, the measured mass (1.75±0.03 MDa) is 6% higher than the bare mass. However, also under these conditions the ion counts of the ds, linear DNA samples were often very low. Therefore, the best practical conditions for analysis of ds, linear DNA samples in the MDa mass range were with the difference between the capillary exit voltage and the ion guide voltage at approximately 90 to 120V, not the conditions of maximum desolvation. It was under these conditions that the data in Table 1 were obtained.

CONCLUSION

We have shown that the mass of the electrospray ions formed from a PCR product, in the 1500-2700 bp range, can be measured by CDMS. With a minimum of sample cleanup, this mass is reproducibly higher than the DNA bare mass, due to the presence of adduct ions and neutral solvent molecules. Other mass spectrometric techniques analyzing large ions typically require more sample cleanup than CDMS, in order to simplify the observed charge state distribution.

These CDMS results can be compared to those obtained with the gel electrophoresis technique employing a 1% agarose gel. With gel electrophoresis, no sample cleanup is required, a $m/\Delta m$ resolution ~30 is attained, ~20 ng per DNA band is needed for analysis, and as many as 100 samples can be run on one gel. However, DNA size is not always predicted from mobility alone, the separation takes ~6 hours, plus another hour for ethidium bromide staining and then additional time is needed to do the gel imaging. With CDMS in the configuration used here, a simple sample cleanup is employed, a lower $m/\Delta m$ resolution of 5 is achieved, and ~200 ng is used for analysis. However, the ion mass is directly measured, one spectrum takes ~15 minutes, and the data can be plotted immediately.

While CDMS in the configuration used here is not a high resolution technique, an upgraded version of the instrument, which was recently described, is anticipated to have an instrumental resolution more than 10 times better than the configuration used to obtain the data reported here.[3] Other instrumental improvements being implemented are expected to extend the range of

detection to somewhat smaller total charges, and thus to somewhat smaller mass ions. Minimizing the intensity of the fragment ion bands and maximizing the intensity of the unfragmented ion peak would enhance the diagnostic information obtained from the CD mass spectrum. Recent work obtaining CD mass spectra of ds, linear DNA (30 MDa λ DNA and a 1 to 5 MDa 1k bp DNA ladder) with an upgraded version of the instrument and several different electrospray ion sources (standard, capacitance[23] and nanospray[24]) indicates that the ion source can have an effect on the unfragmented ion signal level and the amount of DNA fragmentation produced.[25] Furthermore, the use of nanospray electrospray sources could dramatically decrease the amount of sample needed. Thus we expect that by further optimization of the electrospray ion source design and operation, higher resolution mass spectra more representative of the sample can be obtained with a smaller sample size.

PCR is used in many different applications. We used it here to detect the presence of a specific target DNA sequence. Similarly, PCR can be used to detect and identify, for example, bacteria. Such diagnostic tests are scored positive when a PCR product is obtained. Usually the exact size of the resulting PCR product is not very important. It is simply necessary to determine whether or not a PCR product was produced. CDMS might have application in scoring such tests. Only about 500 ions would be needed to score the test and at about 5 ions/sec (count rates we can reasonably expect to reach with further optimization of the electrospray source), this would be accomplished in 2 min total. The turn-around time with CDMS would be much quicker than analysis by gels.

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Table 1. Charge Detection Mass Spectrometric Data

Sample	Bare Mass[1] (MDa)	CD Mass[2] (MDa ± s.d. (n))	% Difference CD vs. Bare Mass
PCR 1525 bp	0.939	1.11 ± 0.04 (2)	+18%
PCR 1982 bp	1.221	1.37 ± 0.01 (3)	+12%
PCR 2677 bp	1.649	1.86 ± 0.01 (2)	+13%
Linear pUC18	1.654	1.82 ± 0.03 (19)	+10%
Linear pBR322	2.687	3.07 (1)	+14%

[1]Bare mass calculated from average value of 615.9 Da per base pair for paired nucleotides without counterion.
[2]s.d. = standard deviation, n = number of measurements

FIGURE CAPTIONS

Figure 1. (a) A positive ion CDMS histogram mass spectrum of electrosprayed 1982 bp PCR product. (b) The associated plot of charge vs. mass, with the short line representing m/z = 2250.

Figure 2. (a) A positive ion CDMS histogram mass spectrum of electrosprayed 2677 bp PCR product. (b) The associated plot of charge vs. mass. Three short lines, representing m/z = 1250, m/z = 1850 and m/z = 2650, are also included.

Figure 3. The mass values for linear pUC18, measured by positive ion CDMS, are plotted against the employed voltage difference between the exit of the glass transfer capillary and the ion guide (the skimmer, located between these two elements, is held at an intermediate voltage). As the voltage difference increases, the measured mass decreases, consistent with increasing desolvation of ions at higher electric fields in the vacuum interface.

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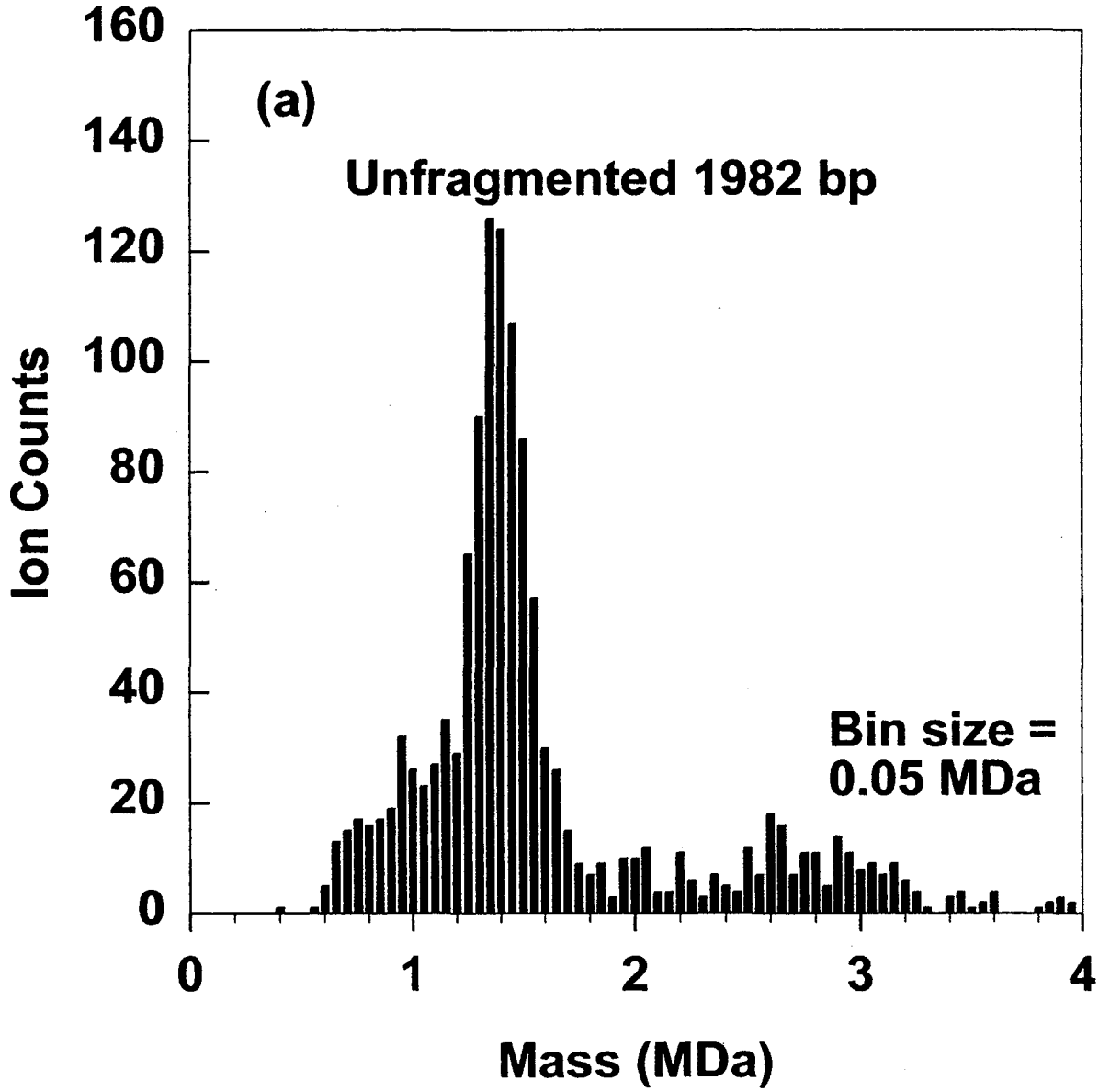
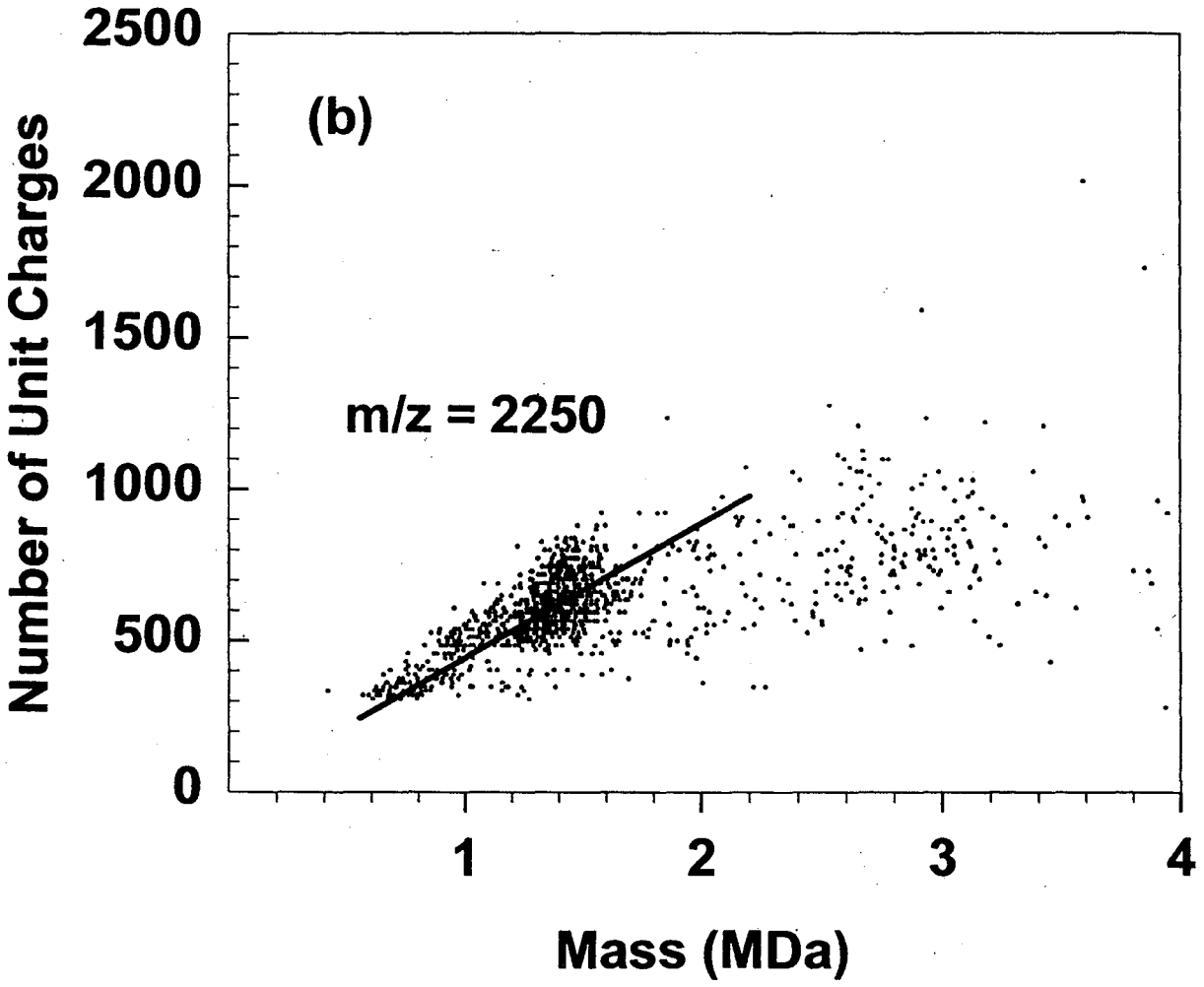
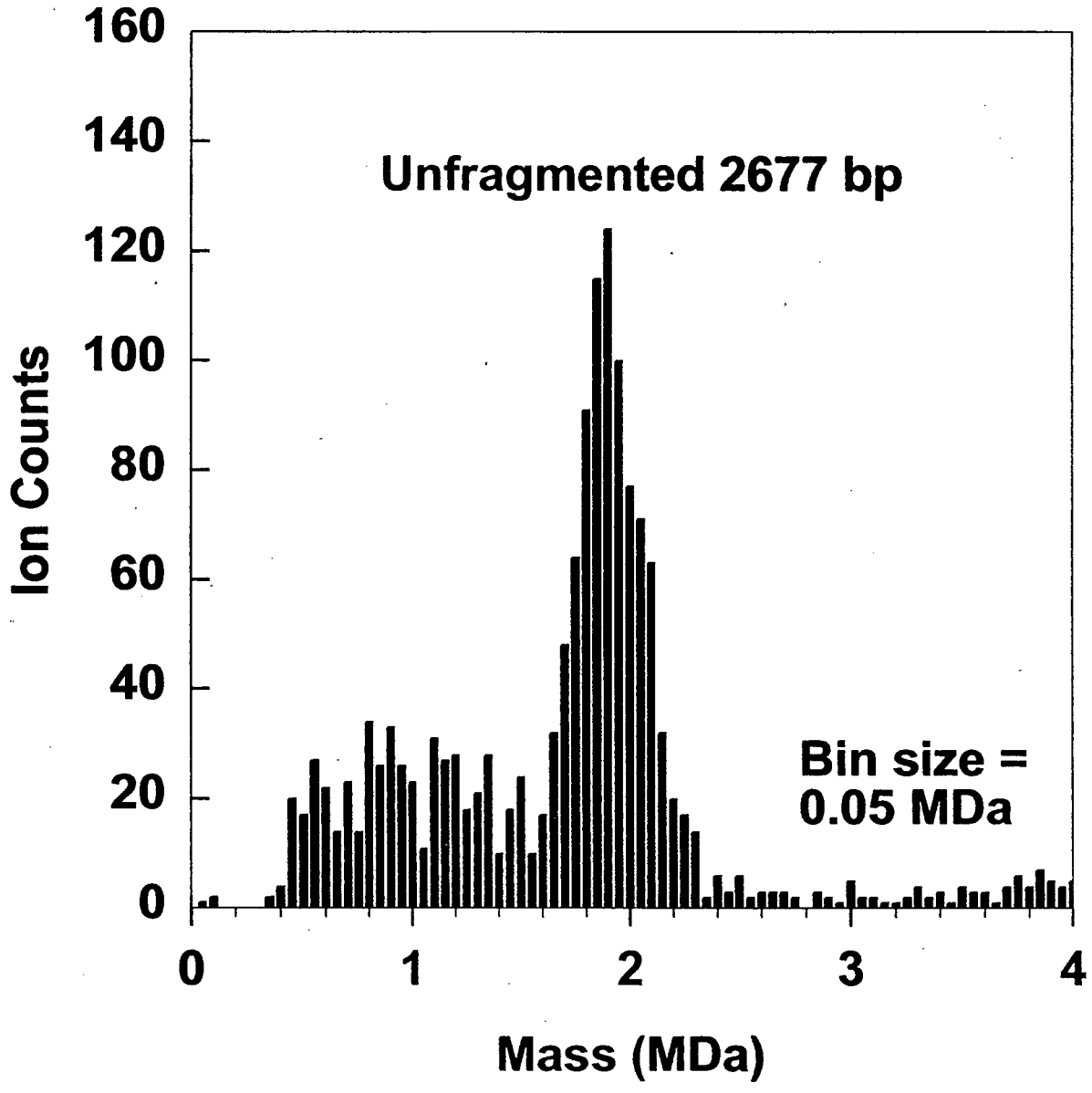
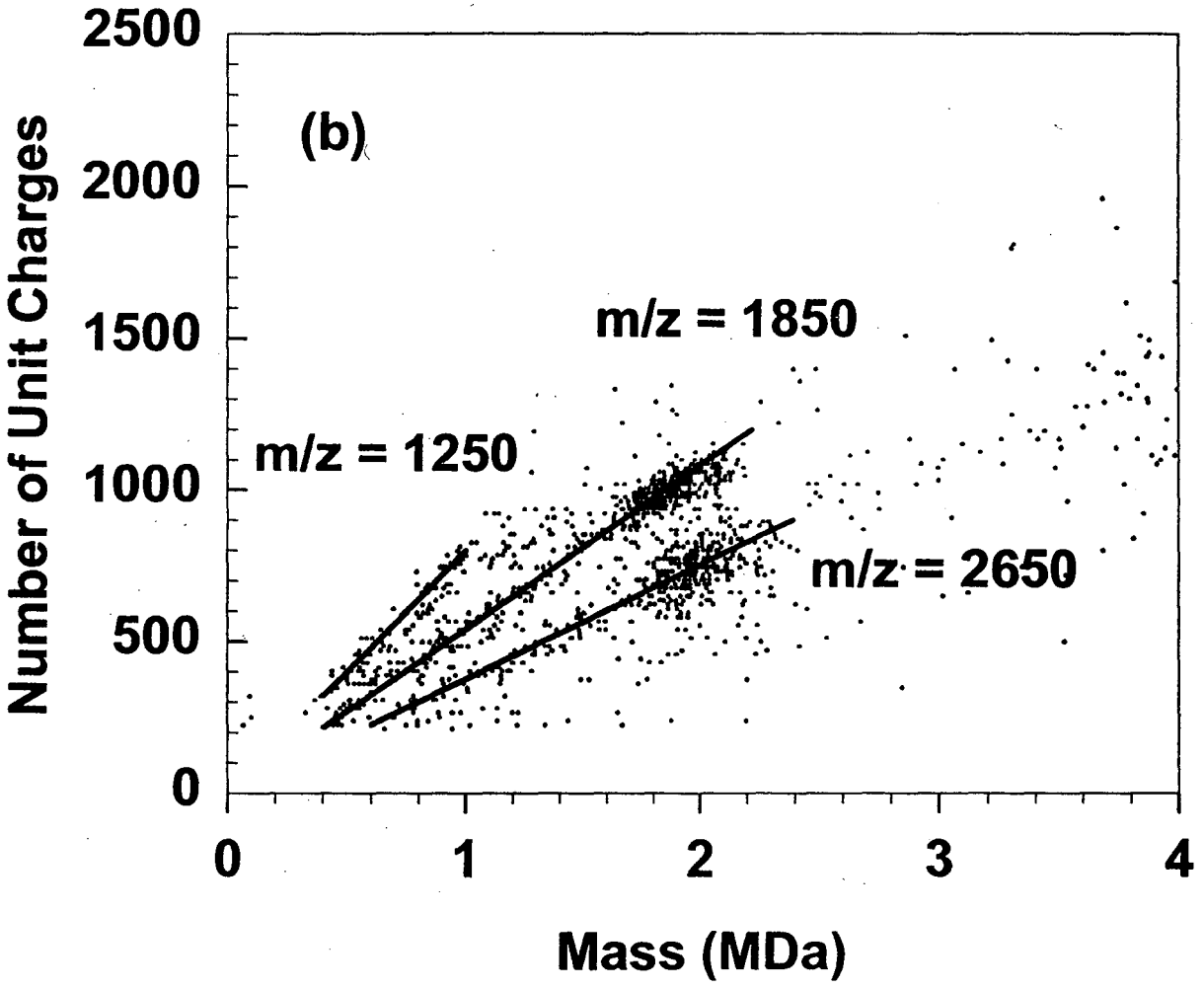
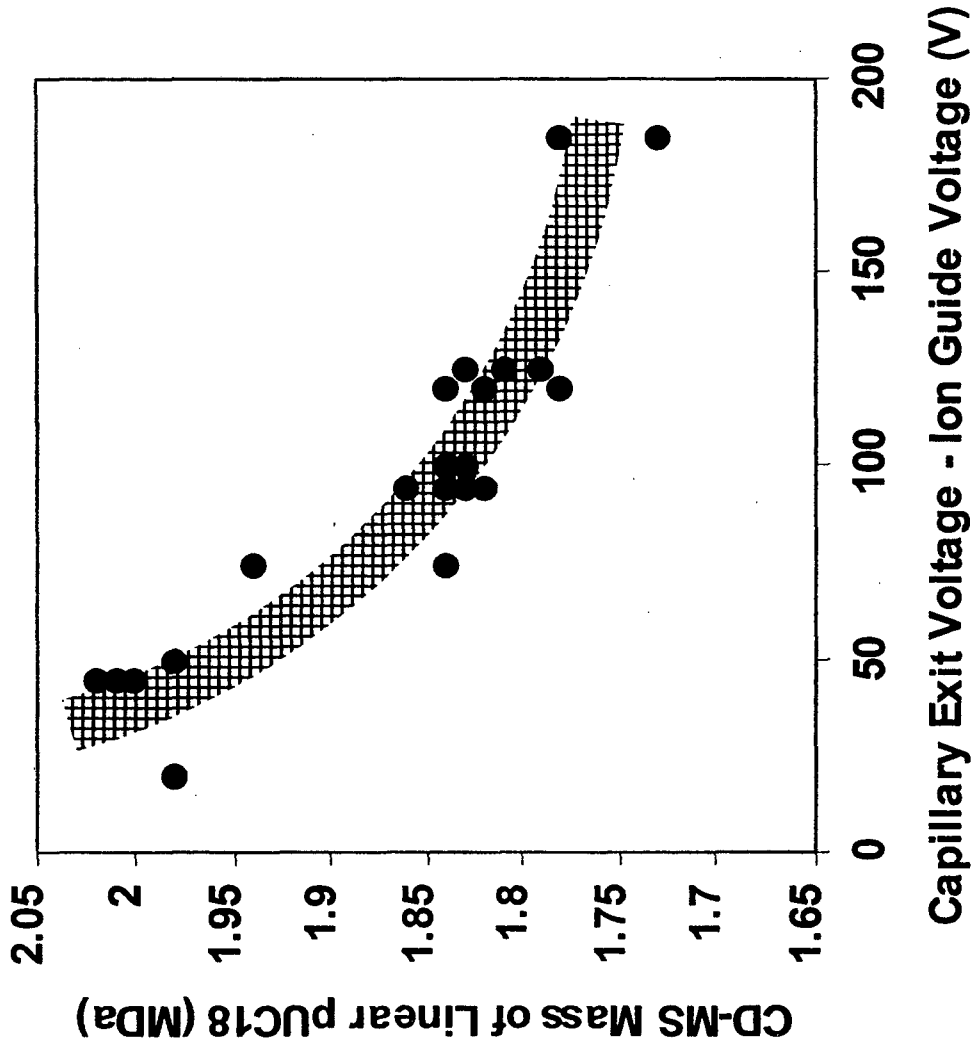

(a)
Unfragmented 1982 bp
Bin size =
0.05 MDa
Ion Counts
Mass (MDa)
160
140
120
100
80
60
40
20
0
0
1
2
3
4


(b)
m/z = 2250
Number of Unit Charges
Mass (MDa)
2500
2000
1500
1000
500
0
1
2
3
4


Unfragmented 2677 bp
Bin size =
0.05 MDa
Ion Counts
Mass (MDa)
0
20
40
60
80
100
120
140
160
0
1
2
3
4


(b)
m/z = 1850
m/z = 1250
m/z = 2650
Number of Unit Charges
Mass (MDa)
2500
2000
1500
1000
500
0
0
1
2
3
4


CD-MS Mass of Linear pUC18 (MDa)
2.05
2
1.95
1.9
1.85
1.8
1.75
1.7
1.65
0
50
100
150
200
Capillary Exit Voltage - Ion Guide Voltage (V)

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