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Retrieval of DNA Using Soft-Landing after Mass Analysis by ESI-FTICR for Enzymatic Manipulation

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The DNA modifications that lead to mutations are of direct interest for, e.g. the understanding of DNA damage recognition and repair.[1] The characterization of the modified oligonucleotides and enzymatic responses to specific DNA modifications are of primary concern, but also present major analytical challenges.[2] Among the available techniques, mass spectrometry has become an increasingly important tool for the study of oligonucleotides, their mutations, and interactions.[3] Conventionally, mass spectrometry provides mass and structural information (e.g. from dissociation experiments and the use of tandem mass spectrometry).[3] However, the small quantities of material analyzed and the destructive nature of conventional mass spectrometric detection (e.g., due to high energy impact on particle multiplier surfaces) have precluded subsequent use of mass separated biopolymers.

We report the use of mass spectrometry in conjunction with "soft-landing"[4] for the high-resolution analysis, separation, and selective collection of oligonucleotides, and their subsequent retrieval for enzymatic manipulation. Fourier transform ion cyclotron resonance mass spectrometry coupled with electrospray ionization (ESI−FTICR) allows nondestructive (i.e., image current) measurement of the mass-to-charge ratios (m/z) of ions with high sensitivity, resolution, and mass accuracy.[5] FTICR methods have been developed for ion selection,[6] manipulation,[7] as well as "in trap" selective ion accumulation of trace level analytes.[8] Here we demonstrate an integrated methodology for ESI-FTICR mass analysis, separation, soft-landing, recovery, and subsequent Polymerase Chain Reaction (PCR) amplification[9] of selected oligonucleotides for further characterization by various means (e.g., gel electrophoresis, mass spectrometry).

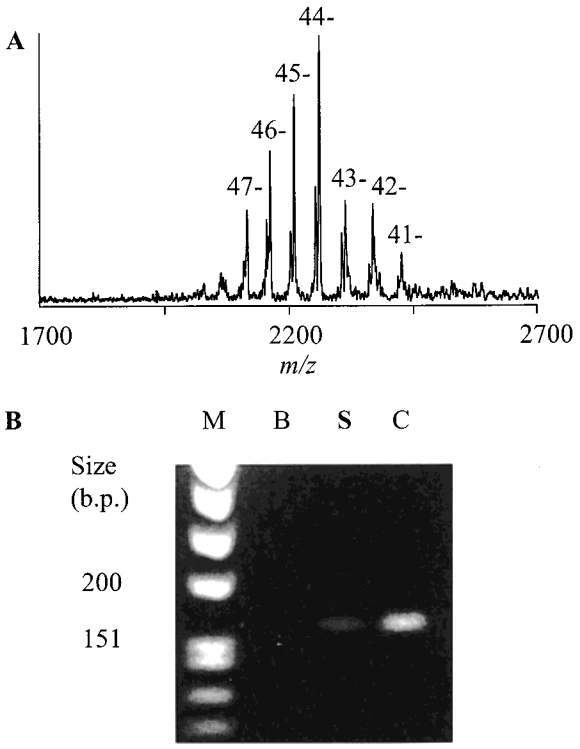

Figure 1. (A) Negative ion ESI-FTICR mass spectrum of the 160-mer ds-DNA studied by soft-landing; charge states for the duplex species are indicated. (B) Agarose gel electrophoresis analysis of the 160-mer soft-landed DNA after PCR amplification. Lane assignment: M, size marker; B, blank, no template added to PCR reaction vial, no amplification; S, soft-landed template amplified; and C, positive control, having 1 attomole of the template directly amplified.

The soft-landing apparatus, which includes a 40-in. long probe with a surface attached at one end, was coupled to previously described 7 T ESI-FTICR instrumentation.[10] The probe assembly allows the transfer of a soft-landing surface in and out of the FTICR instrument without disruption of the high-vacuum environment.[10b] Using the surface probe, a 1.6 cm^2 nitrocellulose membrane surface[11] was positioned 0.5 cm behind the rear trapping plate of the FTICR cell to collect selectively ejected ions. As an initial demonstration, a 160 base pair double-stranded PCR product (160-mer ds-DNA) was electrosprayed and mass analyzed by FTICR (Figure 1A). After the mass analysis, all observed charge states were selected and then axially ejected (achieved by applying a 0.5 V potential difference between the front and rear trapping plates) to be soft-landed on the membrane surface. The selection and soft-landing cycle was repeated 300 times to maximize DNA collection on the surface. The membrane was then retrieved and subjected to PCR for the soft-landed DNA.[12]

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The PCR amplification product was examined by agarose gel electrophoresis (Figure 1B). Compared to both negative (Lane B, Figure 1B) and positive (Lane C, Figure 1B) controls, the experimental results showed that the 160-mer DNA was successfully soft-landed and PCR amplified by this protocol.

We subsequently demonstrated the use of FTICR for selecting one component from a mixture using stored waveform inverse Fourier transform (SWIFT)[13] and for soft-landing the isolated species for enzymatic manipulation. A mixture of two 50-mer single strand DNA segments, differing in mass by only 40 Da (due to a single C to G base substitution) was analyzed by ESI-FTICR (Figure 2A). The low molecular weight component was SWIFT selected and soft-landed at low energy on the membrane (Figure 2B).[14] Gel electrophoresis analysis of the PCR product (not shown) from the retrieved membrane surface indicated amplification of a 50-mer oligonucleotide, but the low resolution (relative to mass spectrometry) is uninformative regarding the single base substitution.[15] Subsequent ESI-FTICR analysis of the purified and concentrated PCR product confirmed that the SWIFT-selected DNA was soft-landed, retrieved, and amplified, since both the soft-landed strand and its complement (not present in the initial sample) were detected (Figure 2C). On the basis of gel electrophoresis results, approximately an attomole of material was retrieved from the membrane and PCR amplified, consistent with estimations based on the FTICR cell charge storage capacity.[16]

In conclusion, we have demonstrated for the first time that oligonucleotides can be retrieved from the mass spectrometer for further manipulation **after** mass analysis and selection. The sensitivity of the approach is limited by the sensitivity of subsequent enzymatic manipulations. In principle, only one DNA molecule is required for PCR.[17] The method will benefit from previously described capabilities for selective accumulation of trace level species.[8] This methodology can potentially supplement conventional strategies for the study of modified or damaged DNA and low abundance sequence variants, where high-sensitivity detection and high-resolution separation with subsequent manipulation are impractical or impossible with conventional means. It also provides a strategy for the study of DNA polymerase responses to modified or damaged DNA segments that can be selected with much greater resolution by FTICR than by electrophoresis. We project it will be feasible to use mass spectrometric methods to prepare biopolymer arrays for hybridization studies (and other applications), where the individual components are selected from otherwise difficult to separate mixtures, and soft-landed to generate the array.

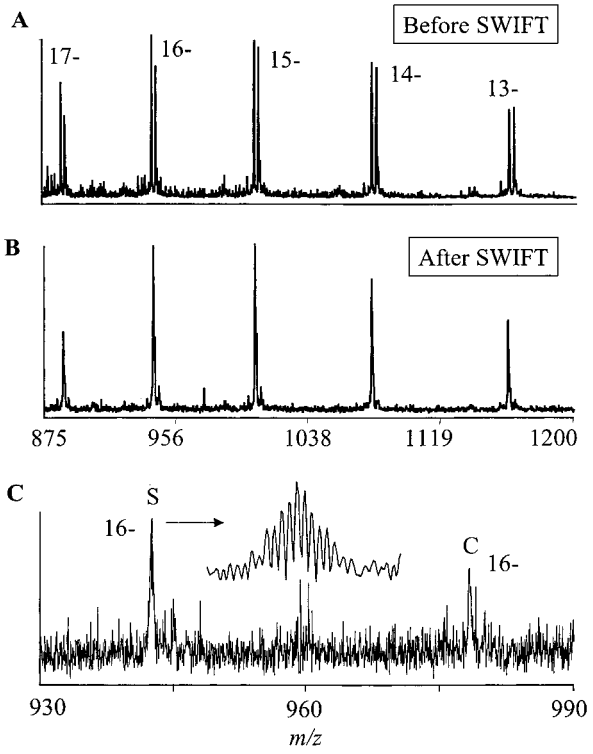

Figure 2. (A) Negative ion ESI-FTICR mass spectrum of the mixture of the two single strand 50-mer DNA (shown 17- to 13-charge states) before SWIFT ion selection. (B) ESI-FTICR mass spectrum of the SWIFT-selected single strand DNA. (C) ESI-FTICR spectrum of soft-landed and PCR amplified DNA (16-charge state shown). Both the selectively soft-landed (S) strand (isotopic distribution shown as an insert) and its complementary (C) strand were observed. Note that the complementary strand was the result of PCR amplification, and not present in either the original solution electrosprayed or the soft-landed species (this provides information on the nucleotide base composition of the selected species).[3f] The measured masses of the strands were 15095.5 (S) and 15699.7 Da (C), in good agreement with expected values of 15096.0 and 15699.3 Da, respectively.

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(12) PCR amplification of soft-landed DNA. The 160-mer double-stranded DNA PCR product in the original electrospray ionization (ESI) solution was amplified from the pGEM 5S plasmid. After soft-landing, the membrane was removed from the mass spectrometer, cut into 5 mm^2 pieces, and vortexed in 100 μL PCR buffer (Stratagene, La Jolla, CA). DNA transferred from the membrane into the buffer served as the PCR template. An aliquot of 10 μL buffer was used, with synthetic primers, *Pfu* DNA polymerase and dNTP, in each PCR amplification, which cycled 40 times (95 °C, 30 s; 54 °C, 30 s; 72 °C, 30 s) in a Perkin-Elmer Thermal Cycler PCR 2400.

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(14) Soft-landing of SWIFT selected DNA was demonstrated for a mixture of two synthetic single strand DNAs. After mass analysis, a SWIFT excitation waveform for ion isolation was generated. An FTICR experimental sequence, which included ion injection, cooling, SWIFT isolation, and soft-landing steps, was repeated for 300 cycles so that all charge states of the selected lower molecular weight DNA[15] were soft-landed at low energy on the membrane. An electrically biased metal grid in front of the membrane prevented unselected species from reaching the surface during ion injection and trapping.

(15) The sequence of the soft-landed single strand DNA is: 5′-ACATCT-TACACATCACCAC TTAAACTGGAATCTTCCCATACATTCAATCC-3′ while the other synthetic DNA in the ESI solution has a G instead of C (at the underlined position). Both DNA and related primers were synthesized in the University of South Carolina oligonucleotide synthesis facility. PCR were carried out using synthetic and commercial reagents (95 °C, 30 s; 46 °C, 30 s; 72 °C, 30 s for 10 cycles, followed by 95 °C, 30 s; 48 °C, 30 s; 72 °C, 30 s for 30 cycles). PCR products were purified and desalted by ethanol precipitation, microdialyzed, and concentrated before analysis by ESI-FTICR.

(16) The amount of one attomole DNA soft-landed was estimated based on the visual relative "spot" intensity on gel electrophoresis (compared to controlled amplification, e.g. Figure 1B). The FTICR cell typically has a charge capacity of 1 million. After 300 cycles of ion injection, manipulation, and soft-landing, tens of millions of ions may be ejected onto the membrane. The cell charge capacity sets an upper limit for the number of soft-landed ions (tens of attomoles) and the experimental results from gel electrophoresis after PCR amplification indicated a maximum of a few percent of ions survived the entire process.

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