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Detection of single macromolecules using a cryogenic particle detector coupled to a biopolymer mass spectrometer

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Macromolecules with masses up to 50 kDa have been detected with a cryogenic particle detector in a MALDI time-of-flight biopolymer mass spectrometer. The cryogenic particle detector was a Sn/Sn-ox/Sn tunnel junction operated at a temperature of 0.4 K. A calibration with 6 keV single photons inferred that the delayed detector pulses corresponded to the absorption of the kinetic energy of a single macromolecule. Time-of-flight spectra of lysozyme proteins are presented. The mass resolution is 100 Da at 14 300 Da. The energy sensitive detection mechanism suggests that cryogenic particle detectors have a high and mass independent detection efficiency for macromolecules. © *1996 American Institute of Physics.* [S0003-6951(96)03524-3]

In this letter, we report on experimental results obtained with cryogenic particle detectors to measure the arrival times of macromolecules in a biopolymer mass spectrometer.[1] Specifically, Sn/Sn-ox/Sn tunnel junctions in the single quasiparticle tunneling mode have been used. The motivation for using cryogenic particle detectors, which are operated at temperatures below 1 K, is the expected high detection efficiency for macromolecules of arbitrary high mass. This is due to the calorimetric detection principle of cryogenic particle detectors, in which the phonons (lattice vibrations) play an essential role.[2,3] A mass spectrometer design using these detectors to efficiently detect large DNA-fragments has been proposed in order to enhance DNA-sequencing throughput.[1]

The detection efficiency of conventional ionizing detectors in biopolymer mass spectrometers decreases rapidly with increasing macromolecule mass,[4] which constitutes a severe limitation. The reason is a threshold effect owing to the small energy transfer of massive macromolecules to electrons which are bound with a finite binding energy to the detector medium.[5] Because macromolecules interact with a detector only on the surface, secondary electron emission detectors are used in biopolymer mass spectrometers. On impact, the charge of the macromolecule ejects an electron out of the surface of the detector which subsequently produces an avalanche of secondary electrons. Secondary electron emission detectors produce fast and large signals, but the quantum efficiency of emitting the first electron decreases rapidly with increasing macro-molecule mass.

In a cryogenic particle detector, the mechanism for detecting a macromolecule is quite different. The kinetic energy, gained by the macromolecule in the electrostatic acceleration field in the mass spectrometer, is deposited onto the surface of the detector. There, in a time scale of less than a nanosecond, phonons (lattice vibrations) with energies of the order of several meV are created, which then propagate into the detector medium. The relaxation times of those excitations are sufficiently long so that they can be collected and converted into an electronic signal with appropriate phonon sensors. Superconducting tunnel junctions are one of the various types of cryogenic phonon sensors which have been developed in the last decade[2,3] and are employed in this study.

In a time-of-flight mass spectrometer, the mass is determined by the time difference between a given start signal and the detector signal. The energy gained by the macromolecule in the electric field is qU, where q is the charge and U the acceleration voltage. On the other hand, the kinetic energy at the position of the detector is $Mv^2/2$, where M is the mass and v the velocity of the macromolecule. Hence, for comparatively short acceleration, the time-of-flight is proportional to both $\sqrt{M}$ and $1/\sqrt{U}$. Equally charged macromolecules have identical kinetic energy when reaching the detector, independent of the value of the macromolecule's mass. Energy sensitive cryogenic particle detectors therefore have no intrinsic mass bias. In addition, because of the direct proportionality of the kinetic energy to the charged state of macromolecule, multiply charged mass spectra could be deconvoluted directly.

The experimental setup is shown in Fig. 1: a custom made MALDI-TOF (matrix assisted laser depletion/ionization time-of-flight) mass spectrometer[4,6] was connected to a helium-3 cryostat (base temperature 0.4 K) on which the Sn-junctions were mounted. The MALDI-probe consisted of a lysozyme protein solution (M=14300 Da) mixed in a sinapic acid matrix (M=224 Da). The lysozyme solution was 1 mg lysozyme (Sigma L-7001) dissolved in 1

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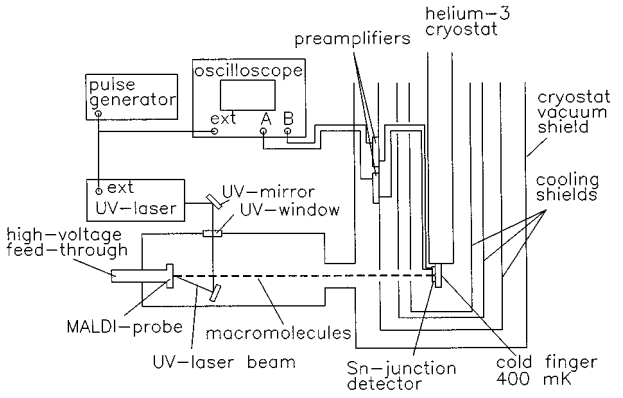

FIG. 1. Experimental setup for detecting macromolecules with a cryogenic particle detector coupled to a custom made MALDI-TOF.

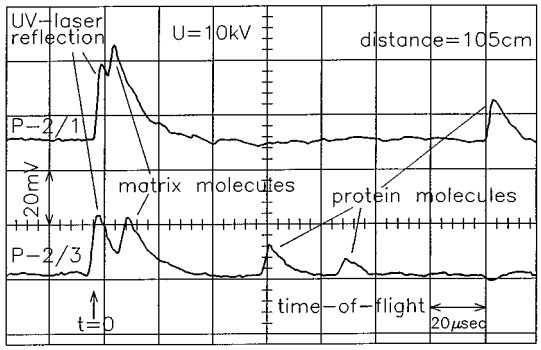

FIG. 2. The response of two Sn-junction detectors to the same molecule releasing UV-laser event as recorded by a dual trace oscilloscope.

ml of HPLC grade H_2O together with 0.1% of trifluoricacid. The matrix solution was 100 mg sinapic acid (Fluka 85430) dissolved in 6 ml ethanol and 4 ml HPLC grade H_2O. A 20 μl aliquot of each solution was mixed and deposited onto a single crystal silicon substrate (12 mm$\times$12 mm) and dried in air under an infrared lamp. The result was a film of small sinapic acid crystals (50–100 μm) covering the entire surface of the silicon substrate. The MALDI-probe was glued with silver paste onto a 5 cm$\times$5 cm metal plate which was mounted on a high vacuum feed-through. An UV-laser (LSI VSL-337) was triggered externally (0.5 Hz) yielding a 3 ns laser pulse of wavelength 337 nm with an energy of 100 μJ per pulse. The laser beam was focused onto the MALDI probe yielding a focal spot of the order of 100 μm^2. In the MALDI-scheme,[6] the high peak power of the laser pulse in the focal spot evaporates matrix crystals, thereby releasing unfragmented proteins. The position of the focal spot on the MALDI-probe could be varied by displacing the beam with a 2-axis UV-mirror. A trajectory calculation indicated that the diameter of the molecular beam was of the order of 5 mm at the detector position (105 cm). The vacuum in the flight path was 10^{-5} mbar.

For this feasibility experiment, superconducting Sn/Sn-ox/Sn tunnel junctions were chosen as cryogenic particle detectors. They can be fabricated with simple methods,[7] are theoretically well understood[8] and have shown an energy sensitivity of the order of 50 eV.[9–11] Their major disadvantage, however, is their limited life time with regards to thermal recycling. Fortunately for future applications, more stable cryogenic particle detectors have been developed in the last decade, producing both robust and sensitive detectors.[2,3] A superconducting tunnel junction consists of two thin superconducting films separated by a thin oxide barrier of the order of a few 10 Å. When energy is deposited into one of the superconducting films, Cooper pairs (coherent electronic states with binding energy of the order of meV) are broken and excess quasiparticles (''normal'' electrons) are produced. This induces an increase in the quasiparticle tunneling current through the insulating barrier of the junction (for more details see Ref. 2). Two identical Sn/Sn-ox/Sn junctions were fabricated by thermal evaporation onto the same single crystal substrate. The Sn-films were patterned by a mechanical mask and the Sn-film was oxidized by using the dc glow discharge method.[8] Each Sn-junction consisted of two Sn-films (thickness=100 nm) with a width of 20 μm and an overlapping region of 80 μm. The two junctions were separated by a distance of 250 μm. Each junction was connected to its own charge sensitive preamplifier (Amptek A250) and the junctions were current biased.

In Fig. 2, the simultaneous recording of the two preamplifier outputs following an UV-laser event are shown (charge sensitive preamplifier output). The waveforms were recorded by a dual-trace oscilloscope which was externally triggered by the same TTL-pulse which triggered the UV-laser (see Fig. 1). At trigger time (t=0), one can see a pulse which is due to the reflected UV-light absorbed in the Sn-junctions (UV-photons have sufficient energy to break Cooper pairs in the Sn-junctions). Delayed pulses are apparent which are due to the slower macromolecules emitted from the MALDI probe. The earlier pulses are from the sinapic acid matrix molecules, and the later pulses due to the more massive, and hence slower, lysozyme proteins. In order to obtain time-of-flight spectra, the preamplifier pulses were shaped by a pulse shaping amplifier (τ=500 ns) and the resulting waveforms were digitized by a PC-based analog/digital waveform analyzer (5 MHz sampling rate and 8-bit pulse height resolution). The 50 μs pretrigger and 200 μs postrigger portion of the digitized waveform of each event was then stored in the PC. Time-of-flight spectra were then obtained by scanning each event and measuring the time difference between the delayed pulse and the UV-light absorption pulse. These time differences were then accumulated into a histogram.

In Fig. 3(a), the time-of-flight histogram is shown for events between t=0 and t=70 μs. The width of a single bin is 350 ns. The first peak corresponds to the sinapic acid molecules which have a time-of-flight of 8 μs for U=20 kV and d=105 cm. The second peak is due to lysozyme proteins with a time-of-flight of 63 μs. In Fig. 3(b), the time-of-flight spectrum for events between 20 μs and 170 μs is shown. The width of a bin is 850 ns. The peak at 63 μs is again due to the single charged lysozyme molecule and the peaks at later times are due to higher mass multiplicities of lysozyme. The position of these higher mass peaks show the expected $\sqrt{M}$ dependence. Data have also been collected at other acceleration voltages and the expected $1/U$ dependence of the peaks was clearly observed.

As can be seen in Fig. 2, the time delayed pulses of the two Sn-junctions are uncorrelated. One of the reasons is that

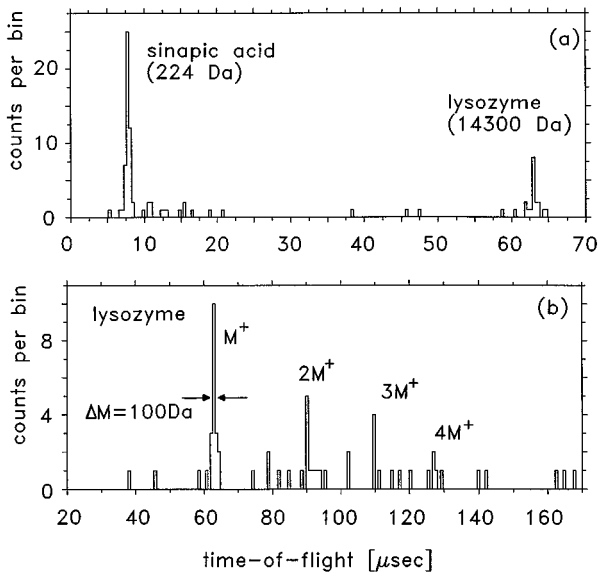

FIG. 3. Time-of-flight histogram (d=105 cm, U=20 kV); (a) time interval 0–70 μs showing sinapic acid and lysozyme peak (bin=350 ns); (b) time interval 20–170 μs showing the lysozyme peak and the peaks due to the higher mass multiplicities of lysozyme (bin=850 ns).

the detector area (20 μm×80 μm) is only 0.008% of the molecular beam (diameter 5 mm). Hence, only a few molecules are expected to hit the detector per UV-laser event. In addition, all of the time delayed events have approximately the same pulse heights indicating that the same kinetic energy is being deposited on the detector regardless of the size of the molecule. The Sn-junction detector was calibrated with a ^{55}Fe source which emits a single 6 keV photon and the observed detector pulses had the same pulse height and pulse shape as the time delayed events produced by macromolecules with a kinetic energy of the order of 10 keV. Futhermore, the estimated reflected UV-light from the 100 μJ pulse reaching the detector at a distance of 105 cm is 70 keV, which is again about the same order of magnitude as the delayed pulses from the macromolecules. We therefore conclude that the delayed pulses shown in Fig. 2 correspond to the deposition of the kinetic energy of a single macromolecule in the Sn-junctions. Most UV-shots produced pulses with a time delay corresponding to the arrival of sinapic acid molecules only. However, sometimes UV-events were recorded showing only lysozyme molecules. This reflects the single counting character of this detector. Hence, the true molecule distribution becomes apparent when adding up the events into a histogram, as shown in Fig. 3. Although the Sn-junction detector signal is fairly slow (Fig. 2), the signal-to-noise is sufficiently large that the arrival time could be measured to a precision of a few 100 ns, leading to a mass resolution of 100 Da at the single lysozyme peak. Cryogenic particle detectors with faster rise times can be made, and it is technologically feasible to fabricate cryogenic particle detectors with areas covering several 10 mm^2.[2,3]

In conclusion, single macromolecules with masses up to 50 kDa have been detected with a cryogenic particle detector coupled to a MALDI-TOF biopolymer mass spectrometer. The detectors were Sn/Sn-ox/Sn superconducting tunnel junctions with a sensitive area of 20×80 μm^2 and the operating temperature was 0.4 K. A time-of-flight spectrum for the protein lysozyme (M=14 300 Da) was obtained showing the single mass peak and the corresponding higher mass multiplicities. A comparison with absorbed 6 keV x rays showed that the delayed pulses correspond to an energy deposition of the order of 10 keV which is the value of the kinetic energy of single macromolecules. This energy sensitive detector mechanism infers that cryogenic particle detectors have no intrinsic mass bias and would yield detectors with a high detection efficiency for massive macromolecules in biopolymer mass spectrometers.

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