Advanced Detectors for Mass Spectrometry

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Mass spectrometry is an instrumental method capable of producing rapid analyses with high mass accuracy. When applied to genome research, it is an attractive alternative to gel electrophoresis. At present, routine DNA analysis by mass spectrometry is seriously constrained to small DNA fragments. Contrasted to other mass spectrometry facilities in which the development of ladder sequencing is emphasized, we are exploring the application of mass spectrometry to procedures that identify short sequences. This approach helps the molecular biologists associated with LBL's Human Genome Center to identify redundant sequences and vector contamination in clones rapidly, thereby improving sequencing efficiency. We are also attempting to implement a rapid mass spectrometry-based screening procedure for PCR products.

The implementation of these applications requires that the performance of matrix-assisted-laser-desorption-ionization (MALDI) and electrospray mass spectrometry is improved. Our focus is the development of new ion detectors which will advance the state-of-the-art of each of these two types of spectrometers. One of the limitations for applying mass spectrometry to DNA analysis relates to the poor efficiency with which conventional electron multipliers detect large ions, a problem most apparent in MALDI-TOF-MS. To solve this problem, we are developing alternative detection schemes which rely on heat pulse detection. The kinetic energy of impacting ions is converted into heat when ions strike a detector and we are attempting to measure indirectly such heat pulses. We are developing a type of cryogenic detector called a superconducting tunnel junction device which responds to the phonons produced when ions strike the detector. This detector does not rely on the formation of secondary electrons. We have demonstrated this type of detector to be at least two orders of magnitude more sensitive, on an area-normalized basis, than microchannel plate ion detectors. This development could extend the upper mass limit of MALDI-TOF-MS and increase sensitivity.

Electrospray ion sources generate ions of mega-Dalton DNA with minimal fragmentation, but the mass spectrometric analyses of these large ions usually leads only to a mass-to-charge distribution. If ion charge was known, actual mass data could be determined. To address this problem, we are developing a detector that will simultaneously measure the charge and velocity of individual ions. We have been able to mass analyze DNA molecules in the 1 to 10 MDa range using charge-detection mass spectrometry. In this technique, individual electrospray ions are directed to fly through a metal tube which detects their image charge. Simultaneous measurement of their velocity provides a way to measure their mass when ions of known energy are sampled. Several thousand ions can be analyzed in a few minutes, thus generating statistically significant mass values regarding the ions in a sample population. We are attempting to apply this technology to the analysis of PCR products.

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Mass Spectrometer for Human Genome Sequencing

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The objective of this program is to develop an innovative fast DNA sequencing technology for the Human Genome Project. It can also be applied to fast screening of genetic and contagious diseases, DNA fingerprinting, and environmental impact analysis.

The approach of this program is to replace conventional gel electrophoresis sequencing methods by using lasers and mass spectrometry for sequencing. The present gel sequencing method usually takes hours to days to acquire DNA analysis or sequencing, since different lengths of DNA segments need to be separated in dense gel. With laser desorption mass spectrometry (LDMS) approach, various sizes of DNA segments are separated in the vacuum chamber of a mass spectrometer. Thus, the time taken to separate various sizes of DNA is less than one second compared to hours using other methods.

Recently, we successfully demonstrated sequencing short DNA segments with this approach. We also have succeeded in using LDMS for fast screening of cystic fibrosis disease. We succeeded in identifying both point mutation and deletion of cystic fibrosis. In addition, we had preliminary success in using LDMS to achieve DNA fingerprinting. Thus, laser desorption mass spectrometry (LDMS) is going to emerge as a new and important biotechnological tool for DNA analysis.

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