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# Novel Mass Spectrometry Mutation Screening for Contaminant Impact Analysis

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Annual Report


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**Research Objective:**

The objective of this program is to develop innovative mass spectrometry technology to achieve fast mutation screening and to reveal the linkage between gene mutation and contaminants. Mass spectrometry has the potential to achieve very fast speed sample analysis. However, the poor mass resolution and low detection efficiency for long DNAs limit the broad application for mutation analysis. New innovative approaches for improving mass resolution and detection sensitivity will be pursued to help to achieve rapid DNA screening. Allele specific polymerase chain reaction (ASPCR) coupled with mass spectrometry for DNA mutation detection will be pursued. This technology is to be applied to wildlife species such as fish for the genotoxic effect of hazardous waste to be assessed at DNA level.

**Research Progress and Implication:**

As of 1 2 year of a 3-year project, we have significant achievement in mass spectrometry DNA analysis technology development. This program is to address the DNA mutation due to the exposure to contaminated media and to promote a better understanding of the relationship between exposure and health impact which are among the top priorities in EMSP program. With the potential of very fast DNA analysis, mass spectrometry technology will be developed to achieve the rapid mutation screening of fish which has been exposed to specific contaminants. Mutations of p53 gene is frequently found in animal tumors and certain human cancers. Analysis of these anti-tumor genes in human cancers as well as laboratory induced animal tumors has indicated that particular carcinogens may be responsible for specific types of tumors. However, the direct linkage of each individual contaminant to specific mutation is usually not known. For genes which are highly conserve, the mutation in animal due to the environmental contaminants can have similar effects on human being. By mutation screening of fish in contaminated water, the relationship of mutation and contaminants can be established. The similar relationship can hold true for human being.

The major achievements during the past 1.5 years are listed and discussed in the follows.

**(1) laser induced acoustic desorption:**

We developed the innovative approach to use laser induced acoustic wave for DNA desorption. In the past, the use of mass spectrometry for DNA detection is by matrix-assisted laser desorption/ionization (MALDI). With MALDI, DNA samples are mixed with small organic compounds. A laser beam with the photon strongly absorbed by the matrix compound but not DNAs is used for desorption and ionization. After the absorption of the laser beam by matrix compounds, the matrix compound gets vaporized and carries DNA molecules into space. The desorbed DNA compounds are subsequently ionized by the protonation or deprotonation process. Although MALDI has had good success in detecting DNAs, the mass resolution is usually poor for large DNA ions due to the broad energy spread caused by the matrix molecules. With laser induced acoustic desorption, no matrix molecules are needed. Thus, better mass resolution can be achieved. With mass resolution improved, rapid mutation detection can be obtained. During the past year and half, we have designed, installed and tested our laser-induced acoustic desorption facility. Mass resolution much better than that from MALDI was obtained.

(2) To couple ASPCR with MALDI for point mutation detection:

Most mutations due to pollutants are point mutation. Since the percentage of cells with mutant DNA is expected to be very small. It is critically important to be able to detect point mutation. We used allele specific primer for PCR amplification of a

mutant p53 template. Then mass spectrometry was subsequently used for the detection of the PCR products. Thus we demonstrated that laser desorption mass spectrometry can be used for mutation detection and screening.

(3) Development of direct sequencing of DNA primers and probes:

DNA primers and short DNA probes are needed for nearly every DNA analysis. However, the sequence of these short DNAs are extremely difficult to check. Since the conclusion of DNA analysis depends on the sequence of these DNA probes or primers, it is critically important to have a method to sequence these short DNAs. We developed matrix-assisted laser desorption/ionization with selected fragmentation (MALDIF) for sequencing short DNAs without the need of DNA ladders. With adequate matrices and laser fluence, short DNAs can be sequenced by selective bond breaking between two DNA bases. We have successfully demonstrated that a large number of short DNA probes can be sequenced.

(4) Successful detection of mutation of p53 gene of medaka fish:

We have analyzed DNA samples from medaka fish which has been exposed to carcinogenic compounds. Since p53 gene in human being is well determined, we used the same sequence for template and select the primers accordingly for PCR reactions for medaka fish DNA samples. We obtained PCR products. It indicates that at least some sections of p53 gene in human and in medaka fish have same sequence. Then we try to design primers for specific point mutation and found DNA samples from some fishes have mutations. It indicates that mutation of p53 gene in medaka fish can be caused by carcinogenic compounds. Furthermore, ASPCR coupled with mass spectrometry can be conveniently used for mutation screening to assess the impact of specific pollutants.

**Planned activities:**

The following tasks will be pursued:

(1) To study the detailed mechanism for laser induced acoustic desorption to further improve the mass resolution and detection sensitivity.
(2) To fabricate ASPCR to extend to more mutation analysis
(3) To develop automation system to increase the analysis speed.
(4) To demonstrate that pollutant-mediated mutation can be used for contaminant impact analysis

**Publications:**

(1) V. V. Golovlev, S. L. Allman , W. R. Garrett, N. I. Taranenko and C. H. Chen, ALaser Induced Acoustic Desorption@, International Journal of Mass Spectrometry and Ion Processes, 169/170, 69-78 (1997)
(2) N. I. Taranenko, V. V. Golovlev, S. L. Allman, N. V. Taranenko, C. H. Chen, J.

Hong and L. Y. Chang, A Matrix-assisted Laser Desorption/Ionization for Short Tandem Repeat Loci@,Rapid Comm. Mass Spectrom. 12, 413-418 (1998)

(3) N. I. Taranenko, S. L. Allman, V. V. Golovlev, N. V. Taranenko, N. R. Isola and C. H. Chen, A Sequencing DNA using mass spectrometry for ladder detection@, Nucleic Acids Research, 26(10), 2488-2490 (1998)

(4) N. I. Taranenko, S. L. Allman, V. V. Golovlev and C. H. Chen, AChemical Cleavage Sequencing of DNA using Matrix-assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry@, Analytical Chemistry (in press)