

Environmental Management Science Program

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Novel Mass Spectrometry Mutation Screening for Contaminant Impact Analysis

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Research Objective

Our objective is to develop innovative mass spectrometry technology to achieve fast mutation screening from contaminated area and to reveal the linkage between gene mutation and contaminants. In this program, we will try innovative approaches to improve mass resolution and detection efficiency for large DNA ions. Allel specific polymerase chain reaction will be coupled with mass spectrometry for rapid DNA mutation detection. The ultimate goal is to lead to the risk analysis of hazardous wastes to be routinely assessed at DNA level at an affordable cost.

Research Progress and Implications

This report is for the work after 7 months of a 3-year project.

I. Research Approach

Mutations of ras proto-oncogenes are frequently found in animal tumors and certain human cancers. Analysis of mutation spectra of ras 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 such as ras, the mutation in animal due to the environmental contaminants may have similar effects on human being. In this work, fishes from controlled contaminated area will be used as a model system to test this hypothesis. The data derived from these experiments can provide a critical linkage between the assessment of environmental contamination and the health effects on human subjected to similar exposure. Since the conventional DNA analysis technology is slow and expensive and a large number of DNA samples need to be analyzed for contaminant assessment, we want to develop new mass spectrometric technology for faster, inexpensive and reliable analysis.

The specific approaches of this program includes the following major tasks:

- (1) To improve mass resolution by soft acoustic desorption;
- (2) To couple with allele specific polymerase chain reaction (PCR) and competitive PCR for quantitative mutation analysis;
- (3) Detection of trace mutatnt DNAs by base specific PCR and mass spectrometry;
- (4) To identify and clone the exon of ras gene by reverse-transcript polymerase chain reaction (RT-PCR); and
- (5) To demonstrate that pollutant-mediated mutations can be used as biological indicators for contaminant impact analysis.

Our major achievements during the past 7 months are in the following:

- (1) Facility set-up for DNA extraction, purification and amplification:

Since our laboratory has been primarily involved in laser and mass spectrometry technology development, little facility for DNA sample preparation is available. During the past 7 month, we have purchased and set up the equipments required for DNA extraction from fish sample analysis. We have designed primers for polymerase chain reaction (PCR) amplification of p53 (tumor suppressor gene) and ras (oncogene) from Medaka fish. These primers were used

to amplify the target sequences and will be subject to conventional heteroduplex analysis methods to detect the presence of point mutations.

(2) Detection of point mutation for p53 with synthetic DNAs:

Since p53 mutation is critically important for several different cancers, the capability to detect the mutation of p53 is very valuable for the assessment of the impact of contaminants. We used allele specific PCR and mass spectrometry to successfully detect point mutation with a simulated p53 gene.

(3) Detection of large DNA fragments from enzyme digestion by matrix-assisted laser desorption/ionization:

Rapid measurements of DNA polymorphism are very valuable for mutation identification. A few DNA samples from microbials have been digested with various enzymes. Laser and mass spectrometry were used to measure these enzyme digested products. These samples were also measured by gel electrophoresis. The results from two different approaches agree each other.

(4) Detection of DNA fragments larger than 1000 nucleotides:

By using new matrices and DNA purification processes, we succeeded in using matrix-assisted laser desorption/ionization for detection of DNA fragments with the size of 1500 base pairs. To our knowledge, it is the largest DNA fragments to be detected by a time-of-flight mass spectrometer. It indicates that mass spectrometer can possibly be used to separate large DNA fragments.

Planned Activities

The following tasks will be pursued in the future.

- (1) Design, install, and test the new detection scheme with laser induced multiphoton ionization of large DNA fragments (10/31/98);
- (2) Design, install, and test DNA desorption by acoustic shaking (12/31/98);
- (3) Develop quantitative PCR mass spectrometry for quantitative DNA measurements (05/31/99);
- (4) Detection of trace gene mutation by ASPCR and mass spectrometry (10/31/99);
- (5) Identify and clone ras gene of fish (03/31/2000); and
- (6) To demonstrate that mutation screening of fish can be used to assess contaminants impact to human health (09/30/2000).