CALIFORNIA STATE SCIENCE FAIR
2007 PROJECT SUMMARY

Name(s)

Steven Chau; Steve Chung

Project Number

S0403

Project Title

Comparative Analysis of Mouse Liver Proteome by One Dimensional and Two Dimensional Gel Electrophoresis

Abstract

Objectives/Goals

To use 1 Dimensional SDS-PAGE along with 2 Dimensional SDS-PAGE to separate proteins in order to see which of the two methods yields a higher resolution of protein.

Methods/Materials

1 Dimensional SDS-PAGE refers to a technique used to count different types of proteins within a sample. Sodium Dodecyl Sulphate, a part of the running buffer, to denature proteins. These denatured, linear molecules are able to pass through the pores in the polyacrylamide gel, but will become trapped according to their molecular masses. After running buffer is added to the polyacrylamide gel, the protein samples are loaded into the gel electrophoresis chamber using a microcapillary pipet. Opposite electrical charges either end of an electrophoresis chamber pull the proteins through the gel at rates dependent on molecular mass.

In 2 Dimensional SDS-PAGE, the same protein mixtures used for the 1 Dimensional SDS-PAGE, mouse liver protein obtained from five weeks old animals, were used. The samples were applied on a specialized strip called an immobilized pH gradient strip and then separated based on their isoelectric points using high voltage. The proteins, located at different parts of on the strip depending on their isoelectric points, were then subjected to second dimensional analysis of the molecular mass using SDS-PAGE. With both Dimensions, the separated proteins were then analyzed using an image analysis system.

Results

When comparing 1D and 2D gel electrophoresis, we found that 2D gel electrophoresis has higher resolving power for different proteins present in a crude mixture. Compared to an average number of 16.5 protein bands obtained by 1D gel electrophoresis, we obtained 367.33 protein spots on an average for the same sample when separated in two dimensions. This is a difference of over 350 proteins, meaning that adding the second dimension increased the number of proteins detected by over 2100%.

Conclusions/Discussion

SDS-PAGE with two dimensions does indeed allow for the detection of more proteins of a given proteome. Two dimensional gel electrophoresis, where proteins are initially separated by isoelectric focusing followed by their separation based on molecular mass, provides a much better resolution. Because the separation was based on two different properties of the protein molecules, 2D SDS-PAGE give a better resolution of individual protein molecules present in the mixture, an over twenty-fold increase.

Summary Statement

This project aims to compare the resolutions of two types of protein resolving techniques in order to determine the more useful method.

Help Received

Used lab equipment at Keck Graduate Institute laboratory under the supervision of Professor Bulbul Chakravarti and Professor Deb Chakravarti.