SBI 4U
J. Kropac

Protein Structure: Making a 'Mystery Protein'

Introduction:

All life forms on Earth contain proteins. Organisms use proteins for two basic functions: making structures(structural proteins) and controlling cell chemistry (enzyme proteins). Both the structural and functional abilities of a protein are determine by the highly specific, three dimensional shape that a protein develops.

Four levels of structure are found in man proteins:

i)**Primary Structure**: The linear sequence of amino acids in the protein chain.
ii) **Secondary structure**: Twisting of amino acids into an alpha helix of beta sheets.
iii)**Tertiary Structure**: The folding over of the helical chain due to chemical interaction of the R- groups on the amino acid chain.
iv) **Quaternary Structure**: Association of two or more polypeptide chains to form a larger protein.

Typically, the techniques that scientists use to work out the structure of proteins are time consuming and expensive. In this lab you will learn about the for levels of protein structure as well as how scientists determine proteins structure and shape. You will be given data from the analysis of a polypeptide, and will determine the likely tertiary shape.

Materials:

40 cm of bare copper wire
colored tabs

Procedure:

In this experiment, we will be looking at a functional protein. Through analysis of a short polypeptide, scientists we able to determine the following amino acids were present:

Amino Acid	Number present in sequence	R-Group
Alanine (ALA)	3	Non-reactive
Aspartic Acid (ASP)	3	Negative R group
Cysteine (CYS)	4	Sulfur containing R group
Glutamine (GLU)	3	Positive R group
Serine (SER)	3	Non-reactive

Primary Sequence

In order to determine protein structure, scientists use digestive enzymes to break apart the protein structure into what we call peptide segments. These protein segments are shorts chains of amino acids. These fragments are like puzzle pieces. By fitting the fragments back together, scientists can determine the primary structure of the protein.

But how?

We can match the sequence of the fragments in the sections where they overlap. For example, in a sample where we had more than one molecule of the proteins, we found the following peptide fragments:

- ala-glu-phe-ser
- tyr-ser-ala-glu
- phe-ser-cys-ala

These sections can only be aligned as follows:

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                phe-ser-cys-ala
                 |   |
          ala-glu-phe-ser
           |   |
   tyr-ser-ala-glu
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Therefore the primary sequence would be:

tyr-ser-ala-glu-phe-ser-cys-ala

Now using the peptide fragments available to you, determine primary structure of your protein:

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Secondary structure

We know that we are analyzing a functional protein (enzyme). Therefore, secondary structure is easy to determine. All functional proteins fold in an alpha helix pattern.

Tertiary Structure

A proteins structure occurs because some of the R groups on amino acids are reactive. How and if they react depends on nearby R groups.

R groups react in many different ways. However, for the purpose of this demonstration, we will be concerned with three reactions:

1)Opposite charges attract each other.
2)Like charges repel.
3)Sulfur atoms strongly attract other sulfur atoms, form <u>disulfide bonds</u>.

Stretch out your piece of wire. Use the page markers provided to mark the position of each amino acid in the primary sequence. Use the following code to track reactive R groups:

- Pink: Positive R Group
- Yellow: Negative R Group
- Green: Sulfur R Group
- Blue: Unreactive R Groups

Now, experiment with folding your protein in such a way that the reactive R groups logically interact. Continue until almost all R groups are reacting.

Quaternary Structure:

Now that we have determined tertiary structure, what would we have to do to create a quaternary structure?