**COURSE BOOK OF**

**CHEMISTRY 2**

**(BIOCHEMISTRY)**

**Desert land and reclamation program**

**Level 1**

**Department of Biochemistry**

**Benha University, Agriculture College**

**Theoretical**

**Theoretical of Chemistry 2**

**BIOCHEMISTRY**

**AC 0102**

**There Are Several Levels of Protein Structure**

For large macromolecules such as proteins, the tasks of describing and understanding structure are approached at several levels of complexity, arranged in a kind of conceptual hierarchy. Four levels of protein structure are commonly defined. A description of all covalent bonds (mainly peptide bonds and disulfide bonds) linking amino acid residues in a polypeptide chain is its primary structure. The most important element of primary structure is the sequence of amino acid residues. Secondary structure refers to particularly stable arrangements of amino acid residues giving rise to recurring structural patterns. Tertiary structure describes all aspects of the three-dimensional folding of a polypeptide. When a protein has two or more polypeptide subunits, their arrangement in space is referred to as quaternary structure.

**Working with Proteins**

Our understanding of protein structure and function has been derived from the study of many individual proteins. To study a protein in detail, the researcher must be able to separate it from other proteins and must have the techniques to determine its properties. The necessary methods come from protein chemistry, a discipline as old as biochemistry itself and one that retains a central position in biochemical research.

**Proteins Can Be Separated and Purified**

A pure preparation is essential before a protein’s properties and activities can be determined. Given that cells contain thousands of different kinds of proteins, how can one protein be purified? Methods for separating proteins take advantage of properties that vary from one protein to the next, including size, charge, and binding properties. The source of a protein is generally tissue or microbial cells. The first step in any protein purification procedure is to break open these cells, releasing their proteins into a solution called a crude extract. If necessary, differential centrifugation can be used to prepare subcellular fractions or to isolate specific organelles. Once the extract or organelle preparation is ready, various methods are available for purifying one or more of the proteins it contains. Commonly, the extract is subjected to treatments that separate the proteins into different fractions based on a property such as size or charge, a process referred to as fractionation. Early fractionation steps in a purification utilize differences in protein solubility, which is a complex function of pH, temperature, salt concentration, and other factors. The solubility of proteins is generally lowered at high salt concentrations, an effect called “salting out.” The addition of a salt in the right amount can selectively precipitate some proteins, while others remain in solution. Ammonium sulfate ((NH4)2SO4) is often used for this purpose because of its high solubility in water.

A solution containing the protein of interest often must be further altered before subsequent purification steps are possible. For example, dialysis is a procedure that separates proteins from solvents by taking advantage of the proteins’ larger size. The partially purified extract is placed in a bag or tube made of a semipermeable membrane. When this is suspended in a much larger volume of buffered solution of appropriate ionic strength, the membrane allows the exchange of salt and buffer but not proteins. Thus dialysis retains large proteins within the membranous bag or tube while allowing the concentration of other solutes in the protein preparation to change until they come into equilibrium with the solution outside the membrane. Dialysis might be used, for example, to remove ammonium sulfate from the protein preparation.

The most powerful methods for fractionating proteins make use of column chromatography, which takes advantage of differences in protein charge, size, binding affinity, and other properties. A porous solid material with appropriate chemical properties (the stationary phase) is held in a column, and a buffered solution (the mobile phase) percolates through it. The protein-containing solution, layered on the top of the column, percolates through the solid matrix as an ever-expanding band within the larger mobile phase. Individual proteins migrate faster or more slowly through the column depending on their properties. For example, in cation-exchange chromatography the solid matrix has negatively charged groups. In the mobile phase, proteins with a net positive charge migrate through the matrix more slowly than those with a net negative charge, because the migration of the former is retarded more by interaction with the stationary phase. The two types of protein can separate into two distinct bands. The expansion of the protein band in the mobile phase (the protein solution) is caused both by separation of proteins with different properties and by diffusional spreading. As the length of the column increases, the resolution of two types of protein with different net charges generally improves. However, the rate at which the protein solution can flow through the column usually decreases with column length. And as the length of time spent on the column increases, the resolution can decline as a result of diffusional spreading within each protein band.

Size exclusion chromatography separates proteins according to size. In this method, large proteins emerge from the column sooner than small ones a somewhat counterintuitive result. The solid phase consists of beads with engineered pores or cavities of a particular size. Large proteins cannot enter the cavities, and so take a short (and rapid) path through the column, around the beads. Small proteins enter the cavities, and migrate through the column more slowly as a result. Affinity chromatography is based on the binding affinity of a protein. The beads in the column have a covalently attached chemical group. A protein with affinity for this particular chemical group will bind to the beads in the column, and its migration will be retarded as a result.

A modern refinement in chromatographic methods is HPLC, or high-performance liquid chromatography. HPLC makes use of high-pressure pumps that speed the movement of the protein molecules down the column, as well as higher-quality chromatographic materials that can withstand the crushing force of the pressurized flow. By reducing the transit time on the column,

HPLC can limit diffusional spreading of protein bands and thus greatly improve resolution.

The approach to purification of a protein that has not previously been isolated is guided both by established precedents and by common sense. In most cases, several different methods must be used sequentially to purify a protein completely. The choice of method is somewhat empirical, and many protocols may be tried before the most effective one is found. Trial and error can often be minimized by basing the procedure on purification techniques developed for similar proteins.

Published purification protocols are available for many thousands of proteins. Common sense dictates that inexpensive procedures such as salting out be used first, when the total volume and the number of contaminants are greatest. Chromatographic methods are often impractical at early stages, because the amount of chromatographic medium needed increases with sample size. As each purification step is completed, the sample size generally becomes smaller making it feasible to use more sophisticated (and expensive) chromatographic procedures at later stages.

**Steps in the Determination of Amino Acids Sequence:**

1. If the protein contains more than one polypeptide chain, the individual chains are fist separated and purified.
2. All the disulfide groups are reduced and the resulting sulfhydryl groups alkylated.
3. A sample of each polypeptide chain is subjected to total hydrolysis, and its amino acids composition is determined.
4. On another sample of the polypeptide chain the N-Terminal and C-Terminal residues are identified.
5. The intact polypeptide chain is cleaved into a series of smaller peptides by enzymatic or chemical hydrolysis.
6. The peptide fragments resulting from step 5 are separated and their amino acid composition and sequences are determined.
7. Another sample of the original polypeptide chain is partially hydrolyzed by a second procedure to fragment the chain at points other than those cleaved by the first partial hydrolysis. The peptide fragments are separated and their amino acid composition and sequence determined (as in steps 5 and 6).
8. By comparing the amino acids sequences of the two sets of peptide fragments particularly where the fragments from the first partial hydrolysis overlap the cleavage points in the second, the peptide fragments can be placed in the proper order to yield the complete amino acid sequence.
9. The positions of the disulfide bonds and the amide groups in the original polypeptide chain are determined.

 

**The Amino Acid Sequences of Millions of Proteins Have Been Determined**

**Short Polypeptides Are Sequenced Using Automated Procedures**

Various procedures are used to analyze protein primary structure. Several protocols are available to label and identify the amino-terminal amino acid residue. Sanger developed the reagent 1-fluoro-2,4-dinitrobenzene (FDNB) for this purpose; other reagents used to label the amino-terminal residue, dansyl chloride and dabsyl chloride, yield derivatives that are more easily detectable than the dinitrophenyl derivatives. After the amino-terminal residue is labeled with one of these reagents, the polypeptide is hydrolyzed to its constituent amino acids and the labeled amino acid is identified. Because the hydrolysis stage destroys the polypeptide, this procedure cannot be used to sequence a polypeptide beyond its amino-terminal residue. However, it can help determine the number of chemically distinct polypeptides in a protein, provided each has a different amino-terminal residue. For example, two residues Phe and Gly would be labeled if insulin were subjected to this procedure.



**Steps in sequencing a polypeptide**. (a) Identification of the amino-terminal residue can be the first step in sequencing a polypeptide. Sanger’s method for identifying the amino-terminal residue is shown here. (b) The Edman degradation procedure reveals the entire sequence of a peptide. For shorter peptides, this method alone readily yields the entire sequence, and step (a) is often omitted. Step (a) is useful in the case of larger polypeptides, which are often fragmented into smaller peptides for sequencing.

**References:**

* Lehninger Principles of Biochemistry (Nelson W. H. Freeman. 4th Ed, 2004).