Exp.6 Quantitative tests of proteins

Introduction:

Spectroscopic methods are routinely used to determine the concentration of protein in a solution, include measurement of the protein's intrinsic UV absorbance and protein-dye complex (table 6.1), such as the Folin-phenol (Lowry), the Bradford dye assay. This requires a series of standard solutions, with a different or same composition as the sample solutions, to construct calibration curve at the same reaction conditions.

Although one or more these methods are used routinely in almost every biochemical laboratory, none of the procedures are particularly convenient and can be used for all proteins. These methods may be affected by other substances frequently present in biochemical solutions, including detergents, lipids, buffers and reducing agents.

A. Biuret test:

The biuret test is given by all compounds that contain **two or more peptide bonds**. Dipeptide and amino acids (except serine and threonine) do not give this reaction. The blue color produced is due to the formation of a coordinate complex of the Cu^{2+} and four nitrogen atoms, to form each of two adjacent peptide chains. The name of the reaction comes from the organic compound biuret which gives a positive test. The intensity of color depends on the peptide bonds present in proteins.

B. Bradford assay:

A simpler, faster, more sensitive and accurate procedure than the Lowry method, described first by Bradford (Bradford et al., 1976), has become preferred method for quantifying protein in many laboratories. Furthermore, when compared with other methods, it is subject to less interference by common reagents and nonprotein components of biological samples, except TritonX-100 and SDS (sodium dodecyl sulfate).

The Bradford assay relies on the binding of the acidic dye Coomassie Blue G-250 to proteins, when it binds to basic and aromatic amino acids resulting in a shift of the absorbance maximum from 465 nm (brownish) to 595 nm (blue). The increase of absorbance at 595 nm is proportional to the amount (concentration) of protein present in the sample. Compare the results with those obtained from known amounts of protein. Thus, the quantity of protein present in the sample can be estimated.

Table 6.1 Methods for determining the concentration of protein

Method	Biuret	Lowry	Bradford	BCA	Spectrophoto-metric(Warburg-Christian)
Sensitivity	Low (1-20mg)	High (~5μg)	High (~1μg)	High (~1μg)	Moderate (50-100μg)
Time	Moderate (20-30min)	Slow (40-60min)	Rapid (~15min)	Slow (~60min)	Rapid (5-10min)
Principle	Peptide bonds + alkaline Cu^{2+} → purple complex	(1) Biuret reaction (2) phosphomolybdate-phosphotungstate by Tyr and Trp	max of Coomassie dye shifts from 465nm to 595nm when protein-bound	(1) Biuret reaction (2) Copper complex with BCA; max=562nm	Absorption of 280nm light by Tyr and Trp residues
Interferences	Good's buffers, Tris, some amino acids	Ammonium-sulfate, glycine, Good's buffers, mercaptans	Strongly basic buffers, Triton X-100, SDS	EDTA, dithiothreitol, ammonium-sulfate	Purines, pyrimidines, nucleic acids
Comments	1)Fast but not sensitive. 2)Similar color with all proteins	1)Time-consuing. 2)Color varies with proteins. 3)Critical timing of procedure.	1)Excellent method 2)only slight interferences 3)stable color which varies with proteins 4)Reagent commercially available.	1)Compatiable with detergents. 2)Reagents commercially available.	1)Useful for monitoring column eluents. 2)Neucleic acid absorption can be corrected.

Reagent:

1. Standard albumin solutions
2. Unknown
3. Biuret reagent
4. Bradford reagent

Procedure:

A. Biuret test:

Pipette 1.0mL of standard albumin solutions, unknown solution and water into separate colorimetric tubes, then drop 2.0mL of biuret reagent into each tube, mix well, stand for 10 min, and read the absorbance at 540nm.

Blank: 1.0mL H_2O + 2.0mL biuret reagent for adjusting 0% T and 0Abs/100%T

Sample	3mg/ml	6mg/ml	9mg/ml	Unknown
Abs_{540}				

B. Bradford test:

Pipette 2.0mL of standard albumin solutions, unknown solution and water into separate test tubes, then drop 2.0mL of bradford reagent into each tube, mix well and drop 3.0mL of the mixed solution into colorimetric tubes, then read the absorbance at 595nm after 20 minutes. Rinse tubes with detergent.

Blank: 2.0mL H_2O + 2.0mL Bradford reagent (then drop 3.0mL of the mixed solution into colorimetric tubes for adjusting 0% T and 0Abs/100%T)

Sample	4 μ g/ml	8 μ g/ml	12 μ g/ml	Unknown
Abs_{595}				

Results：

1. Record your results.
2. Calculate the concentration of unknown in each part.

Discussions：