The lowry method for protein quantitation

Has been the most widely used method to estimate the total level of protein already in solution or easily soluble in dilute alkali in biological samples, the Lowry Assay (Lowry et al., 1951) exhibited a color change of the biological samples in proportion to protein concentration. The color change can be then measured using colorimetric techniques.

With sensitivity down to about 0. 01 mg of protein/mL, the reaction is best used on solutions with protein concentrations ranged in 0. 01-1. 0 mg/mL. The Lowry assay is based on 2 reactions, namely Biuret reaction and the Folin-Ciocalteau reaction. Biuret reaction sees the peptide bonds of proteins, under alkaline reaction, reacts with copper to produce Cu+ ions which react with the Folin reagent. This is called a Biuret chromophore and is commonly stabilized by the addition of tartrate (Gornall et al., 1949).

Folin-Ciocalteau reaction is commonly poorly understood. However, in essence the second reaction is a reduction of the Folin-Ciocalteau reagent (phosphomolybdate and phosphotungstate), primarily by the reduced copper amide bond complex, as well as by tyrosine and tryptophan residues. The reagent is reduced to heteropolymolybdenum blue.

The end product of the two reactions has a strong blue color, which in turn depends partially on the content of tyrosine and tryptophan. The amount of protein in the sample can then be estimated via reading the absorbance (at 750 nm) of the end product of the Folin reaction against a standard curve of a selected protein solution. An example would be Bovine Serum Albumin (BSA) solution.

The Biuret reaction itself is not very sensitive. Lowry protein assay requires more time than other assays and is susceptible to many interfering compounds. While widely used, the Lowry procedure is less preferable an assay than some other protein assays since it is more subject to interference by a wide variety of chemicals. This is one of the major limitations of the assay as many of these interfering substances are commonly used in buffers for preparing proteins or in cell extracts. Known compounds to interfere with the Lowry assay are detergents, carbohydrates, glycerol, EDTA, potassium compounds, sulfhydryl compounds, disulfide compounds, most phenols, uric acid etcetera.

The Lowry assay is also sensitive to variations in the content of tyrosine and tryptophan residues. The assay is linear over the range of 1 to 100Î¼g protein. The absorbance can be read in the region of 500 to 750nm, with 660 nm being the most commonly employed. Other wavelengths can also be used, but it may reduce the effects of contamination.

Using the Folin Ciocalteu reagent to detect reduced copper makes the Lowry assay nearly 100 times more sensitive than the Biure treaction alone. Several useful modifications of the original Lowry assay have been developed to increase the dynamic range of the assay over a wider protein concentration (Hartree, 1972), to make the assay less sensitive to interference by detergents (Dulley and Grieve, 1975), and to first precipitate the proteins to remove interfering contaminants (Bensadoun and Weinstein, 1976).

There is also much protein-to-protein variation in the intensity of color development. Ideally, the standard should be similar to the unknown. For example, if one is measuring IgG concentrations, an immunoglobulin standard would be ideal. For serum, use bovine serum albumin as a standard since albumin is a major component of serum.

An easy and accurate alternative, based on the binding of protein to Coomassie Blue G-250 dye, is the Bradford procedure. In addition, a modification of the Lowry procedure exists based on use of bicinchoninic acid (BCA) in place of the Folin-phenol reagent [Smith et al., Anal Biochem. 150, 76-85 (1985)]. The BCA is less prone to interference than the Lowry procedure and is more sensitive.

Bradford Method

Compared to Lowry Method, Bradford stands out for its simplicity by using only one reagent and greater sensitivity which is four times more than Lowry method. Bradford method is just another common colorimetric method to determine protein concentration in a sample solution. The Bradford method of protein determination is based on the binding of a dye, Coomasie brilliant Blue and shift of absorbance maximum of the dye from 495nm to 595 nm and is proportional to protein concentration when compared to a standard curve.

The Bradford assay is very fast and uses about the same amount of protein as the Lowry assay. It is fairly accurate and samples that are out of range can be retested within minutes. The Bradford is recommended for general

use, especially for determining protein content of cell fractions and assessing protein concentrations for gel electrophoresis.

Assay materials include colour reagent and protein standard. It is sensitive to about 5 to 200 micrograms protein, depending on the dye quality. In assays using 5 ml colour reagent prepared in lab, the sensitive range is closer to 5 to 100 µg protein. Scale down the volume for the “ microassay procedure,” which uses 1 ml cuvettes.

The assay is based on the observation that the shift of the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 when binding to protein occurs. Both hydrophobic and ionic interactions stabilize the anionic form of the dye, causing a visible colour change. The assay is useful since the extinction coefficient of a dye-albumin complex solution is constant over a 10-fold concentration range.

The Bradford reagent is prepared by dissolving 100 mg Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol and added 100 ml 85% (w/v) phosphoric acid. 1 liter of it is dilutes when the dye has completely dissolved and it need to be filtered through Whatman #1 paper just before use. However, there is an optional whereby 1 M NaOH can be used if samples are not readily soluble in the colour reagent). The Bradford reagent should be a light brown in color. Filtration may have to be repeated to rid the reagent of blue components. The Bio-Rad concentrate is expensive, but the lots of dye used have apparently been screened for maximum effectiveness. “ Homemade” reagent works quite well but is usually not as sensitive as the Bio-Rad product.

A standard curve of absorbance versus micrograms protein and determine amounts from the curve is plotted. The concentrations of original samples from the amount protein, volume/sample, and dilution factor are determined through the graph.

The dye reagent reacts primarily with arginine residues and less so with histidine, lysine, tyrosine, tryptophan, and phenylalanine residues. Obviously, the assay is less accurate for basic or acidic proteins. The Bradford assay is rather sensitive to bovine serum albumin, more so than “ average” proteins, by about a factor of two. Immunoglogin G (IgG – gamma globulin) is the preferred protein standard. The addition of 1 M NaOH was suggested by Stoscheck (1990) to allow the solubilization of membrane proteins and reduce the protein-to-protein variation in color yield.

However, it has some limitation (Bergmeyer & Grabl, 1983). The standard curves are not linear for many proteins, especially those with more than 60ug of proteins. This inherent nonlinearity is caused by the reagent itself as there is an overlap in the spectrum of the two different colour form of the dye. The background value of the reagent is continually decreasing when more proteins is bound to the dye. Absorbance may also vary with the age of the reagent. Another serious issue is the variations of the response with different proteins. When tested with 23 different proteins, the standard deviation in estimates of the proteins concentration by Bradford method was twice the value obtained by Lowry method. Hence, it is important to use a standard pprotein that gives a similar colour yield.