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The Lowry Method for Protein Quantitation

Jakob H. Waterborg

1. Introduction

The most accurate method of determining protein concentration is probably acid hydrolysis followed by amino acid analysis. Most other methods are sensitive to the amino acid composition of the protein, and absolute concentrations cannot be obtained *(1)*. The procedure of Lowry et al. *(2)* is no exception, but its sensitivity is moderately constant from protein to protein, and it has been so widely used that Lowry protein estimations are a completely acceptable alternative to a rigorous absolute determination in almost all circumstances in which protein mixtures or crude extracts are involved.

The method is based on both the Biuret reaction, in which the peptide bonds of proteins react with copper under alkaline conditions to produce Cu^{+}, which reacts with the Folin reagent, and the Folin–Ciocalteau reaction, which is poorly understood but in essence phosphomolybdotungstate is reduced to heteropolybdenum blue by the copper-catalyzed oxidation of aromatic amino acids. The reactions result in a strong blue color, which depends partly on the tyrosine and tryptophan content. The method is sensitive down to about 0.01 mg of protein/mL, and is best used on solutions with concentrations in the range 0.01–1.0 mg/mL of protein.

2. Materials

1. Complex-forming reagent: Prepare immediately before use by mixing the following stock solutions in the proportion 100:1:1 (by vol), respectively:
   Solution A: 2% (w/v) Na_2CO_3 in distilled water.
   Solution B: 1% (w/v) $CuSO_4$ $5H_2O$ in distilled water.
   Solution C: 2% (w/v) sodium potassium tartrate in distilled water.
2. 2 *N* NaOH.
3. Folin reagent (commercially available): Use at 1 *N* concentration.

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4. Standards: Use a stock solution of standard protein (e.g., bovine serum albumin fraction V) containing 2 mg/mL protein in distilled water, stored frozen at –20°C. Prepare standards by diluting the stock solution with distilled water as follows:

Stock solution (µL)	0	2.5	5	12.5	25	50	125	250	500
Water (µL)	500	498	495	488	475	450	375	250	0
Protein conc. (µg/mL)	0	10	20	50	100	200	500	1000	2000

3. Method

1. To 0.1 mL of sample or standard (*see* **Notes 1–4**), add 0.1 mL of 2 *N* NaOH. Hydrolyze at 100°C for 10 min in a heating block or boiling water bath.
2. Cool the hydrolysate to room temperature and add 1 mL of freshly mixed complex-forming reagent. Let the solution stand at room temperature for 10 min (*see* **Notes 5** and **6**).
3. Add 0.1 mL of Folin reagent, using a vortex mixer, and let the mixture stand at room temperature for 30–60 min (do not exceed 60 min) (*see* **Note 7**).
4. Read the absorbance at 750 nm if the protein concentration was below 500 µg/mL or at 550 nm if the protein concentration was between 100 and 2000 µg/mL.
5. Plot a standard curve of absorbance as a function of initial protein concentration and use it to determine the unknown protein concentrations (*see* **Notes 8–13**).

4. Notes

1. If the sample is available as a precipitate, then dissolve the precipitate in 2 *N* NaOH and hydrolyze as described in **Subheading 3., step 1**. Carry 0.2-mL aliquots of the hydrolyzate forward to **Subheading 3., step 2**.
2. Whole cells or other complex samples may need pretreatment, as described for the Burton assay for DNA *(3)*. For example, the perchloroacetic acid (PCA)/ethanol precipitate from extraction I may be used directly for the Lowry assay, or the pellets remaining after the PCA hydrolysis step (**Subheading 3., step 3** of the Burton assay) may be used for Lowry. In this latter case, both DNA and protein concentration may be obtained from the same sample.
3. Peterson *(4)* has described a precipitation step that allows the separation of the protein sample from interfering substances and also consequently concentrates the protein sample, allowing the determination of proteins in dilute solution. Peterson's precipitation step is as follows:
   a. Add 0.1 mL of 0.15% deoxycholate to 1.0 mL of protein sample.
   b. Vortex-mix, and stand at room temperature for 10 min.
   c. Add 0.1 mL of 72% trichloroacetic acid (TCA), vortex-mix, and centrifuge at 1000–3000 *g* for 30 min.
   d. Decant the supernatant and treat the pellet as described in **Note 1**.
4. Detergents such as sodium dodecyl sulfate (SDS) are often present in protein preparations, added to solubilize membranes or remove interfering

substances ***(5–7)***. Protein precipitation by TCA may require phosphotungstic acid (PTA) ***(6)*** for complete protein recovery:

a. Add 0.2 mL of 30% (w/v) TCA and 6% (w/v) PTA to 1.0 mL of protein sample.
b. Vortex-mix, and stand at room temperature for 20 min.
c. Centrifuge at 2000 *g* and 4°C for 30 min.
d. Decant the supernatant completely and treat the pellet as described in **Note 1**.

5. The reaction is very pH dependent, and it is therefore important to maintain the pH between 10 and 10.5. Therefore, take care when analyzing samples that are in strong buffer outside this range.
6. The incubation period is not critical and can vary from 10 min to several hours without affecting the final absorbance.
7. The vortex-mixing step is critical for obtaining reproducible results. The Folin reagent is reactive only for a short time under these alkaline conditions, being unstable in alkali, and great care should therefore be taken to ensure thorough mixing.
8. The assay is not linear at higher concentrations. Ensure that you are analyzing your sample on the linear portion of the calibration curve.
9. A set of standards is needed with each group of assays, preferably in duplicate. Duplicate or triplicate unknowns are recommended.
10. One disadvantage of the Lowry method is the fact that a range of substances interferes with this assay, including buffers, drugs, nucleic acids, and sugars. (The effect of some of these agents is shown in Table 1 in Chapter 3.) In many cases, the effects of these agents can be minimized by diluting them out, assuming that the protein concentration is sufficiently high to still be detected after dilution. When interfering compounds are involved, it is, of course, important to run an appropriate blank. Interference caused by detergents, sucrose, and EDTA can be eliminated by the addition of SDS ***(5)*** and a precipitation step (*see* **Note 4**).
11. Modifications to this basic assay have been reported that increase the sensitivity of the reaction. If the Folin reagent is added in two portions, vortex-mixing between each addition, a 20% increase in sensitivity is achieved ***(8)***. The addition of dithiothreitol 3 min after the addition of the Folin reagent increases the sensitivity by 50% ***(9)***.
12. The amount of color produced in this assay by any given protein (or mixture of proteins) is dependent on the amino acid composition of the protein(s) (*see* Introduction). Therefore, two different proteins, each for example at concentrations of 1 mg/mL, can give different color yields in this assay. It must be appreciated, therefore, that using bovine serum albumin (BSA) (or any other protein for that matter) as a standard gives only an approximate measure of

the protein concentration. The only time when this method gives an absolute value for protein concentration is when the protein being analyzed is also used to construct the standard curve. The most accurate way to determine the concentration of any protein solution is amino acid analysis.

13. A means of speeding up this assay using raised temperatures ***(10)*** or a microwave oven (*see* Chapter 5) has been described.

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