Chapter 1

The Lowry Method for Protein Quantitation

Jaap H. Waterborg and Harry R. Matthews

University of California, Department of Biological Chemistry, School of Medicine, Davis, California

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. The procedure of Lowry et al. (*1*) 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 where protein mixtures or crude extracts are involved.

Materials

1. *Complex-forming reagent*: prepare immediately before use by mixing the following 3 stock solutions A, B, and C in the proportion 100:1:1, respectively.

Solution A: 2% (w/v) Na_2CO_3 in distilled water
Solution B: 1% (w/v) $CuSO_4 \cdot 5H_2O$ in distilled water
Solution C: 2% (w/v) sodium potassium tartrate in distilled water

2. *2N NaOH*
3. *Folin reagent* (commercially available): Use at 1*N* concentration.
4. *Standards*: Use a stock solution of standard protein (e.g., bovine serum albumin fraction V) containing 4 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	1.25	2.50	6.25	12.5	25.0	62.5	125	25
Water	μL	500	499	498	494	488	475	438	375	25
Protein concentration	μg/mL	0	10	20	50	100	200	500	1000	200

Method

1. To 0.1 mL of sample or standard, add 0.1 mL of 2*N* NaOH. Hydrolyze at 100°C for 10 min in a heating block or a boiling water bath.
2. Cool the hydrolyzate to room temperature and add 1 mL of freshly mixed complex-forming reagent. Let the solution stand at room temperature for 10 min.
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).
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.

Notes

1. If the sample is available as a precipitate, then dissolve the precipitate in 2*N* NaOH and hydrolyze as in step 1. Carry 0.2 mL aliquots of the hydrolyzate forward to step 2.
2. Whole cells or other complex samples may need pretreatment, as described for the Burton assay for DNA (See Vol. 2). For example, the PCA/ethanol precipitate from extraction I may be used directly for the Lowry assay or the pellets remaining after the PCA hydrolysis (step 3 of the Burton assay) may be used for Lowry. In this latter case, both DNA and protein concentrations may be obtained from the same sample.
3. Rapid mixing as the Folin reagent is added is important for reproducibility.
4. A set of standards is needed with each group of assays, preferably in duplicate. Duplicate or triplicate unknowns are recommended.

References

1. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.

Methods in Molecular Biology

Volume 1

PROTEINS

Edited by

John M. Walker

Humana Press • Clifton, New Jersey

Library of Congress Cataloging in Publication Data

Main entry under title:

Methods in molecular biology.

Includes bibliographies and index.
Contents: v. 1. Proteins.
1. Molecular biology—Technique—Collected works.
I. Walker, John M., 1948–
QH506.M45 1984 574.8'8'078 84-15696
ISBN 0-89603-062-8

Crescent Manor
PO Box 2148
Clifton, NJ 07015

Printed in the United States of America