Protein Methods

Second Edition

DANIEL M. BOLLAG
Merck Research Laboratories
West Point, Pennsylvania

MICHAEL D. ROZYCKI
Department of Chemistry
The Henry H. Hoyt Laboratory
Princeton University
Princeton, New Jersey

STUART J. EDELSTEIN
Department of Biochemistry
University of Geneva
Geneva, Switzerland

WILEY-LISS

A JOHN WILEY & SONS, INC., PUBLICATION

New York • Chichester • Brisbane • Toronto • Singapore

Address all Inquiries to the Publisher
Wiley-Liss, Inc., 605 Third Avenue, New York, NY 10158-0012

Printed in the United States of America.

The text of this book is printed on acid-free paper.

Library of Congress Cataloging in Publication Data

Bollag, Daniel M.
Protein methods / Daniel M. Bollag, Michael D. Rozycki.
Stuart J. Edelstein. — 2nd ed.
p. cm.
Includes bibliographical references and index.
ISBN 0-471-11837-0 (cloth : alk. paper)
1. Proteins—Purification. 2. Proteins—Research—Methodology.
I. Edelstein, Stuart J. II. Rozycki, Michael D. III. Title.
QP551.E23 1996
574.19'245—dc20 96-14083

10 9 8 7 6 5 4 3 2 1

Preface to the Second Edition

The motivation for writing the first edition of this book was to describe techniques of general applicability to proteins. The positive response to *Protein Methods* encouraged us to prepare an updated version and to expand the scope to include procedures that require considering the specific characteristics of the protein under investigation. Hence, in addition to the eight chapters that appeared in the first edition, we have added three chapters on methods of purification that must be tailored to the properties of individual proteins. In view of the burgeoning activity in the determination of protein atomic structure by X-ray crystallography, we also decided to include a chapter on crystallization. In addition, since in many instances, proteins are now purified following expression in bacteria, a new section was added to Chapter 2 concerning the isolation of proteins from inclusion bodies.

In order to tackle the widened range of topics, the authors of the first edition, Daniel M. Bollag and Stuart J. Edelstein, invited Michael D. Rozycki to participate in writing the second edition in order to benefit from his knowledge of protein methods in general and in particular from his extensive experience in protein crystallography. We were fortunate to have comments on the crystallization chapter from Nancy Vogelaar, Rebecca Page, Greg Bowman, Dale Jefferson, and Donatella Pascolini. From Wiley, the encouragement of Peter Brown to begin work on a second edition was decisive and in later stages the help of Susan King and Colette Bean was very much appreciated. The contributions of all those acknowledged in the first edition continue to be appreciated and we reiterate our thanks to friends and colleagues who contributed in various ways. Most of all, we are grateful to our wives, Wendy Miller, Teresa Twomey, and Lynn Edelstein, for their constant support during this effort to expand the scope of *Protein Methods*.

Preface to the First Edition

The revolution in genetic engineering has benefitted greatly from the fact that the behavior of DNA fragments created by restriction enzymes is largely independent of the fragments' precise compositions. Many properties of these fragments therefore depend essentially on their size only. This feature of DNA has permitted various generalized methods to be developed and summarized in extremely useful laboratory manuals, notably *Molecular Cloning* by Maniatis, Fritsch, and Sambrook.

Proteins, from many points of view, have more complicated personalities than DNA fragments. As a result, it has been difficult to design manuals for laboratory methods that can be applied to proteins in general. However, for routine methods concerning the elementary operations of extraction and concentration determination, as well as for certain widely used techniques involving gel electrophoresis, the distinctive properties of different proteins are not of primary importance. Hence, these methods can be applied directly to virtually all proteins.

Our goal in preparing this book has been to assemble the most general methods for protein research and to present them in practical detail in order to provide investigators with all of the information needed to perform these procedures in the laboratory. In addition, we have organized the material in the form of standardized laboratory protocols to facilitate its utilization. This orientation has led us to delve only minimally into the theory of the methods or into the basic concepts of protein biochemistry, but other sources are available for these topics.

Several guidelines have served us in designing the nature of the topics and the manner in which they are presented. The most common theme of protein analysis at the current time is gel electrophoresis, and this serves as the heart of *Protein Methods* (Chapters 5, 6, and 7). The extension of gel electrophoresis involving electroblotting and immunochemical detection of electroblotted proteins has led to some of the most impressive advances in protein analysis in recent years, and these techniques are treated in Chapter 8. Protein analysis by electrophoretic methods requires that the protein sample be extracted from its native cellular environment (Chapter 2), that the *in vitro* protein concentration be estimated (Chapter 3), and sometimes that the sample be concentrated prior to analysis (Chapter 4). Some fundamental principles for handling of proteins are outlined in Chapter 1.

In many cases, the descriptions presented are derived from procedures that have been used and refined in our own laboratory. However, their origins are multiple and are not always accurately traceable. We sincerely

apologize to any uncited investigators who may be responsible for key developments. Overall, we have attempted to provide sufficient references to the original literature to enable each researcher to expand his or her knowledge of the subject and to develop specialized refinements.

We have submitted the material presented here to various colleagues for their critical evaluation, and we particularly wish to express our appreciation to Christine Tachibana and Gideon Bollag who provided valuable comments during the development of the manuscript, as well as to Clement Bordier for his guidance on the sections concerning detergents. We thank Isabelle Tornare and Ann-Marie Paunier Doret in our laboratory for their participation in many of the developments that led to the final protocols. The skillful contributions of F. Pillonel in the preparation of the figures are gratefully acknowledged. For the weak points or errors that may remain, we take full responsibility.

A methods book of this type can always be improved by feedback from users, and we are eager to hear from those who use this book and wish to share with us their comments, criticisms, and suggestions for additions or deletions.

We are most appreciative of the people at the Wiley-Liss Division of John Wiley & Sons, Inc. who helped with this project, starting with Peter Brown, with whom it was conceived, and including Eric Swanson, Eileen Cudlipp, Frederick Siebenmann, and Sonny Fritz. Gary Giulian, Henry Reeves, and Kathleen Dannelly kindly supplied figures used in Chapters 5 and 7.

We also thank our family, friends, and colleagues upon whom we imposed for reading various sections, providing advice, and tolerating our preoccupations during the preparation of this book.

Contents

Chapter 1

Preparation for Protein Isolation

I. Introduction

II. Buffers
- A. Buffer Characteristics
- B. Preparation of Buffers
- C. Concentration Effects of Buffer on pH
- D. Limitations of Certain Buffers
- E. Preventing Buffer Contamination
- F. Water Purity

III. Salts, Metal Ions, and Chelators
- A. Ionic Strength
- B. Divalent Cations
- C. Chelators

IV. Reducing Agents
- A. General Considerations
- B. Specific Recommendations

V. Detergents
- A. Introduction
- B. Classes of Detergents
- C. Protocol for Membrane Protein Solubilization

VI. Protein Environment
- A. Surface Effects
- B. Temperature
- C. Storage

VII. Protease Inhibitors
- A. Common Inhibitors
- B. A Sample Broad Range Protease Inhibitor Cocktail

VIII. References

I. Introduction

This book is devoted to laboratory techniques for the analysis and separation of proteins. Proteins are an extremely heterogeneous class of biological macromolecules. They are often unstable when not in their native environment, which in itself varies considerably among cell compartments and extracellular fluids. Of the many types of proteins, we can distinguish between those that are soluble or membrane-bound, those with catalytic or purely structural roles, and those with various post-translational modifications.

Each protein may have specific requirements once it is extracted from its normal biological milieu. If these requirements are not satisfied, the protein can rapidly lose its ability to carry out specific functions, and an already limited lifetime may be drastically reduced. Thus, determination of these requirements has often been a major hurdle in protein characterization. In some cases, the difficulty has been to stabilize the protein against external proteolysis, while in other cases the problem has been to maintain ligand-binding or enzymatic activity. Solutions to these problems are highly individual. Nonetheless, some fundamental parameters must be considered by anyone studying proteins. In this chapter, we discuss a number of these parameters and attempt to provide general guidelines or sources of information for laboratory work with proteins.

II. Buffers

A. Buffer Characteristics

- A buffer is defined as a mixture of an acid and its conjugate base which can reduce changes in solution pH when acid or alkali are added. The selection of an appropriate buffer is important in order to maintain a protein at the desired pH and to ensure reproducible experimental results. A rudimentary description of key concepts behind buffering, such as pH and pK_a, can be found in the Calbiochem "Buffers" booklet and in Stryer (1988, pp. 41-42).

- There are eight important characteristics to consider when selecting a buffer (adapted from Scopes, 1982):
  1. pK_a value (see Table 1.1)
  2. pK_a variation with temperature
  3. pK_a variation upon dilution
  4. Solubility
  5. Interaction with other components (such as metal ions and enzymes)
  6. Expense
  7. UV absorbance
  8. Permeability through biological membranes

- Some General Observations

  1. Ideally, different buffers with a similar pK_a should be tested to determine whether there are undesired interactions between a certain buffer and the protein under investigation (Blanchard, 1984).

  2. Once a buffer is chosen, it is best to work at the lowest reasonable concentration to avoid nonspecific ionic strength effects. A 50 mM buffer is a good starting point.

3. The useful buffering range diminishes significantly beyond 1 pH unit on either side of the pK_a. Note that many enzymes are irreversibly denatured at extreme pH values (Tipton and Dixon, 1979).

4. The physiological pH in most animal cells is 7.0 - 7.5 at 37°C. Due to the effect of temperature, this value rises to close to 8.0 near 0°C (Scopes, 1982).

5. The buffer of choice also depends on the methods employed:
   - For gel filtration chromatography, almost any buffer can be chosen that is compatible with the protein of interest.
   - For anion exchange chromatography, cationic buffers such as Tris are preferred.
   - For cation exchange or hydroxyapatite chromatography, anionic buffers such as phosphate are preferred (Blanchard, 1984).

6. 'Good's' buffers (for example, MES, PIPES, MOPS) were developed by Good and colleagues (1966) to be biologically inert, to have low UV absorbance, and to be minimally affected by temperature or ionic strength.

7. Buffer mixtures with wide buffering ranges at constant ionic strength are described by Ellis and Morrison (1982).

8. A description of buffers and cryosolvents for low temperature conditions is found in Fink and Geeves (1979).

9. All chemical products should be reagent grade or higher.