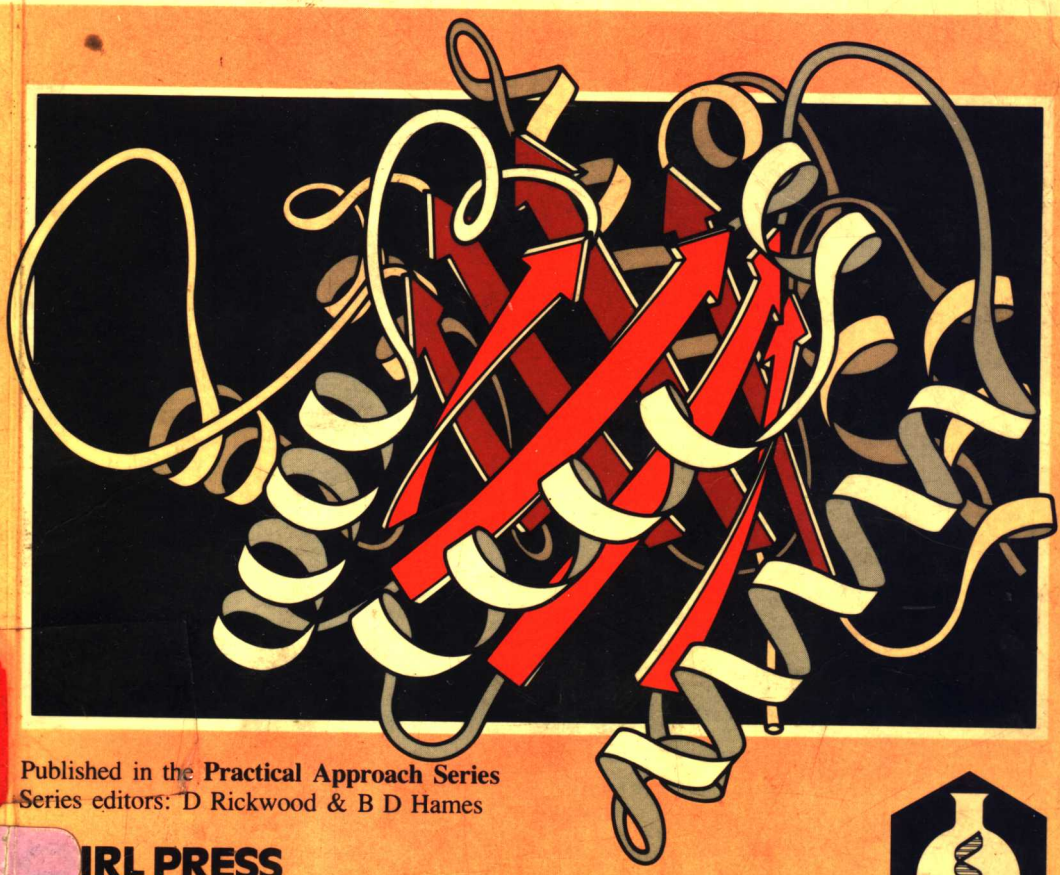
Protein structure

a practical approach

Edited by
T E Creighton



Published in the **Practical Approach Series**
Series editors: D Rickwood & B D Hames

IRL PRESS
at
OXFORD UNIVERSITY PRESS
Oxford New York Tokyo

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Cambridge CB2 2QH, UK

IRL PRESS
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OXFORD UNIVERSITY PRESS
Oxford New York Tokyo

IRL Press
Eynsham
Oxford
England

First Published 1989

British Library Cataloguing in Publication Data

Protein structure
1. Proteins. Structure
I. Creighton, Thomas E., *1940–* II. Series
547.7'5044

Library of Congress Cataloging in Publication Data

Protein structure.
(Practical approach series)
Includes bibliographies and index.
1. Proteins—Analysis. 2. Proteins—Structure.
3. Biochemistry—Technique. I. Creighton, Thomas E.,
1940– . II. Series. [DNLM: 1. Proteins—analysis.
QH 55 P9684]
QP551.P69764 1988 574.19'245 88-31965

ISBN 0 19 963000 3 (hardbound)
0 19 963001 1 (softbound)

Previously announced as:

ISBN 1 85221 137 7 (hardbound)
ISBN 1 85221 138 5 (softbound)

Typeset by Paston Press, Loddon, Norfolk, and printed by
Information Printing Ltd, Oxford, England

Preface

Techniques for the experimental study of protein structure need not be complex, and this volume presents a collection of those most likely to be useful in the average biochemical laboratory. Most of the procedures use relatively simple electrophoretic, chromatographic and spectrophotometric techniques, so all that is required is some simple electrophoresis equipment, a column and a fraction collector, and a UV spectrophotometer. No expensive, complicated items of equipment are required, so the procedures should be generally useful, and the dangers of becoming wedded or addicted to a single instrument or technique are minimal.

With the procedures described here, it is possible to estimate the molecular weight of a specific protein (Chapter 1), to resolve it from all other proteins in virtually any mixture (Chapters 2 and 3), and to identify it given just a portion of its amino acid sequence or a clone of its gene (Chapter 4). The covalent structure can be characterized by the peptide maps it produces (Chapter 5), the numbers of certain amino acid residues present in the protein can be counted (Chapter 6), and the number and identities of any disulphide bonds present can be established (Chapter 7).

The three-dimensional folded conformations of proteins are crucial for their biological properties. Chapter 8 provides guidelines as to what can and cannot be inferred about the structure and properties of a protein from just its gene or amino acid sequence, while the procedures for getting proteins to adopt their appropriate folded conformations are described in Chapter 9. Simple procedures for monitoring the overall conformational properties of protein are described in Chapters 10–12, using electrophoretic, spectral, and immunochemical techniques. Measuring the conformational stability of a protein is described in Chapter 13, and guidelines on how to optimize that stability are given in the final chapter.

Techniques for purifying proteins and determining their primary structures, either directly or by gene cloning, are not given, for they are the subjects of entire volumes in this series (*Protein Purification: A Practical Approach*; *Protein Sequencing: A Practical Approach*; *DNA Cloning: A Practical Approach*). Likewise, procedures for determining the detailed three-dimensional structures of proteins by X-ray diffraction or by nuclear magnetic resonance are not provided here. Neither are techniques that an average molecular biologist would be expected to use directly. Both require items of equipment that are not available in most laboratories and a virtual full-time commitment to just one of the techniques. Therefore, if you want to characterize your protein further, either by determining its complete primary structure or its atomic-resolution three-dimensional structure, you are advised to prepare large quantities of protein, plus perfect single crystals, if possible, and to take them to your nearest, or most friendly, laboratory specializing in these techniques.

In the meantime, use the relatively simple procedures of this volume to obtain a low-resolution 'picture' of your protein that will be useful for understanding its biological function. For more detailed studies of the functional properties of the

protein, you are advised to acquire and use a copy of the companion volume, *Protein Function: A Practical Approach*. For a comprehensive description of the properties of proteins, my volume *Proteins: Structures and Molecular Properties* (W. H. Freeman, New York, 1983) should be useful.

T. E. Creighton

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CHAPTER 1

Estimating molecular weights of polypeptides by SDS gel electrophoresis

YEW PHEW SEE and GEORGE JACKOWSKI

1. INTRODUCTION

Estimation of the molecular weight (M_r) of a polypeptide is of central importance to the characterization of proteins and is finding increasing use in molecular biology. It is our aim in this chapter to discuss very briefly the theoretical aspects of estimating the molecular weights of polypeptides using sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis and to provide a collection of proven procedures and advice on their use.

Molecular weight determination of polypeptides using SDS–polyacrylamide gel electrophoresis (PAGE) was first introduced, empirically, by Shapiro *et al.* (1). It was confirmed and extended by Weber and Osborn (2) and Dunker and Rueckert (3). These authors showed that the relative mobilities of the polypeptides were related to their molecular weights. Explanation for this behaviour of polypeptides in SDS–PAGE was soon provided by Pitt-Rivers and Impiombato (4) and Reynolds and Tanford (5, 6). They found that, under appropriate conditions, all reduced polypeptides bind the same amount of SDS on a weight basis (1.4 g SDS/g polypeptide). Viscosity analysis suggested that the reduced polypeptide–SDS complexes formed rod-like particles, with lengths proportional to the molecular weight of the polypeptides. Unreduced polypeptides containing intact disulphide bonds do not bind the optimum amount of SDS and have different hydrodynamic volumes. Gel filtration analysis showed that these unreduced polypeptide–SDS complexes were smaller than the corresponding reduced polypeptide–SDS complexes (6, 7).

Although viscosity analysis suggested that the reduced polypeptide–SDS complex had a rod-like structure (6), the exact structure of this complex is still unknown. Some protein chemists mistakenly believe that SDS dissociates proteins in the same manner as guanidinium chloride to give a completely random coil. Optical rotatory dispersion analysis showed that the SDS–polypeptide complexes contain significant proportions of α-helical structures (6, 8, 9). Mattice *et al.* (9) had suggested, however, that the 'overall conformation more closely approximates that of a random coil than a rod'. From viscosity analysis, Reynolds and Tanford (6) proposed the prolate ellipsoid model for the SDS–polypeptide structure. Other models such as the 'necklace' model (10), the deformable prolate ellipsoid model (11) and the amino acid-enhanced structural change model (9)

have been proposed. The validity of these models was examined by Mattice *et al.* (9) who concluded that 'none of the models provides a molecular explanation for the ability of diverse proteins to form complexes that contain 1.4 g SDS/g of protein'.

The amount of SDS bound by a protein is dependent on the structure of the protein (reduced or unreduced), the temperature and the ionic strength of the solution (4, 5). Unreduced proteins bind much less SDS than reduced proteins, and increasing ionic strength decreases the amount of SDS bound (4, 5, 12). SDS binds mainly to the hydrophobic regions of polypeptides, whereas the hydrophilic regions bind much less SDS (13). Why proteins with different hydrophobicities bind the same amount of SDS per gram is not understood. Glycoproteins with more than 10% carbohydrate bind much less SDS than other proteins of the same molecular weight (14, 15).

Separation of SDS–polypeptide complexes by SDS–PAGE is analogous to gel filtration. The separations are dependent on the sizes of the SDS–polypeptide complexes and not necessarily on the sizes or molecular weights of the original polypeptides or proteins. The electrophoretic mobility is proportional to the molecular weight of a polypeptide or protein only when the charge/mass ratios and the shapes of all the SDS–polypeptide complexes are the same. Therefore valid molecular weight standards are required for molecular weight determination. (For more detailed discussion, see references 16–18.) Consequently, molecular weight determination of polypeptides in SDS–polyacrylamide gels is still an empirical procedure.

For proper molecular weight determination, the following criteria should be met.

(i) All polypeptides (standard and unknown) must bind the optimum amount of SDS and thus have a constant charge/mass ratio.
(ii) All SDS–polypeptide complexes (standard and unknown) must assume the same conformation or shape.
(iii) Electrophoresis of standard and unknown proteins must be run under the same conditions and at the same time (preferably on the same gel).

Three procedures are available for molecular weight determination using SDS–PAGE. They are:

(i) single gel system—plotting R_f versus log M_r;
(ii) multiple gel system—plotting K_r versus M_r;
(iii) gradient gel system—plotting log (%T) versus log M_r.

Each of these systems will be described and the various problems encountered will be discussed.

2. ELECTROPHORESIS SYSTEMS

SDS–PAGE may be run on either disc or slab gel systems. Presently, the slab gel system is most widely used for molecular weight determination. The advantages of this system are listed below.