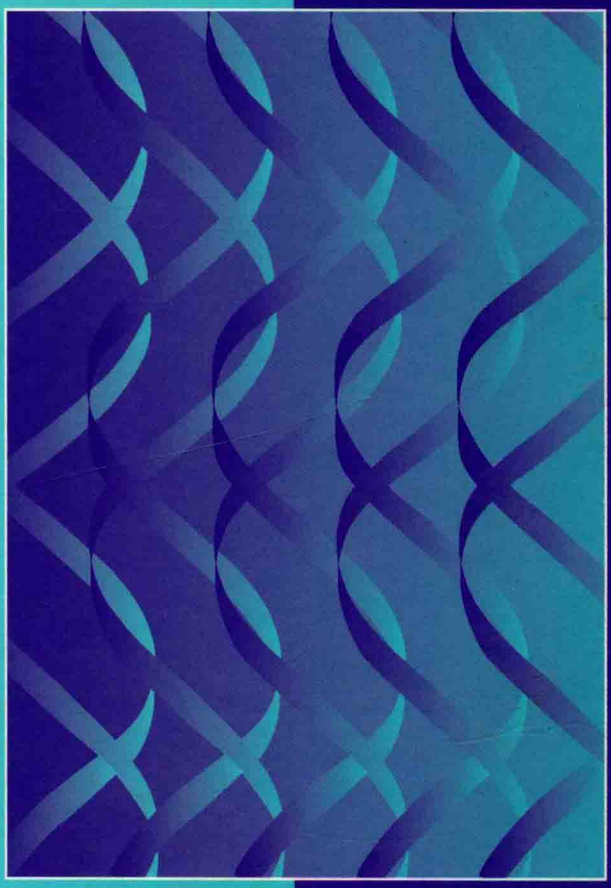
BIOSEPARATION OF PROTEINS

Unfolding/Folding and Validations

Ajit Sadana



VOLUME I

Series Editor Satinder Ahuja

SEPARATION SCIENCE AND TECHNOLOGY

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ACADEMIC PRESS

San Diego London Boston New York Sydney Tokyo Toronto

This book is printed on acid-free paper. ♾

Academic Press
a division of Harcourt Brace & Company
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
http://www.apnet.com

Academic Press Limited
24-28 Oval Road, London NW1 7DX, UK
http://www.hbuk.co.uk/ap/

Library of Congress Card Catalog Number: 97-80234

International Standard Book Number: 0-12-614040-5

PRINTED IN THE UNITED STATES OF AMERICA
97 98 99 00 01 02 QW 9 8 7 6 5 4 3 2 1

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This Is Volume 1 of

SEPARATION SCIENCE AND TECHNOLOGY

A reference series edited by Satinder Ahuja

PREFACE

The biotechnology industry is poised for rapid growth and implementation in diverse areas. However, one major constraint is the need for a more complete understanding of protein separation processes. While progress in the technology of cloning genes to attain high and desired levels of expression has been satisfactory, large-scale production and purification of proteins has, until recently, been rather neglected.

Bioseparation stands at the very center of effective biotechnology development. Interest in protein purification has increased rather dramatically in the past few years and has become the focus of intensive research both at academic institutions and in industry. Several papers and a few books on the general area of bioseparations have recently appeared, but no book, and only a few papers, has emphasized the influence of bioseparation processes on protein inactivation. This information, which is scarce and difficult to find in the open literature, is a critical part of the bioseparation framework: One may ask of what use is a bioseparation process if information on the conformational state and the activity of the recovered protein is not presented in any detail? The aim of this book is to provide that information as part of a critical review and synthesis of the literature.

After the introductory chapter, Chapter 2 describes the three basic steps involved in the bioseparation process: cell disruption, initial fractionation, and high-resolution fractionation. The different high-resolution fractionation steps, as described in Chapter 3, are critical for meeting the stringent requirements set for product purity and effectiveness, including those of the different regulatory agencies.

The quality of the separated product is significantly affected by the inactivation of proteins at interfaces during bioseparation. Chapter 4 analyzes these interfacial protein inactivations. Until now, chromatographic processes have been utilized rather heavily during the bioseparation protocol at different stages of separation. Chapter 5 analyzes protein inactivation during chromatographic methods of separation and includes available information on the mechanistic aspects. Other techniques for effectively separating biological products of interest also need to be developed, keeping both the quantitative and the qualitative aspects in mind. Chapter 6 analyzes protein inactivations during novel bioseparation procedures.

During bioseparation, the biological product of interest must adsorb on an interface. Conformational changes accompanied by subsequent activity changes will presumably result and will significantly influence both the quantitative and the qualitative aspects of biological product recovery. Chapter 7 analyzes the influence of protein adsorption and inactivation during bioseparation. The economics of the downstream process plays a significant role in getting a biological product ready for market. This sort of information is not readily available in the open literature but is presented here in Chapter 8.

Some denaturation during bioseparation is unavoidable. Different renaturation techniques, presumably as "corrective" steps to minimize the extent of denaturation of at least some of the biological products recovered, would be extremely helpful. Some of these techniques may also be used to enhance or improve the quality of the product by facilitating the process by which the product attains the required conformational state(s). Chapter 9 analyzes protein refolding strategies and inactivation during bioseparation.

Consistency in the safety, potency, efficacy, and purity of a biological product is the manufacturer's responsibility. This consistency is the basis of governmental regulation and evaluation. Tests for impurities and contaminants are critical in the development and validation of bioseparation processes and in final product testing. Validation is the "assurance that a process is closely followed during a product's manufacture." Validation protocols or strategies provide written documentation that a process is consistently doing what its manufacturer claims it can accomplish. This validation procedure provides assurance that the process is "under control." Because recombinant techniques are used to make quite a few bioproducts, the importance of this step cannot be overemphasized. This validation process and the protocols and strategies involved therein, along with the appropriate governmental and regulatory guidelines, are presented in Chapter 10.

The text is intended for instruction at the graduate level and even at the senior undergraduate level, as well as for the industrial practitioner who, after examining the "science-based" information in the earlier chapters, will appreciate the focus on the monitoring, validation, and economics of bioseparation processes. The generalized treatment will also interest chemical, biochemical, and biomedical engineers, chemists, biochemists, and those in the medical profession who wish to better understand the fundamentals of bioseparation and its influence on protein/enzyme inactivation. Even venture capitalists will find the book of interest. Biotechnology, by its very nature, is an interdisciplinary area that requires diverse expertise. I hope that this book will foster these in-

teractions, facilitate an appreciation of all perspectives, and help in efforts toward improved economics of bioseparation.

This text is unique in that it provides the appropriate background on bioseparation processes, while emphasizing the extent of, mechanisms of, and control of protein inactivation during these processes and their essential validation. Comparisons of protein inactivation during different bioseparation processes provide valuable information for workers in different areas who are interested in bioseparations. Readers may thus consider this a "second-level" book on bioseparations, with "first-level" books providing the fundamentals of bioseparation processes. This second-level examines and analyzes the control and validation of the product (protein) during these bioseparation processes. Second, and presumably "higher-level," books will be required to pave the way for the emergence and consolidation of protein purification as a discipline rather than as a means to an end.

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