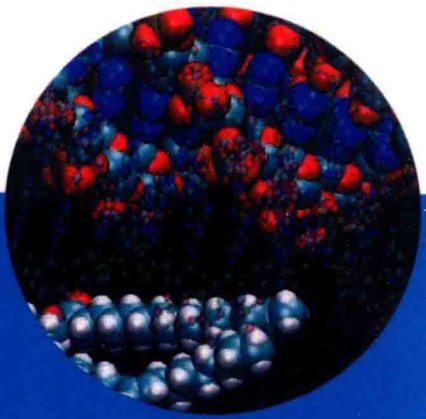
Markus R Wenk
Aaron Zefrin Fernandis



Manuals in Biomedical Research – Vol. 3

A Manual for Biochemistry Protocols

Jan-Thorsten Schantz *Series Editor*

Markus R Wenk
Aaron Zefrin Fernandis

National University of Singapore, Singapore

Manuals in Biomedical Research – Vol. 3

A Manual for Biochemistry Protocols

World Scientific

Published by
World Scientific Publishing Co. Pte. Ltd.
5 Toh Tuck Link, Singapore 596224
USA office: 27 Warren Street, Suite 401-402, Hackensack, NJ 07601
UK office: 57 Shelton Street, Covent Garden, London WC2H 9HE

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Manuals in Biomedical Research — Vol. 3
A MANUAL FOR BIOCHEMISTRY PROTOCOLS

Copyright © 2007 by World Scientific Publishing Co. Pte. Ltd.

All rights reserved. This book, or parts thereof, may not be reproduced in any form or by any means, electronic or mechanical, including photocopying, recording or any information storage and retrieval system now known or to be invented, without written permission from the Publisher.

For photocopying of material in this volume, please pay a copying fee through the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, USA. In this case permission to photocopy is not required from the publisher.

ISBN-13 978-981-270-066-7 (pbk)
ISBN-10 981-270-066-8 (pbk)

Typeset by Stallion Press
Email: enquiries@stallionpress.com

Printed in Singapore by B & JO Enterprise

A Manual for Biochemistry Protocols

MANUALS IN BIOMEDICAL RESEARCH

Series Editor: Jan-Thorsten Schantz
National University of Singapore, Singapore

Published

Vol. 1: A Manual for Primary Human Cell Culture
by Jan-Thorsten Schantz and Ng Kee Woei

Vol. 2: Techniques in Microscopy for Biomedical Applications
by Terje Dokland, Dietmar Werner Hutmacher, Mary Mah-Lee Ng and Jan-Thorsten Schantz

Vol. 3: A Manual for Biochemistry Protocols
by Markus R. Wenk and Aaron Zefrin Fernandis

Forthcoming

Vol. 4: A Manual for Biomaterials/Scaffold Fabrication Technology
by Gilson Khang et al.

Vol. 5: A Manual for Intellectual Property Management
by Maik Brinkmann et al.

此为试读,需要完整PDF请访问: www.ertongbo

Preface

The field of biochemistry is diverse and forms parts of diverse fields including cell biology, molecular biology and medical sciences. Biochemistry is the study of the molecules of life like proteins, lipids, carbohydrates and nucleic acids. Studying the structure, properties and reactions of these important molecules would help in better understanding life as a whole. The practical aspect along with the theoretical background would help in better understanding these mechanisms. This book tries to address and compile some of the routinely used protocols in biochemistry for easy access. The aim of this book is not only to bring together the protocols, but also to understand some of the basics behind following the methodologies. The target is to give students a view of biochemistry, especially those who have just ventured into the field of biochemistry and need a headstart.

The protocols are written as a handy guide that can be carried as a pocket guide for easy reference. The protocols are easy to follow with each step explained in layman terms. Even though the field of biochemistry is exhaustive, an effort has been made to list some of the protocols that could serve as a foundation for starting any biochemical investigation.

We would like to thank all the members of the lab, especially Dr Sravan Kumar Goparaju and Xue Li Guan, whose help in reviewing the manuscript is greatly appreciated. We would also like to thank all the people previously involved in designing these protocols.

Markus R. Wenk
Aaron Z. Fernandis

List of Contributors

Xue Li Guan, BSc
Yong Loo Lin School of Medicine
Department of Biochemistry
Centre for Life Sciences (CeLS)
30 Medical Drive, #04-21
Singapore 117607
Singapore

Sravan Goparaju, PhD
Department of Cellular and Molecular Biology
Division of Biochemistry
Kobe University Graduate School of Medicine
Chuo-Ku, Kobe 650-0017
Japan

Contents

此为试读,需要完整PDF请访问: www.ertongbo

List of Figures

A

Protein Purification

Summary

Protein expression is tightly regulated for normal functioning of a cell or organism. To understand protein structure and function in detail, they often need to be separated from other cellular components (lipids, nucleic acids, sugars, etc.) and isolated to homogeneity. After recovering a protein to near homogeneity, it should retain all its native biological characteristics of structure and activity. To achieve this objective, one needs to take into account the physical and chemical property of proteins (size, charge, solubility, hydrophobicity, precipitation, etc.). These common characteristics of the protein can be exploited to separate it from other components of the cell. With the introduction of recombinant DNA technology, the protein purification technique has been enhanced and also simplified. Purification protocols vary, depending on the precise nature of the protein. General steps include (i) chromatography, (ii) precipitation and/or (iii) extraction.

A.1 Protein Precipitation

Many cytosolic proteins are water-soluble, and their solubility is a function of the ionic strength and pH of the solution. The commonly used salt for this purpose is ammonium sulphate, due to its high solubility even at lower temperatures. Proteins in aqueous solutions are heavily hydrated, and with the addition of salt, the water molecules become more attracted to the salt than to the protein due to the higher charge. This competition for hydration is usually more favourable towards the salt, which leads to interaction between the proteins, resulting in aggregation and finally precipitation. The precipitate can then be collected by centrifugation and the protein pellet is redissolved in a low salt buffer. Since different proteins have distinct characteristics, it is often the case that they precipitate (or "salt out") at a particular concentration of salt.

Requirements

(1) Ammonium sulphate
(2) Ice tray
(3) Magnetic bead and stirrer
(4) Swing-out rotor centrifuge

Protocol 1:

(1) Clarify the protein solution (in most cases the lysates) by centrifugation.
(2) Transfer the supernatant into an ice-cold beaker with a magnetic bead.
(3) Note the exact amount of the supernatant.
(4) Keep the beaker chilled by placing it in an ice tray.
(5) Transfer the beaker with the ice tray onto a magnetic stirrer (Fig. A.1).
(6) Weigh the amount of ammonium sulphate to be added (from Table A.1). The amount depends on the volume of the solution and the percentage saturation of the salt needed. Refer to the precipitation chart. In case of protein purification, a step precipitation is carried out.
(7) Slowly add the ammonium sulphate with stirring. One needs to be careful as the addition of the salt should be very slow. Add a small amount at a time and then allow it to dissolve before further addition.
(8) Keep it on the stirrer for 1 h of precipitation to occur in ice.
(9) Centrifuge at 10 000 g for 15 min at 4°C.
(10) The pellet contains the precipitated protein, which could be dissolved in a suitable buffer for further analysis and purification.
(11) For a second round of precipitation of a different protein, the supernatant is again used and the above same steps are followed.

A.2 Column Chromatography

This method involves passing the protein through a column filled with resins of unique characteristics. Depending on the type of the resin or beads, purification can be achieved through (i) ion exchange, (ii) size exclusion or (iii) affinity chromatography.

A.2.1 Ionic Exchange Chromatography

This is one of the most useful methods of protein purification. Depending on the surface residues on the protein and the buffer conditions, the protein will have a net positive or negative charge

Table A.1 Amount of ammonium sulfate required for protein precipitation.

Initial concentration of ammonium sulfate	Percentage saturation at 0°																
	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
	Solid ammonium sulfate (g) to be added to 1 L of solution																
0	106	134	164	194	226	258	291	326	361	398	436	476	516	559	603	650	697
5	79	108	137	166	197	229	262	296	331	368	405	444	484	526	570	615	662
10	53	81	109	139	169	200	233	266	301	337	374	412	452	493	536	581	627
15	26	54	82	111	141	172	204	237	271	306	343	381	420	460	503	547	592
20	0	27	55	83	113	143	175	207	241	276	312	349	387	427	469	512	557
25		0	27	56	84	115	146	179	211	245	280	317	355	395	436	478	522
30			0	28	56	86	117	148	181	214	249	285	323	362	402	445	488
35				0	28	57	87	118	151	184	218	254	291	329	369	410	453
40					0	29	58	89	120	153	187	222	258	296	335	376	418
45						0	29	59	90	123	156	190	226	263	302	342	383
50							0	30	60	92	125	159	194	230	268	308	348
55								0	30	61	93	127	161	197	235	273	313
60									0	31	62	95	129	164	201	239	279
65										0	31	63	97	132	168	205	244
70											0	32	65	99	134	171	209
75												0	32	66	101	137	174
80													0	33	67	103	139
85														0	34	68	105
90															0	34	70
95																0	35
100																	0

此为试读，需要完整PDF请访问：www.ertongbo