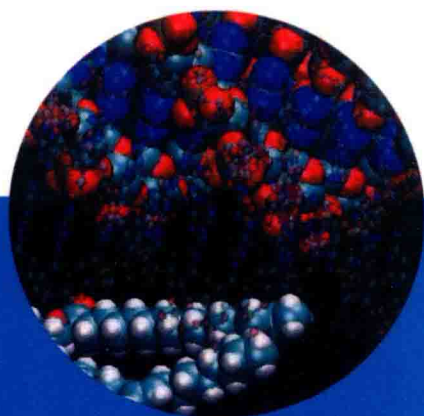


Markus R Wenk
Aaron Zefrin Fernandis



Manuals in Biomedical Research – Vol. 3

A MANUAL FOR BIOCHEMISTRY PROTOCOLS

Jan-Thorsten Schantz *Series Editor*

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National University of Singapore, Singapore

Preface

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**A MANUAL for
Biochemistry
Protocols**

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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.

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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 |