Methods in Molecular Biology

Volume 3

New Protein Techniques

Edited by

John M. Walker

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Molecular Biology

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John M. Walker

The Hatfield Polytechnic, Hatfield, Hertfordshire, UK

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Volume 3

New Protein Techniques

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edited by **John M. Walker**

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Preface

In recent years there has been a tremendous increase in our understanding of the functioning of the cell at the molecular level. This has been achieved in the main by the invention and development of new methodology, particularly in that area generally referred to as "genetic engineering." Although this revolution has been taking place in the field of nucleic acids research, the protein chemist has at the same time developed fresh methodology to keep pace with the requirements of present-day molecular biology. Today's molecular biologists can no longer be content with being experts in one particular area alone. They need to be equally competent in the laboratory at handling DNA, RNA, and proteins moving from one area to another as required by the problem that is being solved. Although many of the new techniques in molecular biology are relatively easy to master, it is often difficult for a researcher to obtain all the relevant information necessary for setting up and successfully applying a new technique. Information is of course available in the research literature, but this often lacks the depth of description that the new user requires. This requirement for in-depth practical details has become apparent by the considerable demand for places on our Molecular Biology Workshops held at Hatfield each summer.

Volume 1 of this series described practical procedures for a range of protein techniques frequently used by research workers in the field of molecular biology. Because of the limitations on length necessarily inherent in producing any

book, one obviously had to be selective in the choice of titles for Volume 1. The production of Volume 3, therefore, allows the development of the theme initiated in Volume 1. This volume contains a further selection of detailed protocols for a range of analytical and preparative protein techniques, and should be seen as a continuation of Volume 1. Companion Volumes 2 and 4 provide protocols for nucleic acid methodology.

Each method is described by an author who has regularly used the technique in his or her own laboratory. Not all the techniques described necessarily represent the state-of-the-art. They are, however, dependable methods that achieve the desired result.

Each chapter starts with a description of the basic theory behind the method being described. The main aim of this book, however, is to describe the practical steps necessary for carrying out the method successfully. The Methods section, therefore, contains a detailed step-by-step description of a protocol that will result in the successful execution of the method. The Notes section complements the Methods section by indicating any major problems or faults that can occur with the technique and any possible modifications or alterations.

This book should be particularly useful to those with no previous experience of a technique and, as such, should appeal to undergraduates (especially project students), postgraduates, and research workers who wish to try a technique for the first time.

John M. Walker

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Chapter 1

Prevention of Unwanted Proteolysis

Robert J. Beynon

1. Introduction

Inescapably, all cells contain proteases, introducing the possibility that disruption of the tissue can bring together a protease and a protein, with the result that the latter suffers hydrolytic damage. To quote Pringle (*1,2*), "Proteolytic artifacts are pervasive, perplexing, persistent and pernicious but with proper precautions, preventable." Autolysis has long been recognized as a problem during protein purification, but methods for its control are still far from perfect. Moreover, there are many circumstances other than during protein purification in which endo- or exopeptidase attack upon a pro-

tein can be at best a frustrating nuisance and at worst an undetected artifact that leads to erroneous conclusions.

The purpose of this chapter is to build upon the excellent papers by Pringle (*1,2*) and to provide updated information on methods for prevention of unwanted proteolysis. (Few of my colleagues have been impressed by my suggestion that an effective general purpose protease inhibitor is 2*M* sulfuric acid!) Unfortunately, no global solution to the problem exists, and to a great extent, an *ad hoc* solution depends upon elucidation of some of the properties of the protease(s) that is (are) suspected to be responsible. This chapter may differ from many others in the volume because I cannot present a "method" as much as a philosophy based upon the advice "know thine enemy." Hence, the methods include a sensitive protease assay in addition to a discussion of the handling of protease inhibitors. Largely, I shall restrict the subject matter to proteolytic artifacts that occur in vitro. Control of proteolysis of proteins in vivo is still difficult, although of increasing importance in studies that aim to express a normal or mutated gene in a foreign cell type.

Critically important but sometimes overlooked is the need to establish that the artifact is truly attributable to proteolysis. Dramatic losses of activity of a protein may be caused by proteases, but may also be caused by, among others, thermal denaturation, dissociation of a cofactor, adsorption onto surfaces, dephosphorylation, or inadvertent modification of the redox status of sulfhydryl/disulfide groups. In a crude homogenate, it may be difficult to assign changes in the properties of a protein to the action of proteases, and often, the only successful approach may require addition of potentially protective protease inhibitors. Limited exoproteolytic attack can combine dramatic changes in the biological

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