Two-Dimensional Gel Electrophoresis of Proteins

Methods and Applications

Edited by
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METHODS AND APPLICATIONS

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Preface

Eight years have elapsed since O'Farrell first introduced two-dimensional gel electrophoresis as a high-resolution technique for the separation of thousands of proteins from a given cell type. Since then there have been many technological advances that have led to improvements in the methods of detecting, quantifying, comparing, characterizing, and storing information contained in the gels. The technique, however, has not been fully standardized, and various laboratories use slightly different gel running conditions and sample preparation procedures. In most fields in which this technique is being applied there is no consensus as to how gels should be presented (e.g., with respect to the direction of the pH gradient). Despite these minor drawbacks it is timely to publish a volume on two-dimensional gel electrophoresis of proteins, especially because no comprehensive compilation of these techniques or data is available. This book attempts to present an overview of current procedures and to review a few areas of research in which this technique is currently being applied.

The book has been divided into three sections: General Methodology, Applications, and Protein Catalogs. The first chapter in Section I describes the methodology of two-dimensional gel electrophoresis. The following chapters describe computerized two-dimensional gel electrophoresis, silver staining, immunoblotting, and one- and two-dimensional peptide mapping. In most cases, a step-by-step guide to the techniques is given so that procedures may be easily repeated. Section II is dedicated mainly to applications of two-dimensional gel electrophoresis. Because of space limitations, it has not been possible to review all fields in which this technique is currently being applied. Rather we have chosen to reflect current research interests by paying special attention to expanding subjects. These include applications in clinical and cancer research, human genetics, protein biosynthesis, and gene expression in plants. Section III presents current protein catalogs of *Escherichia coli* and human HeLa cells. A catalog of mouse fibroblast proteins is also presented in Section I.

The book is suitable for young researchers as well as for senior scientists working with a wide variety of problems in molecular and cell biology, basic biochemistry, genetics, and clinical research.

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J. E. Celis
R. Bravo

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PART I

Methods and Satellite Techniques

CHAPTER 1

Two-Dimensional Gel Electrophoresis: A Guide for the Beginner

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TWO-DIMENSIONAL GEL ELECTROPHORESIS OF PROTEINS

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I. Introduction

The two-dimensional gel electrophoresis system that gives the best resolution for separating a complex mixture of proteins combines the techniques of isoelectrofocusing (IEF) in the presence of urea and a neutral detergent in the first dimension and slab gel electrophoresis under denaturing conditions using sodium dodecyl sulfate (SDS) in the second dimension (Klose, 1975; O'Farrell, 1975; Scheele, 1975; Iborra and Buhler, 1976). The separation makes use of two independent protein characteristics: one is the charge, which is reflected by the isoelectric point (pI), and the other is the molecular weight, which determines the mobility of the SDS–protein complexes in polyacrylamide gels (Weber and Osborn, 1969).

O'Farrell (1975) first demonstrated the great potential of such a technique when using isotopically labeled proteins by resolving more than a thousand polypeptides and detecting components as minor as 0.001% or less of the total cellular protein. This method has been extensively applied in resolving proteins from both prokaryotic and eukaryotic organisms and can be used for the separation of many types of cellular proteins [for references, see special issue of *Clinical Chemistry* (Vol. 28, No. 4, Part II, pp. 737–1092, 1982) and other chapters in this volume]. Unfortunately, basic proteins are not well separated in the IEF/SDS system, because in general they enter the IEF gel poorly. Even when more basic ampholytes are included, the extension of the pH gradient is very small, because in the presence of urea the basic region of the gradient is unstable. Furthermore, the few basic proteins that enter the IEF gel under these conditions always produce streaks. However, it is possible to overcome these problems using a nonequilibrium pH gradient electrophoresis (NEPHGE) in the first dimension as described by O'Farrell *et al.* (1977). The main differences between NEPHGE and IEF are that in the former the samples are applied to in the acidic side of the gel and the voltage × time product is smaller than it is in IEF. Under such conditions the pH gradient does not reach full equilibrium. As a result the proteins are not totally focused at their isoelectric point as they are in IEF gels. Nevertheless, most proteins in NEPHGE gels are separated according to their charge differences.

In general, special, not easily attainable apparatus is needed to improve the resolution of the technique, making an initial start difficult for the beginner (Garrels and Gibson, 1976; Garrels, 1979; N. G. Anderson and N. L. Anderson, 1978; N. L. Anderson and N. G. Anderson, 1978). Nevertheless, it is the author's experience that many studies can be done using the standard equipment found in any laboratory. However,