Advances in Electrophoresis

Edited by A. Chrambach, M. J. Dunn, B. J. Radola

Volume 3

Celis et al.	Protein Databases
Miller	Computer-assisted Analysis of Electrophoretograms
Willard-Gallo	Two-dimensional Electrophoresis in Immunology
Foret and Boček	Capillary Electrophoresis
Preece and Brown	Trends in Particle Electrophoresis
Gill	Forensic Application of DNA Profiling

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Contributions from

J. E. Celis, P. Madsen, B. Gesser, S. Kwee, H. V. Nielsen
H. Holm Rasmussen, B. Honoré, H. Leffers, G. P. Ratz, B. Basse
J. B. Lauridsen, A. Celis, Aarhus
M. J. Miller, Bethesda
K. E. Willard-Gallo, Brussels
F. Foret and P. Boček, Brno
A. W. Preece and A. Brown, Bristol/London
P. Gill, Aldermaston

Dr. Andreas Chrambach
Building 10, Room 6C101
National Institutes of Health
Bethesda MD 20892
USA

Dr. Michael J. Dunn
Department of Surgery
National Heart and Lung Institute
Dovehouse Street
London SW3 6LY
England

Professor Bertold J. Radola
Institut für Lebensmittel-
technologie und
Analytische Chemie
Technische Universität München
D-8050 Freising-Weihenstephan
Federal Republic of Germany

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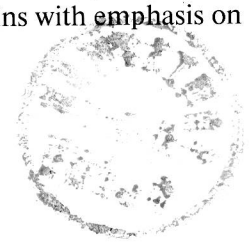
Volume 1

M. J. Dunn, Two-dimensional polyacrylamide gel electrophoresis
C. R. Merril, Detection of proteins separated by electrophoresis
J. M. Gershoni, Protein blotting: a tool for the analytical biochemist
N. C. Stellwagen, Electrophoresis of DNA in agarose and polyacrylamide gels
K. Takeo, Affinity electrophoresis
P. Gebauer et al., Recent trends in capillary isotachophoresis
R. Horuk, Preparative polyacrylamide gel electrophoresis of proteins
J. G. Sutton, Red cell enzyme markers in forensic science: methods of separation and some important applications

Volume 2

A. Görg et al., The current state of electrofocusing in immobilized pH gradients
W. Thormann and *R. A. Mosher,* Theory of electrophoretic transport and separations: the study of electrophoretic boundaries and fundamental separation principles by computer simulation
D. Tietz, Evaluation of mobility data obtained from gel electrophoresis: strategies in the computation of particle and gel properties on the basis of the extended Ogston model
R. J. Cooke, Electrophoresis in plant testing and breeding
C. Damerval et al., Two-dimensional electrophoresis in plant biology
S. M. Hanash, Contribution of protein electrophoretic analysis to cancer research
G. Unteregger, Two-dimensional electrophoresis of nonhistone chromosomal proteins with emphasis on protein prefractionation



Preface

It seems appropriate to reflect on the reasons behind the production of an annual review series on electrophoresis. The monthly journal *Electrophoresis* provides space for reviews and so do many other methodological journals. Moreover, the meetings of the international and some national electrophoresis societies produce proceedings also containing review articles. The purpose of *Advances in Electrophoresis* is to assemble these multiple sources into a central "review bank" that is readily available to everyone using electrophoretic methods.

A central review bank should provide a forum for the authoritative voices in each specialized field of electrophoresis, thereby helping to resolve problems created by discordant advice at different levels of expertise. It should serve to unify research areas whose results are published in a wide range of journals, for example, those of the two most challenging classes of substances – proteins and nucleic acids. Rather than summarizing all available information, the reviews in *Advances in Electrophoresis* present the essence of each topic and demonstrate its potential. The reviews are directed to the great many readers who already use electrophoretic techniques but do not follow their development in the original literature. Also, the reviews should be indispensible to those interested in the application of a new technique or entering a field requiring the use of electrophoresis. Ideally, the reviews will be the key references for the following years in a particular area.

Advances in Electrophoresis contains reviews dealing either with selected techniques or important areas of application of electrophoresis. We have already alluded to the need for reviews on methodological progress. However, we consider it equally essential to provide reviews on important areas of application. Electrophoresis is not an esoteric method employed by only a small group of experts. On the contrary, its range of applications is increasing at an astonishing pace and, in many areas, it is already established as an indispensible tool. By publishing in *Advances in Electrophoresis* a balanced blend of reviews covering applications and techniques we expect a crossfertilizing effect which should stimulate further developments in the field of electrophoresis.

In order to accomplish these aims, we should like to encourage our readers to send us their comments, criticisms and suggestions for important topics to be included in forthcoming volumes. Finally, we wish to thank the authors for the sacrifice they have made in filling these pages and thereby in providing the field of electrophoresis with its first centralized retrieval bank.

October 1989

Andreas Chrambach
Michael J. Dunn
Bertold J. Radola

Contributors

Bodil Basse
Institute of Medical Biochemistry
and Bioregulation Research Centre
Aarhus University
DK-8000 Aarhus C, Denmark
p. 1

Petr Boček
Institute of Analytical Chemistry
Czechoslovak Academy of Sciences
Leninova 82
CS-61142 Brno, Czechoslovakia
p. 271

Alun Brown
Department of Immunology
Rayne Institute
St. Thomas' Hospital
London, UK
p. 349

Ariana Celis
Institute of Medical Biochemistry
and Bioregulation Research Centre
Aarhus University
DK-8000 Aarhus C, Denmark
p. 1

Julio E. Celis
Institute of Medical Biochemistry
and Bioregulation Research Centre
Aarhus University
DK-8000 Aarhus C, Denmark
p. 1

František Foret
Institute of Analytical Chemistry
Czechoslovak Academy of Sciences
Leninova 82
CS-61142 Brno, Czechoslovakia
p. 271

Borbola Gesser
Institute of Medical Biochemistry
and Bioregulation Research Centre
Aarhus University
DK-8000 Aarhus C, Denmark
p. 1

Peter Gill
Central Research and Support
Establishment
Home Office Forensic Science Service
Aldermaston, Reading,
Berkshire, R67 4PN, UK
p. 405

Hanne Holm Rasmussen
Institute of Medical Biochemistry
and Bioregulation Research Centre
Aarhus University
DK-8000 Aarhus C, Denmark
p. 1

Bent Honoré
Institute of Medical Biochemistry
and Bioregulation Research Centre
Aarhus University
DK-8000 Aarhus C, Denmark
p. 1

Sianette Kwee
Institute of Medical Biochemistry
and Bioregulation Research Centre
Aarhus University
DK-8000 Aarhus C, Denmark
p. 1

Jette B. Lauridsen
Institute of Medical Biochemistry
and Bioregulation Research Centre
Aarhus University
DK-8000 Aarhus C, Denmark
p. 1

Henrik Leffers
Institute of Medical Biochemistry
and Bioregulation Research Centre
Aarhus University
DK-8000 Aarhus C, Denmark
p. 1

Peder Madsen
Institute of Medical Biochemistry
and Bioregulation Research Centre
Aarhus University
DK-8000 Aarhus C, Denmark
p. 1

Mark J. Miller
National Institutes of Health
Laboratory of Experimental Carcinogenesis
Division of Cancer Etiology
National Cancer Institute
Bethesda, MD 20892, USA
p. 181

Henrik V. Nielsen
Institute of Medical Biochemistry
and Bioregulation Research Centre
Aarhus University
DK-8000 Aarhus C, Denmark
p. 1

Alan W. Preece
Biophysics Group
Radiotherapy Centre
Horfield Road
Bristol, BS2 8ED, UK
p. 349

Gitte P. Ratz
Institute of Medical Biochemistry
and Bioregulation Research Centre
Aarhus University
DK-8000 Aarhus C, Denmark
p. 1

Karen E. Willard-Gallo
International Institute of Cellular
and Molecular Pathology
Avenue Hippocrate, 75
Brussels, Belgium
p. 219

Contents

Protein databases derived from the analysis of two-dimensional gels
Julio E. Celis, Peder Madsen, Borbala Gesser, Sianette Kwee, Henrik V. Nielsen, Hanne Holm Rasmussen, Bent Honoré, Henrik Leffers, Gitte P. Ratz, Bodil Basse, Jette B. Lauridsen, and Ariana Celis

1 Introduction 3
2 Cellular protein databases 4
3 Databases of secreted proteins 22
4 Databases of tissue proteins 23
5 Databases of body fluid proteins 25
6 Concluding remarks 27
7 References 28
8 Appendix 1: Figures 36
9 Appendix 2: Tables 101

Computer-assisted analysis of two-dimensional gel electrophoretograms
Mark J. Miller

1 Introduction 182
2 Current systems 184
3 Data acquisition 186
4 Data smoothing 189
5 Spot detection and resolution 191
6 Background determination and adjustment 196
7 Calibration 197
8 Spot quantification 198
9 Matching pairs of gels 201
10 Cross matching sets of gels 203
11 Interpretation of results 209
12 Commercial systems 212
13 Conclusions 213
14 References 214

Immunological applications of two-dimensional polyacrylamide gel electrophoresis
Karen E. Willard-Gallo

1	Introduction	221
2	The major histocompatibility complex	221
3	Cellular proteins in human leukocytes	225
4	Proteins in the cell membrane of leukocytes	229
5	Antibodies to proteins separated by two-dimensional gels	236
6	Leukocyte-specific proteins	240
7	Abnormal human lymphoid cells	256
8	Biological response modifiers	261
9	Summary and prospects	265
10	References	266

Capillary electrophoresis
František Foret and Petr Boček

1	Introduction	273
2	Brief history	274
3	Basic concepts	275
4	Migration behavior of the sample zone in an electrolyte system	278
5	Phenomena accompanying electrophoresis	293
6	Capillary zone electrophoresis	301
7	Micellar electrokinetic capillary chromatography	309
8	Capillary isoelectric focusing	313
9	Instrumentation	315
10	Applications	329
11	Addendum	341
12	References	342

Recent trends in particle electrophoresis
Alan W. Preece and K. Alun Brown

1	Introduction	351
2	Origins of surface charge	352
3	Equipment development	353
4	Specialized measurement techniques	361
5	Amplitude weighted phase structuration	375
6	Applications	378
7	References	399

The forensic application of DNA profiling
Peter Gill

1	Introduction	406
2	Hypervariable loci	408
3	Methodology	411
4	DNA 'fingerprinting'	417
5	Alternative probes	427
6	DNA profiles in animals	432
7	DNA profiling in case-work	432
8	DNA analysis in sex determination	436
9	Polymerase chain reaction	439
10	Quality assurance	441
11	Conclusion	441
12	Addendum	442
13	References	442

Index 445

PROTEIN DATABASES DERIVED FROM THE ANALYSIS OF TWO-DIMENSIONAL GELS

Julio E. Celis, Peder Madsen, Borbala Gesser, Sianette Kwee, Henrik V. Nielsen, Hanne Holm Rasmussen, Bent Honoré, Henrik Leffers, Gitte P. Ratz, Bodil Basse, Jette B. Lauridsen, and Ariana Celis

Institute of Medical Biochemistry and Bioregulation Research Centre, Aarhus University, Aarhus C, Denmark

1	Introduction	3
2	Cellular protein databases	4
2.1	Human	4
2.1.1	Transformed human epithelial amnion cells	4
2.1.1.1	Protein name	6
2.1.1.2	Nuclear proteins	6
2.1.1.3	Phosphorylated proteins	6
2.1.1.4	Distribution of proteins in 0.1% Triton X-100 supernatant and cytoskeleton	6
2.1.1.5	Proliferation and transformation sensitive proteins	7
2.1.1.6	Cell cycle specific proteins	7
2.1.1.7	Mitochondrial proteins	7
2.1.1.8	Heat shock proteins	8
2.1.1.9	Proteins affected by interferons	8
2.1.1.10	Cytoskeletal proteins	9
2.1.1.11	Presence of antibody against protein in human sera	9
2.1.1.12	The human epithelial amnion cells protein database, microsequencing and current efforts to map and sequence the entire human genome	9
2.1.2	HeLa cells	10
2.1.3	Normal human embryonal lung MRC-5 fibroblasts	10
2.1.3.1	Transformation and/or proliferation sensitive proteins	11
2.1.3.2	Transformation sensitive proteins whose synthesis increases substantially in transformed MRC-5 V2 fibroblasts	11

2.1.3.3	Transformation sensitive proteins whose synthesis decreases substantially in transformed MRC-5 V2 fibroblasts	13
2.1.4	Normal human diploid fibroblasts (KD strain)	14
2.1.5	Human peripheral blood mononuclear cells	14
2.2	Rat	16
2.2.1	Rat embryonic fibroblasts (REF 52)	16
2.3	Mouse	17
2.3.1	Normal secondary mouse kidney fibroblasts	17
2.3.2	NIH/3T3 cells	18
2.3.3	T Lymphocytes	18
2.4	*Aplysia*	18
2.5	Yeast	19
2.5.1	*Saccharomyces cerevisiae*	19
2.6	Plants	20
2.6.1	Wheat	20
2.6.2	Barley	20
2.6.3	Sorghum	20
2.6.4	*Euglena*	21
2.7	*Escherichia coli*	21
3	Databases of secreted proteins	22
3.1	Human	22
3.1.1	Normal human embryonal lung MRC-5 fibroblasts	22
4	Databases of tissue proteins	23
4.1	Human	23
4.1.1	Brain	23
4.2	Rat	24
4.2.1	Brain	24
4.3	Mouse	24
4.3.1	A systematic approach to the analysis of the total protein complex of mouse	24
4.3.2	Liver	25
5	Databases of body fluid proteins	25
5.1	Human	25
5.1.1	Plasma proteins	25
5.1.2	Cerebrospinal fluid	26
5.1.3	Amniotic fluid	26
5.1.4	Urine	26
5.1.5	Milk	27

6 Concluding remarks 27

7 References 28

8 Appendix 1: Figures 36

9 Appendix 2: Tables 101

Abbreviations: AMA, transformed human epithelial amnion cells; **CNS**, central nervous system; **CSF**, cerebrospinal fluid; **2-D**, two-dimensional; **IEF**, isoelectric focusing; **IFN**, interferon; M_r, relative molecular mass; **NEPHGE**, nonequilibrium pH gradient electrophoresis; **PBMC**, peripheral blood mononuclear cells; **PCA**, principal component analysis; **PCNA**, proliferating cell nuclear antigen; **p*I***, isoelectric point; **REF**, rat embryonic fibroblasts; **TM**, tropomyosin

1 Introduction

High resolution two-dimensional (2-D) gel electrophoresis [1–3] has provided a unique tool (i) to analyze the protein composition of complex samples, and (ii) to examine the global patterns of gene expression of a given cell type ([4–6] and references therein). To date, there have been thousands of reports illustrating the usefulness of this technique in many areas of biology, but only recently, thanks to the development of appropriate computer software ([7–16] see also the chapter by Miller in this volume), it has been possible to scan, assign number to individual polypeptides, compare, quantitate and store the wealth of information contained in the gels. In short, this important development has opened the possibility of establishing computerized databases which in the future are expected to provide an efficient and standardized medium to communicate protein information. Clearly, databases allow easy access to a large body of data: once a protein is identified in a given database (comigration with purified proteins, immunoblotting using specific antibodies, comparison of peptide sequences to sequences stored in protein databanks), all of the information accumulated can be easily retrieved and made available to the researcher.

Obviously, there is no limitation to the type or size of protein databases that can be constructed (manual or computerized, see below), although comprehensive computerized databases, that is those containing information concerning various properties (physical, chemical, biochemical, physiological, genetic, biological, immunological, architectural, *etc.*) of all the proteins of a given cell may turn out to be the most exciting in the long run, as they will permit a global approach to the study of genome organization and function.

The purpose of this review is to give some examples of manual and computerized protein databases established so far, and to underline some satellite technology that may be fundamental in the future development of databases. Given space limitations, we will not comment on various computer softwares used in different laboratories (see Miller's article in this volume). Also, work in the author's laboratory has been deliberately overrepresented, so as to illustrate in detail the potential of comprehensive, computerized protein databases.

2 Cellular protein databases

2.1 Human

To date, a few databases of human cellular proteins have been reported [17–24], but only three have been published in a comprehensive, computerized form (transformed epithelial amnion cells (AMA) [22], peripheral blood mononuclear cells (PBMC) [22] and normal embryonic lung MRC-5 fibroblasts [24]). Interesting features of these databases are described below.

2.1.1 Transformed human epithelial amnion cells

This is perhaps the most comprehensive, computerized mammalian protein database published so far [22], and therefore, it will be reviewed in detail so as to illustrate the potential of human databases and of databases in general. One thousand seven hundred and eighty-one [^{35}S]methionine labeled polypeptides have been separated (1244, IEF, Fig. 1*; 537, NEPHGE, Fig. 2) and recorded by Celis and colleagues using computerized high resolution 2-D gel electrophoresis [22] (QUEST system) [7, 15]. Edited synthetic images of the

* All Figures are collected in Appendix 1, pages 36–100

gels shown in Figs. 1 and 2 are presented in Figs. 3 (IEF) and 4 (NEPHGE), respectively, and computer print-outs displaying all the numbers assigned to the spots are shown in Figs. 5 (IEF) and 6 (NEPHGE). Gel grids that may be used to locate spots in Figs. 5 and 6 are shown in Figs. 7A (IEF) and B (NEPHGE). To facilitate comparison between the gel fluorograms and the computer print-outs, Figs. 1 and 2 display a few proteins which are marked with black in Figs. 5 and 6.

All 1781 proteins are listed in Tables 2 and 3 and a brief explanation of the information entered for a spot in this database is given in Table 1* [22]. In general, categories or entries have been created so as to compile information concerning physical, chemical, biochemical, physiological, genetic, architectural and biological properties of proteins. Information (annotations) contained in entries 1 to 9 and 12 to 15 has been entered by hand. Some of this information has been gathered in other cell types or tissues whose proteins have been matched to AMA proteins. Even though a substantial amount of the information included in Tables 2 and 3 has been generated in AMA cells (entries 4–6, 8, 12–15), some of these data are also available in the manual HeLa protein catalogue [18, 20]. Thus, to facilitate comparison with previously published studies from Celis' laboratory, the HeLa (Tables 2 and 3; entry 2) [18, 20] and mouse (Tables 2 and 3; entry 3) [25] protein catalogue numbers have been included.

Entries 10 and 11 in Tables 2 and 3 list proteins from normal human embryonal MRC-5 fibroblasts [24], and normal PBMC that have been matched (both manually and by the computer) to AMA cell proteins. Fig. 8 shows examples of acidic cellular proteins from human AMA cells (Fig. 8, left) and PBMC (Fig. 8, right) that have been matched by the computer (indicated with the same letters in both IEF synthetic images). Landmarks, which are indicated with a +, have been added manually. Molecular weights of the matched proteins may differ slightly in some cases, and this is due to local distortions in the gels. It should be stressed that some of the proteins have only been matched based on their gel positions with respect to other neighboring proteins, and therefore, additional tests (comigration with purified proteins, immunoblotting using specific antibodies, comparison of peptide sequences) are needed to verify their relatedness.

* For Tables see Appendix 2, pages 101–179