

Anthony P. Nicholas
Sanjoy K. Bhattacharya *Editors*

Protein Deimination in Human Health and Disease

 Springer

Protein Deimination in Human Health and Disease



Protein Deimination

In Human Health and Disease

Edited by
Anthony P Nicholas
Sanjoy K Bhattacharya

Anthony P. Nicholas • Sanjoy K. Bhattacharya
Editors

Protein Deimination in Human Health and Disease

 Springer

Editors

Anthony P. Nicholas
Department of Neurology
University of Alabama at Birmingham
and The Birmingham Veterans
Administration Medical Center
Birmingham, AL, USA

Sanjoy K. Bhattacharya
Bascom Palmer Eye Institute
University of Miami
Miami, FL, USA

ISBN 978-1-4614-8316-8 ISBN 978-1-4614-8317-5 (eBook)

DOI 10.1007/978-1-4614-8317-5

Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013948455

© Springer Science+Business Media New York 2014

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.

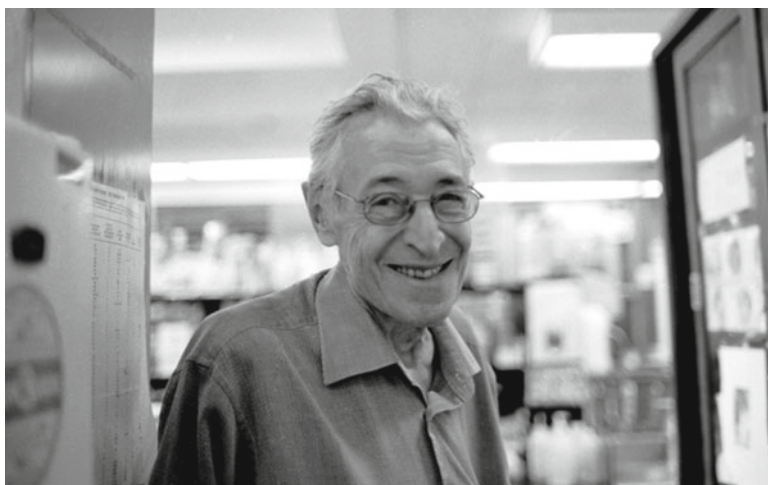
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Inside Cover: Confocal micrograph of immunofluorescent staining in a normal human cerebellum showing co-localization (*yellow/orange*) of glial fibrillary acidic protein (*green*) and deiminated proteins (*red*), using the F95 monoclonal antibody. Modified from Nicholas and Whitaker, *Glia*, Volume 37, pp. 328–336, Copyright 2002, Wiley-Liss, Inc.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)



Professor Mario Moscarello, one of the founders of the deimination field, passed away on Thursday, August 8, 2013, at the age of 83 years, at Toronto Western Hospital, with his family by his side. Mario was a pioneer in myelin research, paving the way for a greater understanding of protein–lipid interactions and the role of post-translational modifications on these interactions throughout his scientific research career of 52 years. At the time of his passing, he was Professor Emeritus at the University of Toronto and Senior Emeritus Scientist at The Hospital for Sick Children. During his career, he supervised more than 80 students from around the world.

Mario received his M.D. degree in 1955 from the University of Toronto and subsequently entered graduate school, obtaining a Ph.D. in biochemistry. His early career involved active research in the biochemistry of amino acids in encephalomyelitis and the encephalomyocarditis virus. Perhaps this foundation, both in amino acid analyses and myelin changes, prepared him for his subsequent discovery of deimination of myelin basic protein.

In 1966, Mario began studying the isolation of acid-soluble proteins from myelin. By 1968, he started an intensive investigation of myelin proteins along with Dr. D. Denise Wood. This led to the discovery of the presence of peptide-bound citrulline in myelin proteins in 1971, coincident with G. E. Rogers' isolation of L-citrulline as a component of proteins from cells in hair fiber medullae and inner root sheaths of hair follicles. Mario showed that citrulline was present in acid hydrolysates of a protein fraction from normal human myelin and in the nonhydrolyzed protein as well, by direct colorimetric analysis. However, stemming from his deep familiarity with biochemistry, he further confirmed the presence of citrulline by protease digestion of myelin, chromatographic separation, and colorimetric confirmation, which was state of the art at that time. This is one of the very first landmark studies that placed L-citrulline within proteins on the map. Arguably and potentially unbeknownst to him this was also one of the early milestones for the field of deimination research.

Dr. Moscarello continued studying myelin, the interaction of myelin proteins with membrane lipids and, in 1976, showed that a nine-peptide sequence derived from myelin basic protein was encephalitogenic, but required more than a linear peptide to induce full encephalitogenic potential. In 1989, he demonstrated the lipid-aggregating properties of citrulline-containing myelin basic protein, another seminal discovery in deimination research. These studies were indicative of an important role for this posttranslational protein in basic biochemical alterations of neuronal membranes. Although Mario's lab had developed an antibody that distinguished citrullinated moieties from arginine, it was during a collaboration with the late John N. Whitaker (then at University of Alabama at Birmingham) in 1992 that they distinguished the MBP C1 isomer from its less-cationic citrullinated isomers and the least-cationic C8 citrullinated isomer. In 1993, Mario began using the term "deimination" when he discovered the ability of the enzyme peptidylarginine deiminase from bovine brain to citrullinate (convert peptidyl-arginine to peptidyl-citrulline) human myelin basic protein. The discovery of this enzymatic activity was first made by Kubilus and Baden in 1983; however, the activity was never tested for modification of myelin basic protein until it was accomplished in the Moscarello laboratory. Another seminal discovery from Mario's group came in 1994, when they showed that myelin in multiple sclerosis was developmentally immature and highly citrullinated. This was the first report, which was published in the *Journal of Clinical Investigation*, describing the paradoxical increased deimination in the brains of infants and patients with multiple sclerosis, when compared with normal adults. Mario also showed similarities in posttranslational modification of myelin basic proteins between models of multiple sclerosis and Pelizaeus-Merzbacher disease, thus establishing a possibility of common denominators in different demyelinating disorders.

We would like to think that Professor Moscarello is survived not only by his family, but also by his work, and we believe that advancing the field of deimination research is the best way to keep his memory alive. With that thought, we dedicate this book to Professor Mario Moscarello, a great mentor and teacher who always instilled in his students the importance of leading a full life and to focus on the work at hand. Ironically, he always telegraphed this by referring to a lyric from the old spiritual entitled *Life's Railway to Heaven*, "Keep your hand upon the throttle, and your eye upon the rail." We will miss him dearly.

Preface

Deimination refers to the posttranslational conversion of protein-bound arginine into protein-bound citrulline. It is often interchangeably termed as “citrullination,” which may also refer to the conversion of free arginine into citrulline. As a result, we have promoted the use of the word “deimination” to exclusively refer to the posttranslational modification (PTM) of protein-bound arginine for ameliorating some confusion for new investigators or researchers from other fields.

Despite being a relatively long-known PTM in mammals and other organisms, deimination has not been subject to rigorous research that some other PTMs have received, such as phosphorylation and glycosylation. Even sumoylation, a relatively newly discovered PTM, has about ten times more recorded published papers today. Currently, for a modification such as phosphorylation there are 10,000-fold more published papers, compared to deimination. In recent major PTM meetings, deimination either records no or only a token presence. For example, during the recent ASBMB-conducted PTM meeting in 2012, deimination was represented only by a single poster.

Two advances are expected to accelerate the pace of research on deimination: (a) the discovery of deiminated proteins with direct relationships to human disease and (b) the development of new reagents for assessment and quantification of deiminated proteins.

Usually the functions of a protein and its involvement in key biological processes spark interest in that protein, especially if a PTM is found to regulate the role of the protein in question. Unfortunately, early detection of deimination occurred in proteins that were primarily structural, during a time in which the study of structural cellular proteins was thought not to be particularly exciting. Although the first deiminated proteins were described in the late 1950s, almost 20 years went by before the enzymes responsible for this PTM, the peptidyl-arginine deiminases (PADs), were first discovered and later confirmed in almost all tissues of the human body.

Although PAD was found in the brain as early as the 1980s, showing to deiminate myelin structural proteins, the largest influx of researchers into the field up until that point did not occur until the late 1990s, after a direct association was found between the presence of deiminated proteins and the occurrence of rheumatoid arthritis. In fact, this disease is now primarily confirmed with a blood test that measures the amount of antibodies against deiminated proteins. As a result, the first chapters (Chaps. 1–6) of this book are dedicated to this topic, covering clinical aspects, the importance of anti-peptidyl-citrulline antibodies, and the roles of gum disease, smoking, and white blood cells themselves in the propagation and detection of this disorder.

Closely related to the joint, deimination is also involved in other related tissues, such as skin (Chap. 7) and hair (Chap. 8), playing important roles in the outer protection of the human body. The next eight chapters are dedicated to the nervous system, including the role of deimination in peripheral nerve development and responses to damage (Chap. 9). Also included are inflammatory diseases of the brain, such as multiple sclerosis (Chaps. 10 and 11), and neurodegenerative diseases, such as Creutzfeldt-Jakob disease (Chap. 12), Alzheimer's disease (Chap. 13), Parkinson's disease, amyotrophic lateral sclerosis, and others (Chap. 14). Also included in the central nervous system is the spinal cord (Chap. 15) and eye (Chap. 16), in which deimination has been linked to several normal processes, as well as disease states.

Recently, increased PAD has been linked to cancer (Chap. 17). But probably the most interesting discovery within the last few years has been the role of deimination as a possible reverser of arginine methylation involved in epigenetic processes controlling the transcription of DNA (Chap. 18), since this mechanism may have ramifications for all of the prior normal processes and disease states linked to deimination. Thus, understanding the place of deimination vis a vis methylation on arginine residues of histone proteins that control the unwinding of the genetic code may be of immense biological significance.

On the other hand, confirmatory detection of deimination still remains a challenge. A rate-limiting step exists with the availability of reliable reagents and methods that enable verifiable detection of this PTM. Compounded with limitations in detection are problems with localization of peptidyl-citrulline moieties, which will need some additional development. For example, a current review on mass spectrometric methods used in this regard summarizes the current state of confirmatory detection (Chap. 19). Also, confounders such as the presence of peptidyl-homocitrulline, a PTM of lysine, must be acknowledged and accounted for, when studying deimination (Chap. 20). However, the most exciting and latest advancement in deimination research is the development and use of the first wave of PAD antagonists (Chap. 21), which is further highlighting how this PTM may be manipulated as new therapeutic interventions for a vast variety of human diseases in which increased deimination is believed to play a critical role. As evidenced in this book, teams of chemists, biologists, engineers, neuroscientists, and physicians have come together, with the promise of integrated collaboration that will hopefully prompt the development of new reagents and methods, as well as possible new treatments for devastating diseases that presently have few therapeutic options.

Ultimately, understanding how protein deimination is involved in human health and disease will hopefully be the focus of a new wave of investigators who will join us in uncovering the secrets of these altered proteins. As a first step, this book summarizes our current knowledge of this exciting and growing research field.

Birmingham, AL, USA
Miami, FL, USA

Anthony P. Nicholas
Sanjoy K. Bhattacharya

Contents

1 Physiological Pathways of PAD Activation and Citrullinated Epitope Generation.....	1
Amanda S. Rohrbach, Sanja Arandjelovic, and Kerri A. Mowen	
2 From Citrullination to Specific Immunity and Disease in Rheumatoid Arthritis.....	25
Aase Haj Hensvold, Gudrun Reynisdottir, and Anca I. Catrina	
3 The Role of Synovial Citrullinated Proteins in the Pathophysiology of Rheumatoid Arthritis.....	41
Joyce J.B.C. van Beers and Ger J.M. Pruijn	
4 Protein Citrullination: The Link Between Rheumatoid Arthritis and Periodontitis?.....	69
M.J. de Smit, J. Westra, W. Nesse, A. Vissink, A.J. van Winkelhoff, and E. Brouwer	
5 From Genes and Environment to Anti-citrulline Immunity in Rheumatoid Arthritis: The Role of the Lungs.....	83
Dimitris Makrygiannakis	
6 Neutrophils and Their Contribution to Autoimmunity in Rheumatoid Arthritis.....	97
Julia Spengler and Dagmar Scheel-Toellner	
7 Deimination in Skin and Regulation of Peptidylarginine Deiminase Expression in Keratinocytes.....	113
Hidenari Takahara, Guy Serre, and Michel Simon	
8 Importance of Citrullination on Hair Protein Molecular Assembly During Trichocytic Differentiation.....	129
Kenji Kizawa, Masaki Unno, Claus W. Heizmann, and Hidenari Takahara	

**9 Deimination in the Peripheral Nervous System:
A Wallflower Existence** 149
Gerburg Keilhoff and Anthony P. Nicholas

**10 Deimination in Multiple Sclerosis and Experimental
Autoimmune Encephalomyelitis** 165
Claire Bradford, Anthony P. Nicholas, Nicola Woodroffe,
and Alison K. Cross

**11 Protein Hypercitrullination in CNS Demyelinating
Disease Reversed by PAD Inhibition** 187
Mario A. Moscarello

12 Deimination in Prion Diseases 219
Eun-Kyoung Choi, Byungki Jang, Akihito Ishigami,
Naoki Maruyama, Richard I. Carp, and Yong-Sun Kim

13 Deimination in Alzheimer’s Disease 237
Akihito Ishigami, Eun-Kyoung Choi, Yong-Sun Kim,
and Naoki Maruyama

**14 Ongoing Studies of Deimination in Neurodegenerative
Diseases Using the F95 Antibody** 257
Anthony P. Nicholas, Liang Lu, Michael Heaven, Inga Kadish,
Thomas van Groen, Mary Ann Accaviti-Loper, Sonja Wewering,
Diane Kofskey, Pierluigi Gambetti, and Michael Brenner

**15 The Role of Deimination as a Response to Trauma
and Hypoxic Injury in the Developing CNS** 281
Patrizia Ferretti, Sigrun Lange, Kin Pong U, and Gennadij Raivich

16 Deimination in Ocular Tissues: Present and Future 295
Di Ding, Mabel Enriquez-Algeciras, Vera L. Bonilha,
and Sanjoy K. Bhattacharya

**17 PAD Enzymes in Female Reproductive Tissues
and Cancer Pathogenesis** 305
Brian D. Cherrington, Sunish Mohanan, and Scott A. Coonrod

18 The Role of Protein Deimination in Epigenetics 327
Jialiang Liang and Patrizia Casaccia

19 Identifying Citrullination Sites by Mass Spectrometry 347
Jennifer E. Grant and Hong Li

20 Homocitrulline: An Analog and Confounder Related to Citrulline	367
Sanna Turunen, Marja-Kaisa Koivula, Anthony P. Nicholas, Leila Risteli, and Juha Risteli	
21 Picking the PAD Lock: Chemical and Biological Approaches to Identify PAD Substrates and Inhibitors	377
Venkataraman Subramanian, Daniel J. Slade, and Paul R. Thompson	
Index	429

Chapter 1

Physiological Pathways of PAD Activation and Citrullinated Epitope Generation

Amanda S. Rohrbach, Sanja Arandjelovic, and Kerri A. Mowen

Keywords Peptidylarginine deiminase • PAD • Calcium • Citrullination • Deimination • Disease • Rheumatoid arthritis

1.1 The Peptidylarginine Deiminase Family

The free amino acid form of citrulline was first isolated from watermelon (*Citrullus vulgaris*) over 70 years ago (Curis et al. 2005), while the peptidyl form of citrulline was first recognized within the hair follicle (Rogers 1962). Peptidylcitrulline is a noncoding amino acid that is generated through hydrolysis of peptidyl-arginine residues by Ca²⁺-dependent peptidylarginine deiminase (PAD) enzymes, with ammonia released as a reaction by-product (Fig. 1.1). This process is referred to as deimination or citrullination. The conversion of arginine to citrulline results in only a small increase in molecular mass (less than 1 Da) but also converts the positively charged guanidino group on an arginine residue into the neutrally charged ureido group on the citrulline amino acid. The small mass difference between arginine and citrulline residues has made identifying sites of deimination challenging, especially on proteins isolated from cellular sources (Hao et al. 2009).

Although an approximate 1 Da change in mass may seem like a relatively minor difference, the conversion of charge from an arginine to a citrulline can have dramatic consequences on protein structure, proteolytic susceptibility, and protein–protein interactions (Vossenaar et al. 2003). For example, filaggrin is a highly basic

A.S. Rohrbach • S. Arandjelovic • K.A. Mowen (✉)
Department of Chemical Physiology, The Scripps Research Institute,
10550 North Torrey Pines Road, La Jolla, CA 92037, USA
e-mail: kmowen@scripps.edu