Modification of Proteins During Aging

MODIFICATION OF PROTEINS DURING AGING

Proceedings of the Mini-Symposium Session "Impact of Aging on Biochemical Function," held during the 75th Annual Meeting of the American Society of Biological Chemists, St. Louis, Missouri, June 3–7, 1984

Editors

Richard C. Adelman

Director of the Institute of Gerontology
Professor of Biological Chemistry
University of Michigan
Ann Arbor, Michigan

Eugene E. Dekker

Acting Chairman
Department of Biological Chemistry
University of Michigan
Ann Arbor, Michigan

Alan R. Liss, Inc., New York

Address All Inquiries to the Publisher
Alan R. Liss, Inc., 41 East 11th Street, New York, NY 10003

Printed in the United States of America.

Library of Congress Cataloging in Publication Data

Main entry under title:

Modification of proteins during aging.

(Modern aging research; v.7)
Includes bibliographies and index.
1. Aging—Congresses. 2. Proteins—Congresses.
I. Adelman, Richard C., 1940- . II. Dekker,
Eugene E. III. American Society of Biological
Chemists. Meeting (75th : 1984 : St. Louis, Mo.)
IV. Title: Impact of aging on biochemical function.
V. Series. [DNLM: 1. Aging—congresses. 2. Enzymes
—metabolism—congresses. 3. Metabolism—in old age—
congresses. W1 M0117 v.7 / WT 104 M692 1984]
QP86.M65 1985 612'.67 85-12922
ISBN 0-8451-2307-6

MODIFICATION OF PROTEINS DURING AGING

MODERN AGING RESEARCH

Series Editors

Richard C. Adelman — George T. Baker III
Jay Roberts — Vincent J. Cristofalo

Volume 1: Neural Regulatory Mechanisms During Aging, Richard C. Adelman, Jay Roberts, George T. Baker III, Steven I. Baskin, and Vincent J. Cristofalo, *Editors*

Volume 2: Aging and Human Visual Function, Robert Sekuler, Donald Kline, and Key Dismukes, *Editors*

Volume 3: Intervention in the Aging Process, William Regelson and F. Marott Sinex, *Editors*. Published in two volumes. Part A: Quantitation, Epidemiology, and Clinical Research. Part B: Basic Research and Preclinical Screening.

Volume 4: Comparative Pathobiology in Major Age-Related Diseases: Current Status and Research Frontiers, Dante G. Scarpelli and George Migaki, *Editors*

Volume 5: Senile Dementia: Outlook for the Future, Jean Wertheimer and Maurice Marois, *Editors*

Volume 6: Altered Endocrine Status During Aging, Vincent J. Cristofalo, George T. Baker III, Richard C. Adelman, and Jay Roberts, *Editors*

Volume 7: Modification of Proteins During Aging, Richard C. Adelman and Eugene E. Dekker, *Editors*

Contributors

Bong Whan Ahn, Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20205 **[39]**

F. Aladar Bencsath, Laboratory of Mass Spectrometry, Rockefeller University, New York, NY 10021 **[83]**

Katherine M. Brooks, Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104 **[109]**

Cathleen R. Carlin, Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104 **[109]**

Anthony Cerami, Laboratory of Medical Biochemistry, Rockefeller University, New York, NY 10021 **[83]**

Jane C.F. Chang, Laboratory of Medical Biochemistry, Rockefeller University, New York NY 10021 **[83]**

Mary Lynne Chapman, Department of Biochemistry, North Texas State University, and Texas College of Osteopathic Medicine, Fort Worth, TX 76107 **[1]**

John K. Cini, Department of Biochemistry, North Texas State University, and Texas College of Osteopathic Medicine, Fort Worth, TX 76107 **[1]**

Vincent J. Cristofalo, Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104 **[109]**

A. Dovrat, Department of Biology, Technion — Israel Institute of Technology, Haifa 32000, Israel **[69]**

Ari Gafni, Institute of Gerontology and Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109 **[19]**

Paul M. Gallop, Laboratory of Human Biochemistry, Children's Hospital Corporation and the Departments of Biological Chemistry and Oral Biology, Harvard Schools of Medicine and Dental Medicine, Cambridge, MA 02115 **[93]**

David Gershon, Department of Biology, Technion — Israel Institute of Technology, Haifa 32000, Israel **[69]**

Robert W. Gracy, Department of Biochemistry, North Texas State University, and Texas College of Osteopathic Medicine, Fort Worth, TX 76107 **[1]**

Caren M. Gundberg, Laboratory of Human Biochemistry, Children's Hospital Corporation and the Departments of Biological Chemistry and Oral Biology, Harvard Schools of Medicine and Dental Medicine, Cambridge, MA 02115 **[93]**

Minoo Jahani, Department of Biochemistry, North Texas State University, and Texas College of Osteopathic Medicine, Fort Worth, TX 76107 **[1]**

Rodney L. Levine, Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20205 **[39]**

H.S. Lu, Department of Biochemistry, North Texas State University, and Texas College of Osteopathic Medicine, Fort Worth, TX 76107 **[1]**

Cynthia N. Oliver, Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20205**[39]**

The number in brackets is the opening page number of the contributor's article.

B. Oray, Department of Biochemistry, North Texas State University, and Texas College of Osteopathic Medicine, Fort Worth, TX 76107 **[1]**

Paul D. Phillips, Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104 **[109]**

Sandor Pongor, Boyce Thompson Institute, Cornell University, Ithaca, NY 14853 **[83]**

A.Z. Reznick, Department of Biology, Technion — Israel Institute of Technology, Haifa 32000, Israel **[69]**

L. Rosenfelder, Department of Biology, Technion — Israel Institute of Technology, Haifa 32000, Israel **[69]**

Morton Rothstein, Department of Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14260 **[53]**

S. Shpund, Department of Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14260 **[69]**

Earl R. Stadtman, Chief, Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20205 **[39]**

J.M. Talent, Department of Biochemistry, North Texas State University, and Texas College of Osteopathic Medicine, Fort Worth, TX 76107 **[1]**

Peter C. Ulrich, Laboratory of Medical Biochemistry, Rockefeller University, New York, NY 10021 **[83]**

T. Franklin Williams, Director, National Institute of Aging, National Institutes of Health, Bethesda, MD 20205 **[ix]**

Mary E. Wittenberger, Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20205 **[39]**

K. Ümit Yüksel, Department of Biochemistry, North Texas State University, and Texas College of Osteopathic Medicine, Fort Worth, TX 76107 **[1]**

Introduction: Opportunities for Research and Training in Aging

The importance and urgency of a better understanding of aging no longer needs to be stressed: we are all aware of the rapid increases in numbers of old and very old persons worldwide, and of the twin facts that many older people are quite healthy and functional but also that severe, debilitating chronic diseases and handicaps increase as people grow older, with increasing burdens of care for families and society. In this current atmosphere, it is very timely that the challenging opportunities for research and training in aging be highlighted.

I would like to list and comment briefly on what seem to my colleagues at the National Institute on Aging and me to be among the most promising and important opportunities at this time. First is the need for us to *distinguish aging from disease.* The more carefully we study older people and older animals, the more it seems to me we find that changes which in the past have been called "normal" aging are in fact due to now-identifiable disease(s), which may in time be prevented, cured or controlled. Let me cite the recently reported example of cardiac function during stress-testing in older healthy human volunteers. In these studies, which were a part of the Baltimore Longitudinal Study of Aging at our Institute, it was found that when the subjects were carefully screened for even occult evidence for coronary heart disease through use of radioactive thallium scanning, the 50% or so in their 70's who had no occult evidence for such disease had on average the same cardiac output on standard stress testing as 25-year olds; there was no evidence of decline with age. There were changes in the nature of the cardiac response: the older subjects showed less of an increase in heart rate with exercise, but adapted adequately through a greater increase in stroke volume. The decreased response in heart rate appears to be due to less responsivity on the part of the myocardium to adrenergic stimuli, a subject that in turn is being further investigated.

Other examples could be cited, including findings indicating preservation of brain metabolism—as measured by 2-deoxyglucose uptake with PET scanning—and preservation of mental function as measured by longitudinal studies of intelligence. Overall, we need to examine very carefully previous views of the purported effects of aging. One aspect related to animal (and human) studies in my view warrants special emphasis: it is essential that distinction be made between developmental or maturational changes, on the one hand—i.e., changes that may be found as an "adolescent" animal grows into full maturity—and on the other hand changes that occur in the senescent period of the lifespan, from full maturity on. Far too many studies in the literature compare young with old animals and ascribe the differences to aging when the changes observed may well be fully expressed by mid-life, with little if any further change.

As a part of understanding aging we need to identify *biomarkers*, measurable biological changes that reflect the aging-related life course and that may vary, from individual to individual, in terms of the chronological course at which the changes appear. Basically, my expectation is that we will find that the truly aging-related changes are structural in nature, i.e., can ultimately be understood in terms of changes in cell membranes, in protein configurations, and in genes.

A second, related research area of high priority should be that of the application of *molecular genetics* to aging and aging-related diseases. The remarakable developments in recombinant DNA and related technologies, as illustrated by the advances in understanding oncogenes and in identifying the gene locus in Huntington disease, present the challenges to identify specific gene changes that may be influential in the aging process, and to identify the genetic abnormality that must be present in at least some families with Alzheimer disease.

Among the common and serious diseases which affect older persons, research on *Alzheimer disease* and related neuroscience research, continues to be one of the highest priorities of our Institute, with fortunately rapid growth in research efforts and many promising leads. Here I can simply cite some of the exciting areas: studies showing regenerative potential for nerve dendrites and successful transplantation of nerve cells; studies on the protein chemistry of the amyloid and neurofibillary tangles in Alzheimer tissues; studies on the role of changes in the oxidative metabolic pathways in brain in producing changes in neurotransmitters; neuropharmacology applied to this condition.

Other very important functional disabilities, limitations on independence, that older people face are *loss of mobility* and *urinary incontinence*, conditions that are just now beginning to be carefuly studied. Osteoarthritis, the most common cause of loss of mobility, is being found to be characterized

by familial or genetic differences from non-affected persons, together with different characteristics of the cartilage in joints. A related problem is that of osteoporosis, the subject of intensive research on mechanisms and possible preventive steps. With respect to urinary incontinence, studies in the Gerontology Research Center of our Institute have shown that biofeedback and behavioral techniques can improve or control the incontinence in most older women living in the community.

The area of *maintenance of health and functioning* is obviously important. Everyone is in favor of *exercise* and *good nutrition* and good health habits but very little is actually documented, in animals or humans, about what can be accomplished through exercise, or what is a properly nutritious diet for older people (or animals). These areas are open for further research.

The breadth of issues related to aging precludes going into details in this short introduction but as you well know they include immunological changes with aging, the endocrinological systems, and behavioral and social aspects and impacts on the aging process. In what I have included, I have tried to illustrate with examples that may be closer to the interests of this group.

Let me close by stating the importance and urgency of *our training more investigators and teachers* in the field of aging. Estimates from a study just completed by our Institute and other federal agencies indicate that we need something like 10 times as many investigators and faculty members as we have now, in basic and clinical sciences, to take leading roles in the aging field. To move towards meeting this need, the NIA is giving high priority to the support of training and career development in research related to aging. Among our new approaches is an arrangement to "piggyback" support for traineeship positions for persons whose career goals are in the aging field, onto existing research training programs in a variety of areas related to aging. We would be very glad to discuss these and other mechanisms of support for training and research in this challenging, growing field. Thank you.

T. Franklin Williams, MD
Director, National Institute on Aging
National Institutes of Health
Bethesda, MD

Contents

Modification of Proteins During Aging, pages 1-18

IMPAIRED PROTEIN DEGRADATION MAY ACCOUNT FOR THE ACCUMULATION OF "ABNORMAL" PROTEINS IN AGING CELLS

R.W. Gracy, K.Ü. Yüksel, M.L. Chapman, J.K. Cini, M. Jahani, H.S. Lu, B. Oray and J.M. Talent
Dept. of Biochemistry, N.T. St. Univ./Texas College of Osteop. Med.
Fort Worth, Texas 76107

INTRODUCTION

Within the past decade many laboratories have examined changes in the biological properties of macromolecules during the aging process. Studies in various aging systems have revealed that many enzymes appear to be modified. Virtually any easily measurable parameter has been reported to be altered in aging (Kanungo 1980, Wilson 1981) but, no clear picture has emerged. Moreover, it has not been established if these changes are a cause or effect of aging or if they have any significant effects on the biological process of aging.

While there are exceptions, many enzymes appear to exist with lowered activities, lowered stability or altered catalytic and regulatory properties (Table 1). The identification of these specific changes requires detailed and highly sensitive protein structural analyses because:

a) the old, "defective enzymes" are very similar to their respective young counterparts;

b) the amounts of "altered" enzymes are variable and in some cases very low;

c) there is no single acceptable aging-model system.

Our laboratory has been involved in examination of the age-related accumulation of abnormal proteins at the molecular level. The present study reviews the specific changes in

enzymes in aging systems which include:

a) fibroblasts aged in vivo and in vitro from normal, aging donors and from donors with premature aging diseases,

b) cells of various ages from the lens of the eye.

c) lymphocytes aged in vivo,

We have used the enzyme triosephosphate isomerase (EC 5.3.1.1; TPI) as a marker protein to evaluate changes during aging. This enzyme is essential for both glycolysis and gluconeogenesis and required for all living cells. Since the entire sequence of the human enzyme has been completed by our laboratory (Lu et al 1984), we were able to identify the subtle changes associated with aging. In addition, this report will also consider possible parallels with other enzymes.

TABLE 1. EXAMPLES OF ENZYME CHANGES WITH AGING

TYPE OF CHANGE/ENZYME	AGING SYSTEM	REFERENCES
ISOZYME SHIFTS:		
Alanine aminotransferase	Rat liver cytosol	Kanungo & Patnaik (1975); Patnaik & Kanungo (1976)
Aldolase	Rat lens	Dovrat & Gershon (1981)
Glucose 6-phosphate dehydrogenase	Rat liver Human & bovine lens	Wang & Mays (1978) Skala-Rubinson et al. (1976)
Hexokinase	Human RBC Rabbit RBC	Magnani et al. (1980) Rijksen et al. (1977)
Lactate dehydrogenase	Rat heart	Kanungo & Singh (1965) Singh & Kanungo (1968)

TABLE 1. EXAMPLES OF ENZYME CHANGES WITH AGING (cont'd)

TYPE OF CHANGE/ENZYME	AGING SYSTEM	REFERENCES
ISOZYME SHIFTS:		
Nucleoside Phosphorylase	Human & bovine lens	Skala-Rubinson et al. (1976)
Triosephosphate isomerase	Human & bovine lens	Skala-Rubinson et al. (1976)
	Human fibroblasts	Tollefsbol et al. (1982)
DECREASED ACTIVITY:		
Aminopeptidase A	Rabbit reticulocyte	Melloni et al. (1981)
Carboxypeptidase	Rabbit reticulocyte	Melloni et al. (1981)
Citrate Synthase	Rat heart	Vitorica et al. (1981)
Glucose 6-phosphate dehydrogenase	Rat adrenal ovary	Stewart & Hunter (1969); Leatham & Apple (1977)
Glutamine synthetase	Rat liver & kidney	Wu (1977)
Hexokinase	Human RBC	Magnani et al. (1980)
	Rabbit RBC	Rijksen et al. (1977)
Isocitrate dehydrogenase	Rat heart	Vitorica et al. (1981)
Leucine aminopeptidase	Rabbit reticulocyte	Melloni et al. (1981)
Ornithine aminotransferase	Rat kidney & liver	Wu (1977)

TABLE 1. EXAMPLES OF ENZYME CHANGES WITH AGING (cont'd)

TYPE OF CHANGE/ENZYME	AGING SYSTEM	REFERENCES
DECREASED ACTIVITY:		
Pyruvate kinase	Rat heart	Vitorica et al. (1981)
Superoxide dismutase	Mouse liver, heart, brain rat liver rat lens	Reiss & Gershon (1976a,b); Dreyfus et al. (1978) Dovrat & Gershon (1981)
ACCUMULATION OF INACTIVE FORMS:		
Aldolase	Nematode Mouse liver Rabbit liver Mouse heart & muscle	Zeelon (1973) Gershon & Gershon (1973) Anderson (1974) Chetsansa & Liskiwskyi (1977)
Cathepsin D	Rat liver, heart, kidney	Wiederanders & Oelke (1984)
Enolase	Turbatrix aceti	Rothstein (1979)
DECREASED STABILITY:		
Glucose 6-phosphate dehydrogenase	Rat brain, kidney, spleen, liver, lung Human liver, lung, fibroblasts	Wulf & Cutler (1975) Kahn et al. (1977)
Glutathione reductase	Eye lens	Harding (1973)
Superoxide dismutase	Mouse liver, heart, brain; rat liver	Reiss & Gershon (1976a,b); Dreyfus et al. (1978)

TABLE 1. EXAMPLES OF ENZYME CHANGES WITH AGING (cont'd)

TYPE OF CHANGE/ENZYME	AGING SYSTEM	REFERENCES
INCREASED STABILITY:		
Phosphoglycerate kinase	Rat liver	Sharma & Rothstein (1980)
CHANGE IN AGGREGATION:		
Alpha-crystallin	Vertebrate eye lens	Bloemendal (1977)
REGULATORY CHANGES:		
Glutamine synthetase	Rat kidney & liver	Wu (1977)
Ornithine amino-transferase	Rat kidney & liver	Wu (1977)
KINETIC CHANGES:		
Hexokinase	Human RBC Rabbit RBC	Magnani et al. (1980) Rijksen et al. (1977)
CHANGES IN COENZYME BINDING:		
Glyceraldehyde 3-phosphate dehydrogenase	Rat muscle	Gafni (1981)
CONFORMATIONAL CHANGES:		
Phosphoglycerokinase	Rat liver	Sharma & Rothstein (1980)
Enolase	Turbatrix aceti	Rothstein (1979)
DECREASED RESPONSE TO STIMULI:		
Glucokinase	Rat liver	Gold et al. (1976)

RESULTS AND DISCUSSION:

Figure 1 shows the accumulation of labile forms of four enzymes in human skin fibroblasts. The labile enzymes accumulate in cells from old donors, as well as from subjects with the premature aging diseases, progeria and Werner's syndrome.

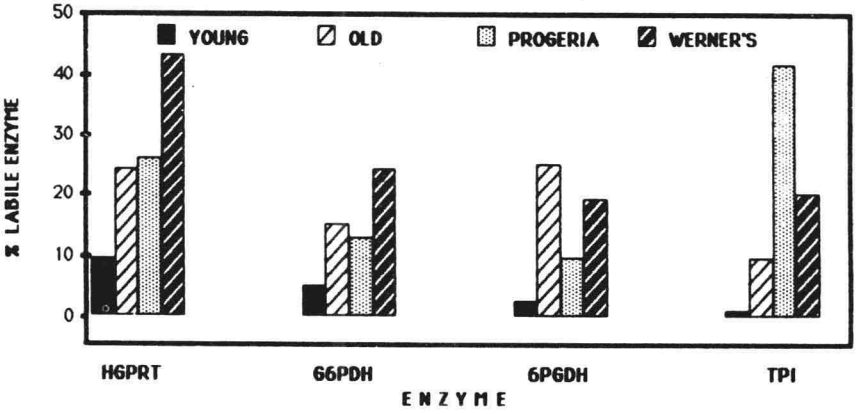

Figure 1. Accumulation of unstable enzymes in aging and premature aging diseases. Abbreviations are: triosephosphate isomerase (TPI); glucose 6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6PGDH), and hypoxanthine phosphoribosyl transferase (HGPRT). The values were taken from the following references: Goldstein and Singal (1974) G6PDH (young and Werner's); Goldstein and Moerman (1975) G6PDH (progeria), 6PGDH (progeria), HGPRT (progeria); Holliday and Tarrant (1972) G6PDH (young and old), 6PGDH (old); Holliday, Porterfield and Gibbs (1974) G6PDH (Werner's); Tollefsbol, Zaun and Gracy (1982) TPI (all forms); Tollefsbol and Gracy (1983) 6PGDH (young, old and Werner's), HGPRT (all forms).

In an attempt to better understand the molecular basis for such changes we have examined triosephosphate isomerase in detail. This enzyme was chosen because it has a relatively simple structure. TPI was found to exhibit several isozymes in many tissues and species (Snapka et al. 1974; Naidu et al. 1984). The more acidic isozymes accumulate in aging cells and tissues (Yuan et al. 1981 a; Yuan et al.