

**NUTRITIONAL AND
TOXICOLOGICAL
SIGNIFICANCE OF ENZYME
INHIBITORS IN FOODS**

ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

Editorial Board:

NATHAN BACK, *State University of New York at Buffalo*

NICHOLAS R. DI LUZIO, *Tulane University School of Medicine*

EPHRAIM KATCHALSKI-KATZIR, *The Weizmann Institute of Science*

DAVID KRITCHEVSKY, *Wistar Institute*

ABEL LAJTHA, *Rockland Research Institute*

RODOLFO PAOLETTI, *University of Milan*

Recent Volumes in this Series

Volume 195A

PURINE AND PYRIMIDINE METABOLISM IN MAN V, Part A: Clinical Aspects

Including Molecular Genetics

Edited by W. L. Nyhan, L. F. Thompson, and R. W. E. Watts

Volume 195B

PURINE AND PYRIMIDINE METABOLISM IN MAN V, Part B: Basic Science Aspects

Edited by W. L. Nyhan, L. F. Thompson, and R. W. E. Watts

Volume 196

STEROID HORMONE RESISTANCE: Mechanisms and Clinical Aspects

Edited by George P. Chrousos, D. Lynn Loriaux, and Mortimer B. Lipsett

Volume 197

BIOLOGICAL REACTIVE INTERMEDIATES III: Mechanisms of Action in
Animal Models and Human Disease

Edited by James J. Kocsis, David J. Jollow, Charlotte M. Witmer,
Judd O. Nelson, and Robert Snyder

Volume 198A

KININS IV, Part A

Edited by Lowell M. Greenbaum and Harry S. Margolius

Volume 198B

KININS IV, Part B

Edited by Lowell M. Greenbaum and Harry S. Margolius

Volume 199

NUTRITIONAL AND TOXICOLOGICAL SIGNIFICANCE OF
ENZYME INHIBITORS IN FOODS

Edited by Mendel Friedman

Volume 200

OXYGEN TRANSPORT TO TISSUE VIII

Edited by Ian S. Longmuir

Volume 201

LIPOPROTEIN DEFICIENCY SYNDROMES

Edited by Aubie Angel and Jiri Frohlich

A Continuation Order Plan is available for this series. A continuation order will bring delivery of each new volume immediately upon publication. Volumes are billed only upon actual shipment. For further information please contact the publisher.

NUTRITIONAL AND TOXICOLOGICAL SIGNIFICANCE OF ENZYME INHIBITORS IN FOODS

Edited by

Mendel Friedman

U.S. Department of Agriculture
Berkeley, California

PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

American Institute of Nutrition FASEB Symposium on Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods (1985: Anaheim, Calif.)
Nutritional and toxicological significance of enzyme inhibitors in foods.

(Advances in experimental medicine and biology; v. 199)

"Proceedings of the American Institute of Nutrition FASEB Symposium on Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods, held April 21-26, 1985, in Anaheim, California"—T.p. verso.

Includes bibliographies and indexes.

1. Digestive enzymes—Inhibitors—Congresses. 2. Food—Protein content—Congresses. 3. Nutritionally induced diseases—Congresses. I. Friedman, Mendel. II. American Institute of Nutrition. III. Federation of American Societies for Experimental Biology. IV. Title. V. Series.

QP609.D52A44 1985

612/.398

86-15151

Proceedings of the American Institute of Nutrition FASEB Symposium on Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods, held April 21-26, 1985, in Anaheim, California, with additional invited contributions

© 1986 Plenum Press, New York
Softcover reprint of the hardcover 1st edition 1986

A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise, without written permission from the Publisher

ISBN 978-1-4757-0024-4 ISBN 978-1-4757-0022-0 (eBook)
DOI 10.1007/978-1-4757-0022-0

PREFACE

Soybean proteins are widely used in human foods in a variety of forms, including baby formulas, flour, soy protein concentrates, soy protein isolates, soy sauces, textured soy fibers, and tofu. The presence of inhibitors of digestive enzymes in soy proteins impairs nutritional quality and possible safety of this important legume. Normal processing conditions based on the use of heat do not completely inactivate these inhibitors, so that residual amounts of plant protease inhibitors are consumed by animals and man.

Inhibitors of digestive enzymes are present not only in legumes, such as soybeans, lima beans, and kidney beans, but also in nearly all plant foods, including cereals and potatoes, albeit in much smaller amounts. The antinutritional effects of inhibitors of proteolytic enzymes have been widely studied and can be ameliorated by processing and/or sulfur amino acid fortification. A more urgent concern is reports that rats fed diets containing even low levels of soybean-derived inhibitors, which are found in foods such as soy-based baby formulas, may develop over their lifespan pancreatic lesions leading eventually to neoplasia or tumor formation. On the other hand, recent studies suggest that certain enzyme inhibitors from plant foods may prevent cancer formation in other tissues. A key question, therefore, is whether inhibitors from plant foods constitute a human health hazard.

Since the interaction of plant-derived inhibitors with proteases and other enzymes including trypsin, chymotrypsin, elastase, amylase, and lipase is a subject of increasing importance in animal and human nutrition, food chemistry, and food safety, I organized a symposium broadly concerned with the nutritional and toxicological significance of enzyme inhibitors in foods. The symposium was sponsored by the American Institute of Nutrition - Federation of American Societies for Experimental Biology (FASEB) for its annual meeting in Anaheim, California, April 21-26, 1985. Invited speakers were asked to develop at least one of the following topics:

1. Mechanism of action of inhibitors - biofeedback mechanisms, role of hormones, differentiation of antinutritional from pancreatic effects, etc.
2. Inhibitors as inducers or promoters of pancreatic lesions.
3. Pancreatic response to inhibitors in different animal species.
4. Beneficial effects of inhibitors.
5. The importance of inhibitors in the human diet.

6. Inactivation and removal of inhibitors from plant foods.

The most important function of a symposium, I believe, is dissemination of insights and exchange of ideas so as to catalyze progress by permitting synergistic interaction among related disciplines. I hope that the reports presented at the symposium fulfilled this purpose. In addition, a number of scientists who could not participate in the symposium accepted invitations to contribute papers to this volume on the theme of the symposium. Indeed, about half the papers are especially written invited contributions. This book is, therefore, a hybrid between symposium proceedings and a collection of invited papers.

Brought together here are outstanding international authors from nine countries, who discuss the multidisciplinary aspects of the nutritional and toxicological significance and related agronomic, biochemical, and food processing aspects of naturally occurring inhibitors of digestive enzymes. Although a comprehensive summary is not possible in a preface, I wish to call special attention to several manuscripts. First, Weder's comprehensive review of the comparative potencies of plant inhibitors against digestive enzymes from animal and human sources is especially welcome, since it directly relates to the importance of enzyme inhibitors in human nutrition. The related report by Toskes on biofeedback effects in human patients should stimulate needed studies on the role of inhibitors in human health. The manuscripts by Green, Howatson, Morgan, and Roebuck describe pioneering studies on the relationship between the amounts of soybean inhibitors in the diet and the induction of pancreatic hypertrophy, hyperplasia, and adenoma. These nicely complement the comprehensive paper by Gumbmann and colleagues on long-term effects of soybean inhibitors and dietary protein levels on pancreatic histology and pathology. The papers by Schneeman, Liener, Nitsan, and Harwood and their colleagues on the observed species-variation of the pancreatic effects suggest that the rat may be especially sensitive and the monkey much less so or not at all. If these observations turn out to be generally true, then primates including humans may be more tolerant of inhibitors than rodents. The papers by Green and colleagues, Temler and Mettraux, Howatson, and Toskes strikingly demonstrate the key role of cholecystokinin and other hormones in the mechanism of action of inhibitors. The comprehensive paper by Rackis and collaborators on the widespread occurrence of inhibitors in the plant kingdom and on possible approaches to inactivate or remove them from foods offers an authoritative and critical assessment of the subject. This is complemented by the paper authored by Hymowitz on the development of new genetic varieties of soybeans with low trypsin inhibitor content. These studies, and related aspects described in the paper by Friedman and Gumbmann, could serve as the basis for possible new or improved ways to completely eliminate inhibitors from our food supply. The invited paper by Buonocore and Silano on amylase inhibitors and the two papers on effects of processed foods on digestive and metalloenzymes broaden the scope of the book from dealing with purely protease inhibitors to enzyme inhibitors in general. Other papers provide valuable insight into biochemical, immunochemical, and nutritional aspects of enzyme inhibitors from plant sources.

The described multidisciplinary studies reveal a complex and fascinating interplay between evolution, chemistry, nutrition, and toxicology of plant foods.

I am particularly grateful to all contributors for excellent cooperation, to Prof. James A. Olson of the American Institute of

Nutrition for helpful correspondence on the theme and contents of the symposium, to Prof. Barbara O. Schneeman and Dr. Michael R. Gumbmann for co-chairing the symposium sessions, and to Glenda M. Dugan for editorial assistance.

Plenum Press is publishing the papers under the title NUTRITIONAL AND TOXICOLOGICAL SIGNIFICANCE OF ENZYME INHIBITORS IN FOODS, as a volume in the series Advances in Experimental Medicine and Biology. This book is intended to complement the following published volumes which I edited for the same series: PROTEIN-METAL INTERACTIONS (1974); PROTEIN CROSSLINKING: BIOCHEMICAL AND MOLECULAR ASPECTS (1977); PROTEIN CROSSLINKING: NUTRITIONAL AND MEDICAL CONSEQUENCES (1977); NUTRITIONAL IMPROVEMENT OF FOOD AND FEED PROTEINS (1978); and NUTRITIONAL AND TOXICOLOGICAL ASPECTS OF FOOD SAFETY (1984).

I very much hope that these volumes will be a valuable resource for further progress in agriculture, protein chemistry, food chemistry, food safety, animal and human nutrition, pharmacology, physiology, toxicology, and medicine; all areas of world-wide urgency.

Mendel Friedman
April, 1986

The eyes of all look to you expectantly,
and you give them their food when it is due,
You give it openhandedly, feeding every
creature to its heart's content.

Psalm 145: 15-16

Eat nothing that will prevent you from eating.

Ibn Tibbon, c. 1190

CONTENTS

1. PROTEIN INHIBITORS OF SERINE PROTEINASES - MECHANISM AND CLASSIFICATION..... 1
Michael Laskowski, Jr.
2. THE BIOLOGICAL FUNCTIONS AND PHYSIOLOGICAL EFFECTS OF INGESTED INHIBITORS OF DIGESTIVE REACTIONS..... 19
S. S. Rothman
3. SAFETY OF TRYPSIN INHIBITORS IN THE DIET: EFFECTS ON THE RAT PANCREAS OF LONG-TERM FEEDING OF SOY FLOUR AND SOY PROTEIN ISOLATE..... 33
Michael R. Gumbmann, William L. Spangler,
Glenda M. Dugan, and Joseph J. Rackis
4. DOSE EFFECTS OF RAW SOYABEAN FLOUR ON PANCREATIC GROWTH..... 81
R. G. H. Morgan, R. A. Crass, and P. S. Oates
5. ENHANCEMENT OF PANCREATIC CARCINOGENESIS OF RAW SOY PROTEIN ISOLATE QUANTITATIVE RAT MODEL AND NUTRITIONAL CONSIDERATIONS 91
B. D. Roebuck
6. PANCREATIC CARCINOGENESIS - THE POTENTIAL OF CHOLECYSTOKININ AS A COCARCINOGEN IN THE HAMSTER - NITROSAMINE MODEL.....109
Allan G. Howatson
7. INTERACTION OF DIETARY PROTEIN AND TRYPSIN INHIBITOR ON PLASMA CHOLECYSTOKININ AND PANCREATIC GROWTH IN RATS.....123
Gary M. Green, Van H. Levan, and Rodger A. Liddle
8. GASTRIN AND CHOLECYSTOKININ LEVELS IN RATS FED SOYA BEAN TRYPSIN INHIBITOR.....133
Roman S. Tember and Christine Mettraux
9. NEGATIVE FEEDBACK INHIBITION OF PANCREATIC EXOCRINE SECRETION IN HUMANS.....143
P. P. Toskes

10. PROTEASE INHIBITORS: THEIR ROLE AS MODIFIERS OF CARCINOGENIC PROCESSES.....	153
Walter Troll, Krystyna Frenkel, and Rakoma Wiesner	
11. NUTRITIONAL AND METABOLIC RESPONSE TO PLANT INHIBITORS OF DIGESTIVE ENZYMES.....	167
Daniel Gallaher and Barbara O. Schneeman	
12. AMONG SPECIES SPONSE TO DIETARY TRYPSIN INHIBITOR: VARIATION SPECIES.....	185
Barbara O. Schneeman and Daniel Gallaher	
13. THE EFFECT OF THE LONG-TERM FEEDING OF RAW SOYFLOUR ON THE PANCREAS OF THE MOUSE AND HAMSTER.....	189
Irvin E. Liener and Aharon Hasdai	
14. ACCENTUATED RESPONSE TO DIETARY RAW SOYFLOUR BY MEAL-FEEDING IN VARIOUS SPECIES.....	199
Zafrira Nitsan and Israel Nir	
15. EFFECT OF LONG-TERM FEEDING OF SOY-BASED DIETS ON THE PANCREAS OF CEBUS MONKEYS.....	223
J. P. Harwood, L. M. Ausman, N. W. King, P. K. Sehgal, R.J. Nicolosi, I.E. Liener, D. Donatucci, and J. Tarcza	
16. INHIBITION OF HUMAN PROTEINASES BY GRAIN LEGUMES.....	239
Juergen K. P. Weder	
17. REGULATION OF PROTEINASE INHIBITOR GENES IN FOOD PLANTS.....	281
Willis E. Brown, John S. Graham, Jong S. Lee, and Clarence A. Ryan	
18. GENETICS AND BREEDING OF SOYBEANS LACKING THE KUNITZ TRYPSIN INHIBITOR.....	291
Theodore Hymowitz	
19. PROTEASE INHIBITORS IN PLANT FOODS: CONTENT AND INACTIVATION.....	299
J. J. Rackis, W. J. Wolf, and E. C. Baker	
20. PREPARATION OF UNHEATED SOY PROTEIN ISOLATE WITH LOW TRYPSIN INHIBITOR CONTENT.....	349
E. C. Baker and J. J. Rackis	
21. NUTRITIONAL IMPROVEMENT OF LEGUME PROTEINS THROUGH DISULFIDE INTERCHANGE.....	357
Mendel Friedman and Michael R. Gumbmann	

22. RELEVANCE OF MULTIPLE SOYBEAN TRYPSIN INHIBITOR FORMS TO NUTRITIONAL QUALITY.....	391
Anna L. Tan-Wilson and Karl A. Wilson	
23. ANTINUTRITIONAL AND BIOCHEMICAL PROPERTIES OF WINGED BEAN TRYPSIN INHIBITORS.....	413
Benito O. de Lumen and Jones Chan	
24. PROTEASE INHIBITORS OF THE MARAMA BEAN.....	429
Barry Starcher, Leah Bryant, and Michael Elfant	
25. TRYPSIN/CHYMOTRYPSIN INHIBITORS FROM MILLETS.....	439
Thillaisthanam N. Pattabiraman	
26. ANTIGENICITY OF NATIVE AND MODIFIED KUNITZ SOYBEAN TRYPSIN INHIBITORS.....	449
David L. Brandon, Sakhina Haque, and Mendel Friedman	
27. PHOTOREACTIVE, ACTIVE DERIVATIVES OF TRYPSIN- AND CHYMOTRYPSIN-INHIBITORS FOMR SOYBEANS AND CHICK PEAS.....	469
Yehudith Birk, Patricia Smirnoff, and J. Ramachandran	
28. BIOCHEMICAL, NUTRITIONAL, AND TOXICOLOGICAL ASPECTS OF ALPHA-AMYLASE INHIBITORS FROM PLANT FOODS.....	483
Vincenzo Buonocore and Vittorio Silano	
29. THE INHIBITION OF DIGESTIVE ENZYMES BY POLYPHENOLIC COMPOUNDS.....	509
David Wynne Griffiths	
30. EFFECT OF SEVERELY ALKALI-TREATED CASEIN ON GASTROINTESTINAL TRANSIT AND SELECTED INTESTINAL ENZYME ACTIVITY.....	517
Bernard Possompes and Jacques Berger	
31. INACTIVATION OF METALLOENZYMES BY LYSINOALANINE, PHENYLETHYLAMINOALANINE, ALKALI-TREATED FOOD PROTEINS, AND SULFUR AMINO ACIDS.....	531
Mendel Friedman, Ok-Koo K. Grosjean, and James C. Zahnley	
INDEX.....	561