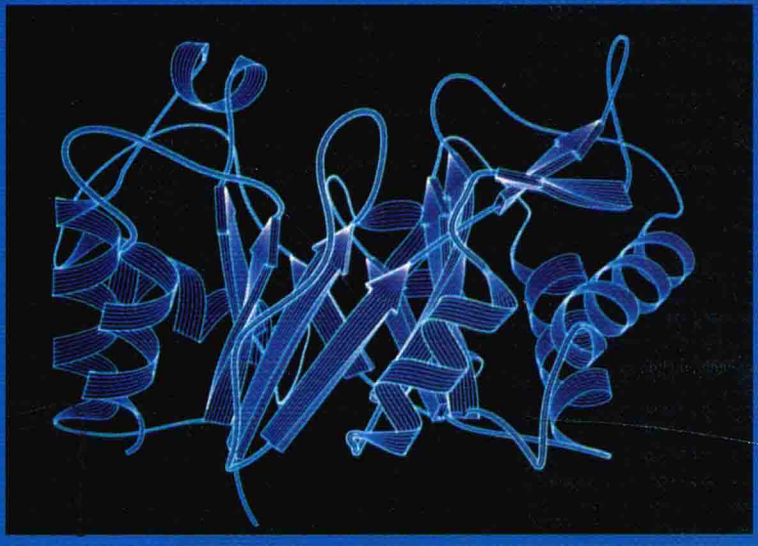
Food Proteins

Structure and Functionality

Edited by
K. D. Schwenke and R. Mothes



VCH

Food Proteins
Structure and Functionality

Edited by K. D. Schwenke and R. Mothes

Developed from the 4th Symposium on Food Proteins „Structure-Functionality Relationships“ held at Reinhardsbrunn, Germany, 5. - 8. October 1992

Weinheim · New York · Basel · Cambridge · Tokyo

Editors
Prof. Dr. rer. nat. habil. Klaus Dieter Schwenke
Dr. rer. nat. Ralf Mothes
Forschungsgruppe Pflanzenproteinchemie
Universität Potsdam
(gefördert durch KAI e.V. im WIP)
Arthur-Scheunert-Allee 155
D-14558 Bergholz-Rehbrücke
Federal Republic of Germany

Published jointly by
VCH Verlagsgesellschaft, Weinheim (Federal Republic of Germany)
VCH Publishers, New York, NY (USA)

Editorial Director: Dr. Hans F. Ebel
Production Manager: Dipl.-Wirt.-Ing. (FH) Bernd Riedel

Library of Congress Card No. applied for.

A catalogue record for this book is available from the British Library

Die Deutsche Bibliothek – CIP-Einheitsaufnahme

Food proteins : structure and functionality ; developed from the 4th Symposium on Food Proteins „Structure, Functionality, Relationships", held at Reinhardsbrunn, Germany, 5. – 8. October 1992 / ed. by K. D. Schwenke and R. Mothes. – Weinheim ; New York ; Basel ; Cambridge ; Tokyo : VCH, 1993
ISBN 3-527-30037-6
NE: Schwenke, Klaus Dieter [Hrsg.]; Symposium on Food Proteins <04, 1992, Reinhardsbrunn>

Printing: strauss offsetdruck gmbH, D-69493 Hirschberg
Bookbinding: J. Schäffer, D-67269 Grünstadt
Printed in the Federal Republic of Germany

Food Proteins
Structure and Functionality

K. D. Schwenke and R. Mothes

Distribution
VCH, P. O. Box 10 11 61, D-69451 Weinheim (Federal Republic of Germany)
Switzerland: VCH, P. O. Box, CH-4020 Basel (Switzerland)
United Kingdom and Ireland: VCH (UK) Ltd., 8 Wellington Court, Cambridge CB1 1HZ (England)
USA and Canada: VCH, 220 East 23rd Street, New York, NY 10010-4606 (USA)
Japan: VCH, Eikow Building, 10-9 Hongo 1-chome, Bunkyo-ku, Tokyo 113 (Japan)

ISBN 3-527-30037-6 (VCH, Weinheim)
ISBN 1-56081-769-0 (VCH, New York)

Preface

This book comprises contributions to the 4th Symposium on Food Proteins continuing the fruitful tradition of the series of protein symposia organized by one of the editors (K.D.S.) under the auspices of the former Central Institute of Nutrition, Potsdam-Rehbrücke.
It is the functionality – or more precisely the complexity of techno-functional properties – which has become the main topic of discussion at these symposia since the second one was held in 1989. The exponential increase in the knowledge of protein structure and insight into their functionality encouraged us to choose "Structure Functionality Relationships" as the topic of the fourth food protein symposium held in 1992. This subject reflects an actual trend in protein research of elucidating the function of a protein by its structure.
Establishing such structure-functionality relationships for food proteins is highly complicated because of the complexity of food systems and the interaction of different food components with proteins. Nevertheless, the knowledge about the influence of structure on the functional properties of food proteins is essential for their rational use in food systems.
The contributions to the present volume are arranged in three sections. Section one is devoted to those contributions dealing with structural investigations of proteins in the native and modified state. It is divided into two subsections, depending on the origin of the proteins. Animal proteins, i.e. egg-white lysozyme, ovalbumin, casein, and whey proteins, are the subject of discussion in the first part. The storage proteins from legumes, oil seeds and cereals and also ribulose-1,5-biphosphate carboxylase (RUBISCO), the principal protein of green leafs, are the respective subjects of the second part. Chemical and enzymatic modifications as well as genetic engineering approaches are used for guided alterations of structure and functional properties and to derive relationships between both. Moreover, the isolation and resynthesis of biologically active peptides in hydrolysates of casein fractions on the one hand and the induction of special bactericidal activity of egg-white lysozyme on the other hand forces the way of looking at food protein functionality to be modified.
Section two comprises contributions dealing with the problem of food protein interactions. The study of interactions with different food components such as polysaccharides and lipids or their reaction products, as well as those with vegetable non-protein compounds, supplies valuable information about protein functionality in complex food systems.
Interactions are also essential for the formation of food emulsions and foams as well as for gel-like systems, and this is the topic of section three of this book reflecting the sizeable progress which has been made in studying the surface functionality of proteins. The considerable methodical variety used provided the basis for the physico-chemical definition of a so highly complicated phenomenon as functionality. The potential practical applications of scientific findings are shown and supplemented by short contributions dealing with food technological topics.
Because of the financial support of the Deutsche Forschungsgemeinschaft Bonn a number of outstanding scientists from Eastern Europe and Asia were able to attend the conference and contribute to this book. In this way, this 4th symposium really became an international forum of food protein chemists, where the climate, like a "Western-Eastern Divan" created an exceptionally open and fruitful scientific discussion.
The help and support of the following sponsors was essential for the successful outcome of this conference: Ministerium für Wissenschaft, Forschung und Kultur des Landes Brandenburg,

Thüringer Ministerium für Wissenschaft und Kunst, Stiftungsfonds Unilever im Stifterverband für die Deutsche Wissenschaft, Nestlé Deutschland AG, Fa. L. & C. Kebelmann GmbH &Co. Stahnsdorf, KONTRON Instruments GmbH, Deutsches Institut für Ernährungsforschung Potsdam-Rehbrücke. Special thanks are due to Helga Beyer, Christoph Schneider and Jens-Peter Krause for their help in organizing this symposium. In addition, we are grateful to VCH, who gave us the opportunity to publish the papers as a conference-proceedings book.

May 1993

K.D. Schwenke
R. Mothes

Contents

Structure and Modification - Plant Proteins

Interactions

Surface Functional and Rheological Properties

Structure and Modification

Animal Proteins

Genetic Engineering Approaches to Relationships between Structure and Functionality of Hen Egg-White Lysozyme

Akio Kato

Department of Biochemistry, Yamaguchi University, Yamaguchi 753, Japan

Summary

Genetic engineering approaches were introduced to elucidate the relationships between structure and surface functional properties of hen egg-white lysozyme (HEWL). Various amino acid replacements were carried out by site-directed mutagenesis in the cDNA of HEWL to deamidate (Asn103,106→Asp), cleave the S-S bond (Cys94→Ala), and delete the salt linkage between Lys13 and Leu129 (Lys13→Asp). The cDNAs of mutant lysozymes were inserted into a yeast expression vector (PYG100) and expressed in *Saccharomyces cerevisiae*. The mutant lysozymes were secreted in yeast medium and purified by two steps of cation-exchange chromatography. All mutant lysozymes revealed almost the same lytic activity as wild type. The conformational stabilities of lysozyme were significantly decreased by cleaving S-S bond and deleting salt linkage, while those of the deamidated mutant lysozymes were almost the same as wild type. The flexibility of deamidated lysozyme was enhanced, although the stability was not decreased. The surface properties (foaming and emulsifying properties) of lysozyme were significantly enhanced by deamidation, cleavage of S-S bond, and deletion of salt linkage. These results support our hypothesis that the conformational stabilities and flexibilities are important structural factors for the surface functional properties of proteins.

Introduction

We have reported that the surface hydrophobicity [1], conformational stability [2], flexibility [3], and protein-protein interaction [4] are the structural factors governing the surface functional properties of proteins. Nevertheless, it remains to be solved to get clear-cut explanation of the relationship between the structural and functional properties of proteins. To estimate the role of conformational parameter in the surface properties, the same protein should be used where only the conformational factor differs from one mutant to another. In this way the influence of the structural factor on the surface properties may be precisely addressed. For this purpose, genetic engineering is one of the most promising approach. In 1988, we reported in the Journal, Protein Engineering [2], that the surface properties of proteins showed good correlation with the conformational stability of tryptophan synthases which were point-mutated at the position 49 by genetic engineering. By this genetic approach, it was completely elucidated that the conformational stability is the most important structural factor governing the surface properties of proteins. In this report, I will describe the similar genetic approaches to elucidate the relationships between structural and functional properties of hen egg white lysozyme.

Materials and Methods

Materials. T4DNA ligase, alkaline phosphatase and restriction enzymes were purchased from Takara Shuzo Co. (Kyoto). The Takara 7-DEAZA sequence kit for sequencing, and Takara Blunting kit for blunting were also purchased from Takara Shuzo Co. (Kyoto). The oligonucleotide-directed *in vitro* mutagenesis system (version 2) for site-directed mutagenesis and α-^{32}P dCTP (800Ci/mmol) were purchased from Amersham. *Micrococcus lysodeikticus* cells for lysozyme assay were from Sigma, and CM-Toyopearl resin was a product of Toso (Tokyo). All other chemicals were of analytical grade for biochemical use.

Plasmids. The yeast expression plasmid, pYG-100 [5], was supplied by K. Matsubara, University of Osaka. The recombinant plasmid (pKK-1) contains 16 bp of the 5'-noncoding region, 440 bp of the coding region, and about 120 bp of the 3'-noncoding region of hen pre-lysozyme cDNA in the same orientation as *lacZ* in pUC18 [6].

Oligonucleotide-directed mutagenesis of HEWL cDNA. The conversion of Asn 103 and 106 codon to Asp was carried out by site-directed mutagenesis with bacteriophage vector M13mp19. The *Eco*RI/*Hin*dIII fragment of the pKK-1 plasmid containing almost full-length cDNA encoding HEWL was subcloned into the *Eco*RI/*Hin*dIII site of bacteriophage vector M13mp19. The mutant HEWL cDNAs were constructed in the recombinant M13mpl9 vector by the Amersham oligonucleotide-directed mutagenesis system (version 2), as recommended by supplier. For the deamidation mutant, the mutagenic oligonucleotide primers, 5'-AGCGATGGAGATGGCATGGAAC-3' and 5'-GAAACGGCATGGACGCGTGGG-3', which were synthesized by the phosphoamidate method with an Applied Biosystem 381A DNA synthesizer, were used to convert Asn 103 (AAC) and Asn 106 (AAC) to Asp (GAT) and Asp (GAC) codon, respectively. For the SS-deletion mutant, the mutagenic oligonucleotide primer, 5'-AGCGTGAACGCCGCGAAGAAG-3' were used to convert Cys 94 (TGC) to Ala (GCC) codon. For the salt-linkage cleavage mutant, the mutagenic oligonucleotide primer, 5'-GCAGCTATGGACCGTCACGG-3' were used to convert Lys 13 (AAG) to Asp (GAC) codon. The DNA sequence of each mutant was confirmed by the dideoxy sequencing method [7].

Construction of expression vector for HEWL in yeast. The wild-type or mutant HEW pre-lysozyme cDNA fragment was isolated by double digestion of M13mp19 with *Eco*RI/*Sal*I. The cDNA fragments were ligated to pYG-100, which had been digested with *Sal*I. The non-ligated *Eco*RI site in cDNA fragments and *Sal*I site in PYG-100 were blunt-ended by the blunt-end kit and self-ligated. The recombinant plasmid was propagated in *E. coli* JM 107 (*rec*A) in LB medium (1% Bacto trypton 5% yeast extract, 0.5% NaCl). The expression plasmid, which had the cDNA insert located downstream of the GPD promoter in the correct orientation, was identified by a digestion analysis with restriction