



Food Immunochemistry and Immunology

食品免疫化学与分析

LIBING WANG • CHUANLAI XU



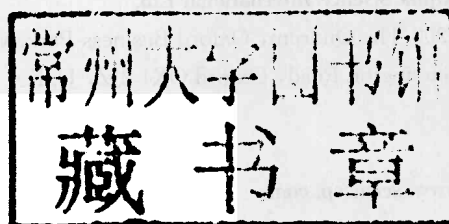
SCIENCE PRESS
Beijing



Alpha Science International Ltd.
Oxford, U.K.

Food Immunochemistry and Immunology

Libing Wang
Chuanlai Xu



SCIENCE PRESS
Beijing



Alpha Science International Ltd.
Oxford, U.K.

Copyright © 2012, Science Press and Alpha Science International Ltd.

Authors

Libing Wang

Chuanlai Xu

Responsible Editors

Haiguang Wang

Hao Wang

Co-Published by:

Science Press

16 Donghuangchenggen North Street

Beijing 100717, China

and

Alpha Science International Ltd.

7200 The Quorum, Oxford Business Park North

Garsington Road, Oxford OX4 2JZ, U. K.

www.sciencep.com

ISBN 978-7-03-033817-4 (Science Press, Beijing)

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without prior written permission of the copyright owner.

Printed in Beijing, China

Preface

Rapid urbanization and corresponding lifestyle changes mean that more and more people purchase processed food, eat in restaurants, or buy meals from street vendors. Food production is increasingly industrialized. This introduces multiple opportunities for contamination to occur and makes populations more vulnerable to lapses in food safety at any point along the production chain.

China is a big country for food production and consumption, and also a big trading partner for food. So far, there are 448,000 food enterprises of various varieties in China with a huge annual output. The food industry faces great pressure in developing systems such as total quality management and hazard analysis to identify and manage key steps in food production. Food safety authorities in force a major control. The Chinese government, as it has always done, attaches great importance to food quality and safety. It has been making unremitting efforts to improve food quality and to ensure food safety since the "10th National Food Safety Management 5-year Plan". Quality control analysis is an essential tool in food production. Immunoassay, and related immunochemical analytical procedures [e. g. , ELISA, fluorescence polarization immunoassay (FPIA) and immuno-chromatographic assay (ICA)] have been widely used to detect various residues in foods. *Food Immunochemistry and Immunology* summarizes the most representative immunochemical technologies applied in food detection. It consists of 13 chapters. Chapter 1 provides the reader with a concise overview of immunology. Chapter 2 describes the binding properties of an antibody to an antigen. Chapters 3 and 4 introduce the common procedure for antibody preparation, purification and modification. Chapter 5 summarizes the labelling technology for antibody with tracers. Chapters 6-9 outline the primary knowledge of time-resolved fluorescence immunoassay, techniques in the molecular immunology and immunogenetics, biomimics (molecular imprinting technique) and immuno-electron microscopy technique. Chapters 10-13 give state-of-the-art information about the application of immunoassay in the following topics: pesticide, veterinary drug, biological toxin and other persistent pollutants detections. This book is of special benefit to researchers on antibody production of chemicals and immunoassay development in food analysis.

I greatly appreciate the help from my teammates (Dr. Chifang Peng, Dr. Hua Kuang, Dr. Wei Ma, Dr. Liguang Xu, Dr. Yingyue Zhu, Dr. Yuan Zhao, Dr. Liqiang Liu and others in preparing this edition of the book). Many more people collaborated in the project, directly and indirectly, and I would like to acknowledge my gratitude to all of them.

Libing Wang

Colored Figures

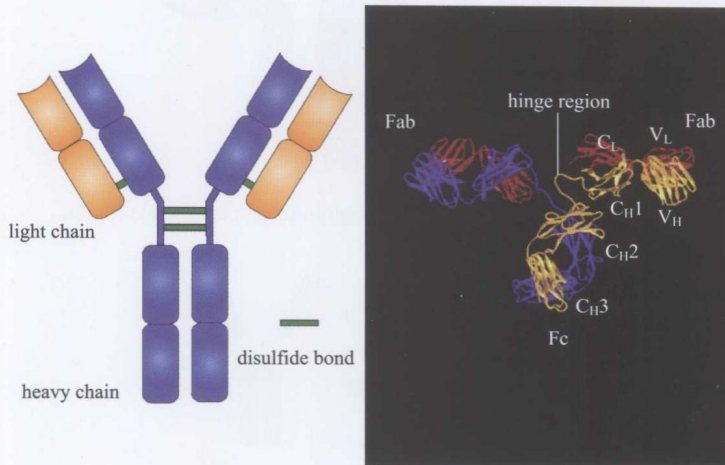


Figure 2-1 Structure model of immunoglobulin (IgG)

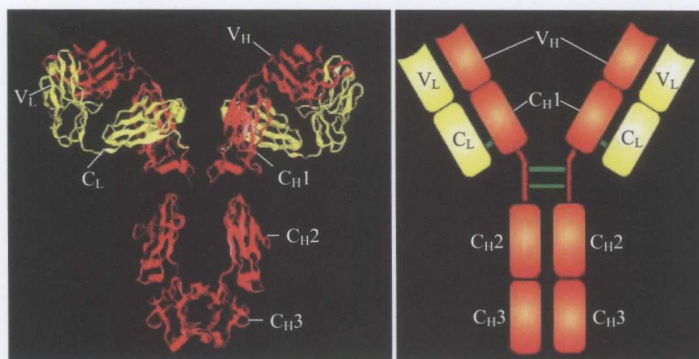


Figure 2-2-A A model of immunoglobulin ribbon

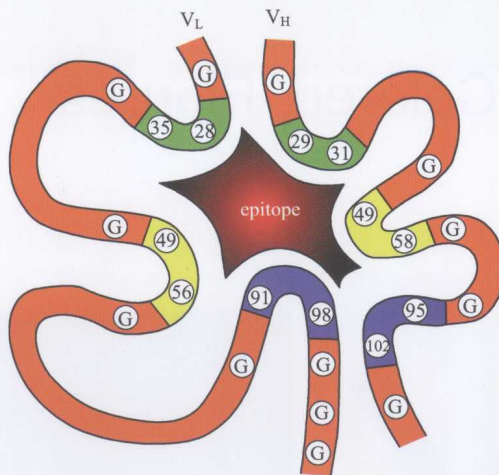


Figure 2-2-B A model of immunoglobulin hypervariable region

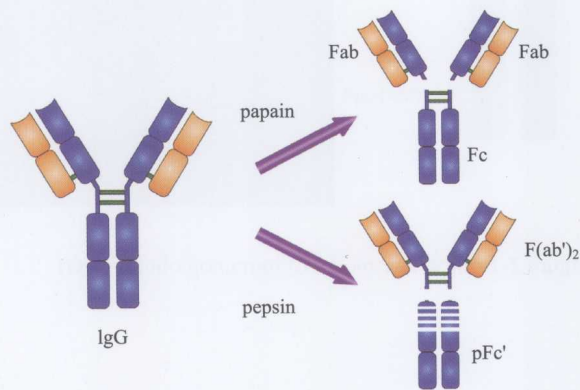


Figure 2-3 Diagram of IgG molecules hydrolysis fragments

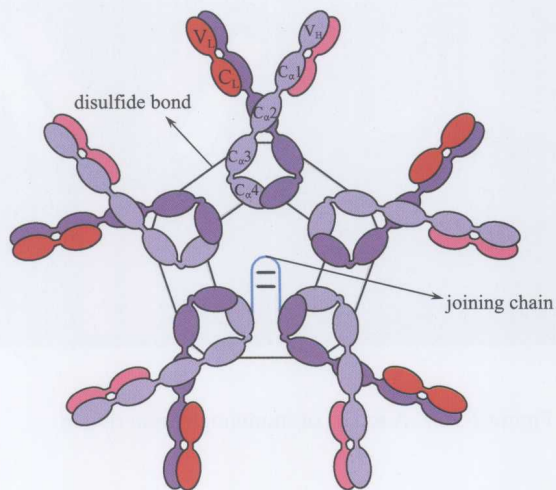


Figure 2-4 IgM pentamer

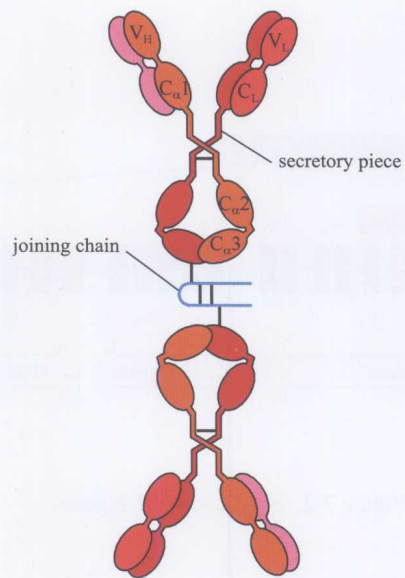


Figure 2-5 Structure of immunoglobulin Ig A dimer

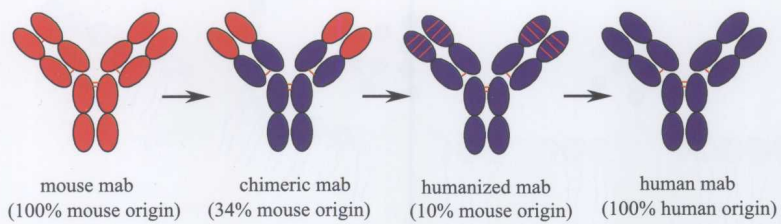


Figure 4-1 Humanization progress of mouse mab

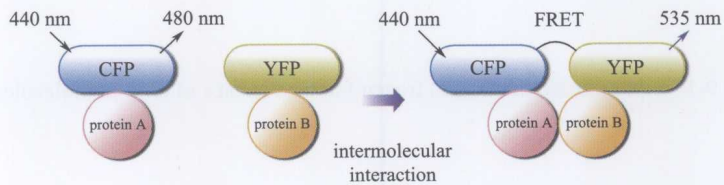


Figure 6-1 Schematic diagram of the FRET principles

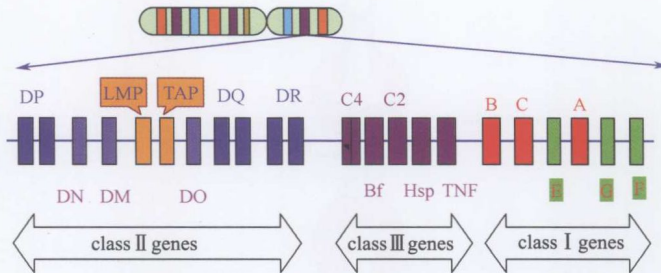


Figure 7-2 Structure of HLA gene

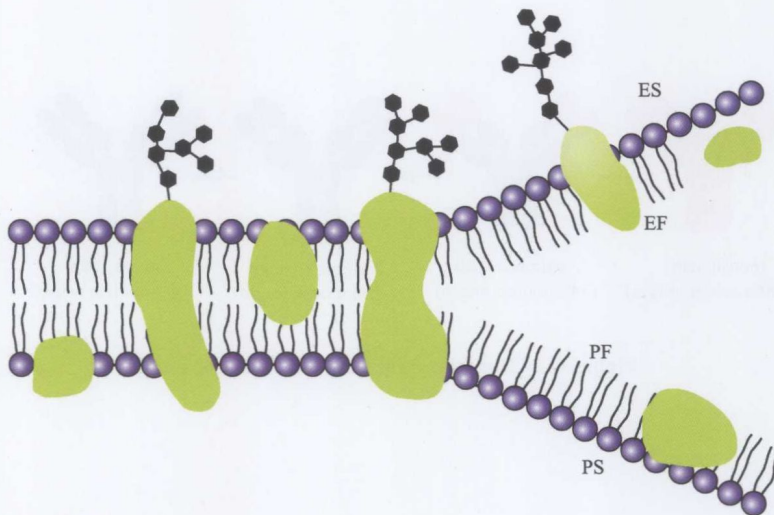


Figure 9-1 Lipid mosaic model and frozen fracture surface of biofilm molecules

Contents

Preface

Chapter 1 Introduction	1
1.1 History of immunology and immunological techniques	1
1.1.1 The empirical period of immunology	1
1.1.2 The period of classical immunology	3
1.1.3 The period of early modern immunology	7
1.1.4 The period of modern immunology	9
1.2 The extensive application of immunological techniques	13
1.2.1 Immunology and medicine	13
1.2.2 Immunology and biology	14
1.2.3 Immunology and the development of biotechnology	14
1.3 Present situation and prospects of immunology	15
1.3.1 The in-depth development of immunology at molecule level	15
1.3.2 The interaction of immune system and neuroendocrine system	16
1.3.3 The infiltration of immunology into biology, basic medicine, clinical medicine and preventive medicine	16
1.3.4 The applied research of immunology promoted the development of biotechnology	16
References	17
Chapter 2 The Basic Principles of Antigen-antibody Reaction	19
2.1 The general principles of antigen-antibody binding	19
2.1.1 Antigen	19
2.1.2 Antibody	29
2.1.3 The general principles of antigen-antibody binding	40
2.1.4 The characteristics of antigen-antibody reaction	43
2.2 The factors affecting Ag-Ab binding	45
2.2.1 Antibody	45
2.2.2 Antigen	46
2.2.3 Electrolytes	46
2.2.4 pH	46
2.2.5 Temperature	47
2.3 The main application of Ag-Ab binding reaction	47
2.3.1 The detection of complement	47

2.3.2	The detection of immune globulin	47
2.3.3	The detection of immune complexes	48
2.3.4	The detection of cytokines	49
	References	49
Chapter 3	Fabrication and Purification of Antibodies	51
3.1	Polyclonal antibodies	51
3.1.1	Preparation principle	51
3.1.2	The choice of species	52
3.1.3	Processing the antigen	52
3.1.4	Immunization of animals	53
3.2	Monoclonal antibodies	53
3.2.1	Principle	53
3.2.2	The choice of animal and immunization	55
3.2.3	Cell fusion	55
3.2.4	Screening of hybridoma cell and detecting antibody	56
3.2.5	Subcloning of hybridoma cell	56
3.2.6	Hybridoma cell freezing and thawing	57
3.2.7	Mass production of monoclonal antibody	57
3.2.8	Identification of monoclonal antibody	58
3.3	Purification of antibody	59
3.3.1	Principle	59
3.3.2	Purification methods	59
	References	61
Chapter 4	Antibody Engineering	62
4.1	Cell engineering antibody	62
4.1.1	Animal immunizing	62
4.1.2	Cell fusion	62
4.1.3	Screening of hybridoma cell	63
4.2	Genetic engineering antibody	63
4.2.1	Chimeric antibody	65
4.2.2	Humanized antibody	67
4.2.3	Fully humanized antibody	67
4.2.4	Small molecule antibodies and antibody fusion protein	68
4.2.5	Bispecific diabody	71
4.3	Antibody library technology	72
4.3.1	Application of antibody library	73
4.3.2	Phage display human antibody library	76
4.3.3	The construction of phage display human antibody	77

4.4	Applications of antibody	84
	References	85
Chapter 5	Labelled Immunoassay Technique	87
5.1	Radioimmunoassay	87
5.1.1	Technical evolvement	88
5.1.2	Principle	89
5.1.3	Basic reagents and technical methods	92
5.2	Enzyme immune techniques	98
5.2.1	Summary	98
5.2.2	Preparation of enzyme-labelled antibody	100
5.2.3	Amplification of enzyme immunoassay	104
5.2.4	Application and development trend of enzyme immunoassay	107
5.3	Fluoroimmunoassay	114
5.3.1	Brief review	114
5.3.2	Labelling method	118
5.4	Chemiluminescence immunoassay	118
5.4.1	Principle	118
5.4.2	Classification of CLIA method	120
5.4.3	Applications and prospects	123
	References	128
Chapter 6	Time-Resolved Fluorescence Immunoassay	130
6.1	Principles	130
6.1.1	Homogeneous fluorescence analysis	131
6.1.2	Time-resolved fluorescence and rare earth element	132
6.1.3	Adjustment of fluorescence resonance energy transfer(FRET).....	133
6.2	Applications	133
6.2.1	Application in immunology	133
6.2.2	Applications in microbiology	133
6.2.3	Applications in molecular biology	134
6.2.4	Applications in hormone detection	134
6.2.5	Other applications	134
6.3	Multi-labelled time-resolved fluorescence immunoassay	134
6.3.1	Principle	134
6.3.2	Application	138
6.4	Enzyme-amplified time-resolved fluoroimmunoassay	139
6.4.1	Principles	139
6.4.2	Application	140
	References	141

Chapter 7 Molecular Immunology and Immunogenetics	143
7.1 DNA isolation and purification	143
7.1.1 Introduction	143
7.1.2 Several common methods for enrichment and purification of DNA	144
7.2 Nucleic acid probe technology	150
7.2.1 Overview	150
7.3 Nucleic acid molecular hybridization	154
7.3.1 Principles and types	154
7.3.2 Application	157
7.4 Construction and screening of cDNA library	159
7.4.1 cDNA library and construction	159
7.4.2 cDNA library screening	166
7.5 HLA gene matching and typing technology	171
7.5.1 HLA gene matching	171
7.5.2 Matching methods for HLA-II genes	175
References	177
Chapter 8 Immunoblotting Technique	179
8.1 Introduction	179
8.1.1 SDS-PAGE	179
8.1.2 Transfer	181
8.1.3 Enzyme immunoassay	183
8.1.4 Notices of Western blot	187
8.2 Molecular imprinting technique (MIT)	187
8.2.1 Overview of molecular imprinting	188
8.2.2 Methyl testosterone and molecular imprinting technique	199
8.2.3 The problem and outlook of molecularly imprinting technique	200
8.3 Applications of immunoblotting test	201
References	202
Chapter 9 Immunolectron Microscopy	206
9.1 Overview	206
9.1.1 Tissue fixation and sampling	206
9.1.2 Immune staining	206
9.1.3 Embedding	208
9.2 Ferritin immune technology	211
9.2.1 Basic principles	211
9.2.2 Extraction and purification of ferritin	211
9.2.3 Combination of ferritin and immunoglobulin	212
9.2.4 Preparation of electron microscope specimens	213

9.3	Other immunoelectron microscopy	214
9.3.1	Colloidal gold labelled method for immunoelectron microscopy	214
9.3.2	Scanning immunoelectron microscopy	216
9.3.3	Freeze-etching immunoelectron microscopy	218
9.4	Situ hybridization for electron microscopy	219
9.4.1	Application of isotope-labelled cRNA probe with situ hybridization electron microscope in chromosome	220
9.4.2	Application of biotin-labelled DNA probe with situ hybridization electron microscopy	221
9.4.3	Application of digoxin-labelled rRNA probe in situ hybridization electron microscope	224
	References	226
Chapter 10	Pesticide Immunoassay	228
10.1	Organophosphorus pesticides	228
10.1.1	Overview	228
10.1.2	Immunoassay of organophosphorus pesticide	235
10.2	Immunoassay for organochlorine pesticides	239
10.2.1	Overview	239
10.2.2	Analysis method	245
10.3	Carbamates	246
10.3.1	Overview	246
10.3.2	Immunoassay for carbamates	254
10.4	Pyrethroid insecticides	257
10.4.1	Overview	257
10.4.2	Immunoassay for pyrethroids	264
10.5	Herbicides pesticides	267
10.5.1	Overview	267
10.5.2	Immunoassay for herbicides	274
10.6	Other pesticides	277
10.6.1	Triazole pesticides	277
10.6.2	Benzimidazole pesticides	279
10.6.3	Immunoassay	279
10.7	Immunoassay based on class specific antibody	282
	References	285
Chapter 11	Immunoassay for Veterinary Drugs	288
11.1	Aminoglycoside antibiotics	288
11.1.1	Introduction	288
11.1.2	Immunoassay	292

11.2	Macrolide antibiotics	293
11.2.1	Introduction	293
11.2.2	Immunoassay of MALs	297
11.3	Chloramphenicols drugs	298
11.3.1	Introduction	298
11.3.2	Immunoassay	299
11.4	β -lactam antibiotics	301
11.4.1	Introduction	301
11.4.2	Analytical method	307
11.5	Nitrofurans drugs	309
11.5.1	Introduction	309
11.5.2	Immunoassay for nitrofurans	311
11.6	Quinolones	312
11.6.1	Introduction	312
11.6.2	Immunoassay for QNs	319
11.7	Sulfonamides	322
11.7.1	Introduction	322
11.7.2	Immunoassay for SAs	324
11.8	Tetracyclines	326
11.8.1	Introduction	326
11.8.2	Immunoassay for TCs	328
11.9	Anthelmintic (worm) agents	329
11.9.1	Introduction	329
11.9.2	Immunoassay for anthelmintics	330
11.10	Anticoccidial drugs	333
11.10.1	Introduction	333
11.10.2	Immunoassay for anticoccidial drugs	334
11.11	Anabolic hormones	335
11.11.1	Introduction	335
11.11.2	Immunoassay for ASs	344
11.12	β -Agonist	349
11.12.1	Introduction	349
11.12.2	Immunoassay for β -agonist	349
11.13	Other drugs	350
11.13.1	Benzodiazepines	350
11.13.2	Retention analysis for polyether antibiotics	352
11.13.3	Immunoassay for olaquinox and its metabolites	355
11.14	Multi-residual immunoassay for veterinary drugs	355

References	357
Chapter 12 Biological Toxin Immunoassay	364
12.1 Animal toxins	364
12.1.1 Overview	364
12.1.2 Immunoassay of biological toxins	366
12.2 Fungi and mycotoxins	367
12.2.1 Overview	367
12.2.2 Analytical methods	372
12.3 Bacteria and bacterial toxins	374
12.3.1 Overview	374
12.3.2 Immunoassay for bacterial toxins	376
12.4 Plant toxins	377
12.4.1 Introduction	377
12.4.2 Immunoassay for plant toxins	380
12.5 Algae and phycotoxins	381
12.5.1 Overview	381
12.5.2 Immunoassay	383
References	384
Chapter 13 Immunoassay for Other Residues	386
13.1 Dioxins	386
13.1.1 Introduction	386
13.1.2 Immunoassay for dioxins	387
13.2 Polybrominated biphenyl	388
13.2.1 Introduction	388
13.2.2 Immunoassay for PBDEs	389
13.3 Sudan red and para red	389
13.3.1 Introduction	389
13.3.2 Immunoassay for Sudan red	392
13.4 Malachite green	393
13.4.1 Introduction	393
13.4.2 Immunoassay for MG	394
13.5 Melamine	394
13.5.1 Introduction	394
13.5.2 Immunoassay of MEL	395
13.6 Phthalate(ester of phthalic acid)	396
13.6.1 Introduction	396
13.6.2 Immunoassay for PAE	396
13.7 Bisphenol A	398

13.7.1	Introduction	398
13.7.2	Immunoassay for BPA	399
13.8	Heavy metal contamination	400
13.8.1	Introduction	400
13.8.2	Immunoassay	400
	References	401

Colored Figures