

CONTENTS

Abbreviations

CHAPTER 1

Lactoferrin

CHAPTER 2

Review

2.1	Transferrins: Biological and Phylogenetic Distribution	6
2.2	Physical Structure of Lactoferrin	6
2.2.1	Primary Structure: Evolutionary Conservation	6
2.2.2	Tertiary Folding: A Universal Pattern	7
2.2.3	Domains and Lobes: Rigidity vs Flexibility	7
2.2.4	Metal Binding: The Synergy	9
2.2.5	Iron Lodging and Releasing: Intriguing Mechanisms	10
2.3	Folding Stability	12
2.4	Glycosyl Moieties of Lactoferrins	12
2.5	Functions of Lactoferrin	14
2.5.1	Iron Absorption	14
2.5.2	Anti-inflammatory Effects	15
2.5.3	Immunomodulatory Roles	15
2.5.4	Antiviral Properties	16
2.5.5	Antimicrobial Efficiency and Infant Food Formula	17
2.5.6	Antifungal Potencies	17
2.5.7	Antitumour Activity	18
2.5.8	Lactoferrin in Pathology and Clinical Applications	18
2.5.9	Recombinant Lactoferrin and Mass Production	19
2.6	Functional Fragments of Lactoferrin	20
2.7	Porcine Lactoferrin: Studies Done So Far	21

CHAPTER 3

Comparative Sequence Analysis of the Transferrin Family of Proteins

3.1	Introduction	24
3.2	Resources	24
3.3	Sequence Conservation Among Lactoferrins	25
3.4	Transferrin Proteins Together: The Sequence Variability	28
3.5	Phylogeny of Transferrin Proteins: Complex Patterns	36
	Conclusions	39

CHAPTER 4

Porcine Lactoferrin Purification, Isolation of Monomers and Functional Assays of Reassociated N- and C- Lobe Complex

4.1	Introduction	41
4.2	Materials and Methods.....	41
4.2.1	Purification of Porcine Lactoferrin	41
4.2.1.1	Isolation of Porcine Lactoferrin	41
4.2.1.2	Ion-exchange Chromatography	41
4.2.1.3	Gel Filtration Chromatography	42
4.2.1.4	SDS-PAGE.....	42
4.2.2	Preparation of N- and C- Lobes of PLf.....	42
4.2.2.1	Digestion with Proteinase K.....	42
4.2.2.2	Ion-exchange Chromatography	42
4.2.2.3	Gel Filtration Chromatography	42
4.2.2.4	SDS-PAGE.....	43
4.2.2.5	N-Terminal Sequencing.....	43
4.2.2.6	Determination of pI	43
4.2.3	Preparation of Reassociated N- and C- Lobe Complex	43
4.2.3.1	Formation of Reassociated N- and C- Lobe Complex	43
4.2.3.2	Gel Filtration Chromatography	43
4.2.3.3	SDS-PAGE.....	44
4.3	Results.....	44
4.3.1	Purification of Porcine Lactoferrin	44
4.3.1.1	Isolation of Porcine Lactoferrin	44
4.3.1.2	Ion-exchange Chromatography	44
4.3.1.3	Gel Filtration Chromatography	45
4.3.1.4	SDS-PAGE.....	45
4.3.2	Preparation of N- and C- Lobes of PLf.....	45
4.3.2.1	Digestion with Proteinase K.....	45
4.3.2.2	Ion-exchange Chromatography	45
4.3.2.3	Gel Filtration Chromatography	46
4.3.2.4	SDS-PAGE.....	46
4.3.2.5	N-Terminal Sequencing.....	47
4.3.2.6	Determination of pI	48
4.3.3	Preparation of Reassociated N- and C- Lobe Complex	48
4.3.3.1	Formation of Reassociated N- and C- Lobe Complex	48
4.3.3.2	Gel Filtration Chromatography	48
4.3.3.3	SDS-PAGE.....	49
4.4	Discussion	49
	Conclusions.....	53

CHAPTER 5

Iron Release Mechanism and the Nature of Domain-Domain Interactions of Porcine Lactoferrin

5.1	Introduction	55
5.2	Materials and Methods.....	55
5.2.1	pH Induced Iron Release from Intact PLf.....	56
5.2.2	pH Induced Iron Release from C- Lobe.....	56

5.2.3	pH Induced Iron Release from N- Lobe.....	56
5.2.4	pH Induced Iron Release from Reassociated N _{Fe} - C _{Fe} Lobe.....	56
	Complex	
5.2.5	pH Induced Iron Release from Reassociated N _{Fe} - C _{Apo} Lobe	57
	Complex	
5.3	Results.....	57
5.3.1	pH Induced Iron Release from Intact PLf.....	57
5.3.2	pH Induced Iron Release from C- Lobe.....	57
5.3.3	pH Induced Iron Release from N- Lobe.....	57
5.3.4	pH Induced Iron Release from Reassociated N _{Fe} - C _{Fe} Lobe	57
	Complex	
5.3.5	pH Induced Iron Release from Reassociated N _{Fe} - C _{Apo} Lobe	58
	Complex	
5.4	Discussion	58
	Conclusions	63

CHAPTER 6

Crystallization of Human Lactoferrin

6.1	Introduction.....	66
6.2	Materials and Methods.....	66
6.2.1	Preparation of Iron saturated Lactoferrin.....	66
6.2.2	Preparation of Apo Lactoferrin	66
6.2.3	Deglycosylation.....	67
6.2.4	Primary Screening.....	67
6.2.5	Optimization Screening.....	67
6.3	Results.....	67
6.3.1	Preparation of Iron saturated Lactoferrin.....	67
6.3.2	Preparation of Apo Lactoferrin	68
6.3.3	Deglycosylation.....	68
6.3.4	Primary Screening.....	68
6.3.5	Optimization Screening.....	68
6.4	Discussion	69
	Conclusions.....	72

CHAPTER 7

Thermal Folding of Porcine Lactoferrin: Differential Scanning Calorimetric Studies

7.1	Introduction.....	74
7.2	Structure and Unfolding of Protein.....	74
7.2.1	Stability of the Folding.....	74
7.2.2	Techniques Used to Monitor Protein Unfolding.....	75
7.3	Differential Scanning Calorimetry and Protein Unfolding	75
7.4	Materials and Methods.....	80
7.4.1	Materials.....	80
7.4.2	Differential Scanning Calorimetric Measurements.....	80
7.5	Results.....	80
7.5.1	Scanning of DPLf.....	80

7.5.2	Scanning of Apo-intact Protein.....	83
7.5.3	Scanning of Ferric and Apo-N- Lobes.....	84
7.5.4	Scanning of C- Lobe	86
7.5.5	Scanning of Reassociated N- and C- Lobe complex.....	86
7.6	Discussion	86
	Conclusions... ..	92
	References	94