

The Evolution of a Physiological System: The Pulmonary Surfactant System in Diving Mammals

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Thesis summary	i
Declaration	iii
Acknowledgements	iv
Bibliography	vi
List of abbreviations.....	viii

Chapter 1

Mammalian pulmonary surfactant: Diving and the lung

1.1. Overview: The mammalian pulmonary surfactant system	1
1.2. The lungs of diving mammals.....	3
1.2.1. <i>Morphology</i>	3
1.2.1.1. <i>Cetacea</i>	3
1.2.1.2. <i>Phocidae</i>	5
1.2.1.3. <i>Otariidae</i>	5
1.2.2. <i>Respiratory patterns</i>	7
1.2.3. <i>Diving and the lung</i>	8
1.3. Diving mammal behaviour.....	9
1.4. Composition of pulmonary surfactant	12
1.4.1. <i>Lipids</i>	12
1.4.2. <i>Proteins</i>	17
1.4.2.1. <i>Hydrophilic proteins</i>	17
1.4.2.2. <i>Hydrophobic proteins</i>	18
1.4.3. <i>Variations in methods</i>	21
1.5. Functions of pulmonary surfactant	22
1.5.1. <i>Compliance</i>	22
1.5.2. <i>Surface activity</i>	23
1.5.2.1. <i>Stability ratio</i>	24

1.5.2.2.	<i>Langmuir-Wilhelmy surface balance</i>	24
1.5.2.3.	<i>Enhörning pulsating bubble surfactometer</i>	27
1.5.2.4.	<i>Captive bubble surfactometer</i>	29
1.5.3.	<i>Acting as an anti-adhesive</i>	32
1.5.4.	<i>Prevention of pulmonary oedema</i>	32
1.5.5.	<i>Immune functions</i>	33
1.5.6.	<i>Surfactant alterations and disease</i>	34
1.5.7.	<i>Anti-oxidant functions</i>	36
1.5.8.	<i>Airway patency</i>	37
1.6.	Regulation of surfactant secretion	38
1.6.1.	<i>Autonomic neurotransmitters</i>	38
1.6.2.	<i>Hormones</i>	39
1.6.3.	<i>Other factors</i>	40
1.6.4.	<i>Stretch</i>	40
1.6.5.	<i>Pressure</i>	42
1.7.	Evolution and phylogenetics	43
1.7.1.	<i>Diving mammal evolution</i>	43
1.7.2.	<i>Molecular evolution and phylogenetic relationships</i>	45
1.7.2.1.	<i>Molecular evolution at the nucleotide level</i>	46
1.7.2.2.	<i>Molecular evolution at the amino acid level</i>	48
1.7.3.	<i>Evolution and conservation of pulmonary surfactant</i>	48
1.8.	Allometry and relationships between various characteristics	50
1.9.	Directions of the thesis	52
1.9.1.	<i>Species selection</i>	52
1.9.2.	<i>Aims of the thesis</i>	53

Chapter 2

Surfactant protein C: Positive selection and functional adaptations in diving mammals

2.1.	Introduction	54
2.2.	Methods	57
2.2.1.	<i>Animals</i>	57
2.2.2.	<i>DNA extraction</i>	60
2.2.3.	<i>Designing of primers</i>	63
2.2.4.	<i>Polymerase chain reaction (PCR).....</i>	63
2.2.4.1.	<i>Amplification of sp-c gene.....</i>	63
2.2.4.2.	<i>Detection of PCR products</i>	64
2.2.5.	<i>DNA sequencing</i>	65
2.2.6.	<i>Cloning.....</i>	65
2.2.7.	<i>Phylogenetic analysis.....</i>	66
2.2.7.1.	<i>Designing a user tree and sequence alignment.....</i>	66
2.2.7.2.	<i>Identifying selection at the nucleotide level.....</i>	68
2.2.7.3.	<i>Identifying selection at the amino acid level.....</i>	70
2.3.	Results	73
2.3.1.	<i>Selection at the nucleotide level.....</i>	73
2.3.2.	<i>Selection at the amino acid level.....</i>	88
2.4.	Discussion	103
2.5.	Summary.....	112
2.6.	Future directions	113

Chapter 3

The composition of pulmonary surfactant from diving mammals

3.1.	Introduction	114
3.2.	Methods	115
3.2.1.	<i>Animals</i>	115
3.2.2.	<i>Lavage protocol and surfactant extraction</i>.....	115
3.2.3.	<i>Transmission electron microscopy</i>.....	116
3.2.4.	<i>Electrospray ionisation mass spectroscopy</i>	116
3.2.5.	<i>High performance liquid chromatography</i>.....	119
3.2.6.	<i>Enzyme-linked immunosorbent assay</i>	120
3.2.6.1.	<i>Surfactant protein A</i>	120
3.2.6.2.	<i>Surfactant protein B</i>	121
3.2.7.	<i>Allometric regressions</i>	122
3.2.8.	<i>Statistics</i>	122
3.3.	Results	123
3.3.1.	<i>Validation of methods</i>.....	123
3.3.1.1.	<i>Transmission electron microscopy</i>	123
3.3.1.2.	<i>ESI-MS and tandem MS/MS</i>	123
3.3.2.	<i>Phospholipids</i>	133
3.3.3.	<i>Cholesterol</i>	143
3.3.4.	<i>Proteins</i>.....	145
3.3.5.	<i>Allometric regressions of PC molecular species</i>	148
3.4.	Discussion	151
3.4.1.	<i>Validation of methods</i>.....	151
3.4.1.1.	<i>Transmission electron microscopy</i>	151
3.4.1.2.	<i>Electrospray ionisation mass spectrometry</i>	151
3.4.1.3.	<i>High performance liquid chromatography</i>	153
3.4.2.	<i>Composition of surfactant in diving mammals</i>	155
3.4.2.1.	<i>Phospholipids</i>	155
3.4.2.2.	<i>Allometry of surfactant phospholipids</i>	158
3.4.2.3.	<i>Cholesterol</i>	160

3.4.2.4. <i>Proteins</i>	163
3.5. Summary	165
3.6. Future directions	166

Chapter 4

The surface activity of pulmonary surfactant from diving mammals

4.1. Introduction	168
4.2. Methods	169
4.2.1. <i>Animals</i>	169
4.2.2. <i>Lavage protocol and surfactant extraction</i>	170
4.2.3. <i>Captive bubble surfactometry</i>	170
4.2.4. <i>Bubble measurements</i>	170
4.2.4.1. <i>Adsorption</i>	170
4.2.4.2. <i>Quasi-static and dynamic compression</i>	171
4.2.4.3. <i>Compressibility</i>	171
4.2.5. <i>Statistical analysis</i>	172
4.3. Results	172
4.3.1. <i>Adsorption</i>	172
4.3.2. <i>Minimum surface tension</i>	173
4.3.3. <i>Film surface area reduction</i>	174
4.3.4. <i>Film compressibility</i>	175
4.4. Discussion	176
4.4.1. <i>Validation of Methods</i>	176
4.4.2. <i>Surface properties of pinniped surfactant</i>	177
4.4.2.1. <i>Equilibrium surface tension (γ_{eq})</i>	178
4.4.2.2. <i>Minimum surface tension (γ_{min})</i>	180
4.4.2.3. <i>Film compressibility</i>	181
4.4.3. <i>Functions of Pinniped Surfactant</i>	182

4.4.3.1.	<i>Alveolar stability</i>	182
4.4.3.2.	<i>Static lung compliance</i>	182
4.4.3.3.	<i>Acting as an anti-adhesive</i>	183
4.4.3.4.	<i>Controlling fluid balance</i>	185
4.5.	Summary	185
4.6.	Future directions	186

Chapter 5

The development of the pulmonary surfactant system in the California sea lion, *Zalophus californianus*

5.1.	Introduction	188
5.2.	Methods	189
5.2.1.	<i>Animals</i>	189
5.2.2.	<i>Experimental protocols</i>	189
5.3.	Results	189
5.3.1.	<i>Phospholipids</i>	189
5.3.2.	<i>Cholesterol</i>	193
5.3.3.	<i>Proteins</i>	193
5.3.4.	<i>Surface activity</i>	195
5.4.	Discussion	197
5.5.	Summary	199
5.6.	Future directions	201

Chapter 6

Pulmonary surfactant secretion from alveolar type II cells isolated from California sea lions, *Zalophus californianus*

6.1.	Introduction	203
6.2.	Materials and Methods.....	204
6.2.1.	<i>Animals</i>	204
6.2.2.	<i>ATII cell isolation</i>	204
6.2.3.	<i>Statistical analyses</i>	205
6.3.	Results	205
6.3.1.	<i>Cell culture</i>	205
6.3.2.	<i>Mechanical stimulation</i>	208
6.3.3.	<i>Chemical stimulation</i>	214
6.4.	Discussion	216
6.4.1.	<i>Validation of methods</i>	216
6.4.2.	<i>Mechanical stimulation</i>	217
6.4.3.	<i>Chemical stimulation</i>	219
6.5.	Summary.....	221
6.6.	Future directions	222

Chapter 7

Evolution of a Physiological System: Pressure as a Selection Variable on the Mammalian Pulmonary Surfactant System

7.1.	The pulmonary surfactant system: Importance and evolution ...	224
7.1.1.	<i>Selection at the molecular level</i>	226
7.1.2.	<i>Selection at the compositional level</i>	227
7.1.3.	<i>Selection at the functional level</i>	228
7.1.4.	<i>Selection during development</i>	228

7.1.5. Selection at the cellular level	229
7.2. Conclusion.....	230

APPENDICES

Appendix 1: The genetic code and degenerate primer design	232
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Appendix 2: Phylogenetic Analysis by Maximum Likelihood (PAML) ...	234
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REFERENCES	248
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Thesis summary

Pulmonary surfactant is a complex mixture of lipids and proteins that lowers surface tension, increases lung compliance, and prevents the adhesion of respiratory surfaces and pulmonary oedema. Pressure can have an enormous impact on respiratory function, by mechanically compressing tissues, increasing gas tension resulting in increased gas absorption and by increasing dissolved gas tensions during diving, resulting in the formation of bubbles in the blood and tissues.

The lungs of diving mammals have a huge range of morphological adaptations to enable them to endure the extremely high pressures associated with deep diving. Here, I hypothesise that surfactant will also be modified, to complement the morphological changes and enable more efficient lung function during diving. Molecular adaptations to diving were examined in surfactant protein C (SP-C) using phylogenetic analyses. The composition and function of pulmonary surfactant from several species of diving mammals was examined using biochemical assays, mass spectrometry and captive bubble surfactometry. The development of surfactant in one species of diving mammal (California sea lion), and the control of surfactant secretion using chemical and mechanical stimuli were also determined.

Diving mammals showed modifications to SP-C, which are likely to lead to stronger binding to the monolayer, thereby increasing its fluidity. Phospholipid molecular species concentrations were altered to increase the concentration of more fluid species. There was also an increase in the percentage of alkyl molecular species, which may increase the stability of the monolayer during compression and facilitate rapid respreading. Levels of SP-B were much lower in the diving species, and cholesterol was inversely proportional to the maximum dive depth of the three species. Surface activity of surfactant from diving mammals was very poor compared to surfactant from terrestrial mammals. The newborn California sea lion surfactant was similar to terrestrial mammal surfactant, suggesting that these animals develop the diving-type of surfactant after they first enter the water. The

isolated cells of California sea lions also showed a similar response to neuro-hormonal stimulation as terrestrial mammals, but were insensitive to pressure.

These findings showed diving mammal surfactant to have a primarily anti-adhesive function that develops after the first entry into the water, with a surfactant monolayer, which would be better suited to repeated collapse and respreading.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

The author consents to this thesis being made available for photocopying and loan when deposited in the University Library.

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List of abbreviations

AFM – atomic force microscopy

atm - atmospheres

ATII – alveolar type II

BF – breathing frequency

BLES – bovine lipid extract surfactant

BM – body mass

BSA – bovine serum albumin

CARB – carbamylcholine chloride

C_{15} – compressibility at 15mN/m

C_{25} – compressibility at 25mN/m

CBS – captive bubble surfactometer

CHOL – cholesterol

CPCT – cholinephosphate cytidyltransferase

CRD – carbohydrate recognition domain

D# – number of a particular dynamic compression-expansion cycle

DEX – dexamethasone

d_N – rate of non-synonymous substitutions

d_S – rate of synonymous substitutions

DPM – disintegrations per minute

DPPC – dipalmitoylphosphatidylcholine

DSP – disaturated phospholipid

ELISA – enzyme linked immunosorbent assay

ESI-MS – electrospray ionisation mass spectroscopy

F_{codon} – codon frequency parameter assuming high level of codon usage bias

F_{equal} – codon frequency parameter assuming no codon usage bias

γ (gamma) – surface tension

γ_{eq} – equilibrium surface tension

γ_{\max} – maximum surface tension

γ_{\min} – minimum surface tension

HPLC – high performance liquid chromatography

HRP – horseradish peroxidase

ISOP – isoproterenol

LB – Luria-Bertani broth

LPC – lysophosphatidylcholine

LnL – log likelihood

$2\Delta\ln L$ – twice the difference in log likelihoods

LRT – likelihood ratio test

m/z – mass/charge ratio

PAML – Phylogenetic Analysis by Maximum Likelihood

PC – phosphatidylcholine

PCR – polymerase chain reaction

PE – phosphatidylethanolamine

PG – phosphatidylglycerol

PI – phosphatidylinositol

PL – phospholipid

PS – phosphatidylserine

Q – quadropole (of the mass spectrometer)

QS# – number of a particular quasi-static compression-expansion cycle

RDS – respiratory distress syndrome

SIDS – sudden infant death syndrome

SP-A – surfactant protein A

SP-B – surfactant protein B

SP-C – surfactant protein C

SP-D – surfactant protein D

Sph – sphingomyelin

SRT – surfactant replacement therapy

T3 – triiodothyronine

T4 – thyroxine

TEM – transmission electron microscopy

TLC – thin layer chromatography

USP – unsaturated phospholipid

UV – ultraviolet

ω (omega) – ratio of d_N/d_S

ω_c – ratio of d_N/d_S for different classes of amino acid substitutions

ω_p – ratio of d_N/d_S for different properties of amino acids