
Proteomic Analysis of Liver Membranes through an Alternative Shotgun Methodology

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Table of Contents

Abstract	5
Declaration	6
Acknowledgments	7
Publications Contributing to the Production of the Thesis	8
Abbreviations	9
Chapter 1: Introduction	11
1.1 Background	12
1.2 Membrane Proteomics	15
1.2.1 Membrane Protein Structure and Sample Preparation	15
1.2.1.1 Membrane Protein Structure	15
1.2.1.2 Membrane Protein Isolation and Purification	19
1.2.1.3 Digestion of Membrane Proteins	23
1.2.2 Shotgun Proteomics Analysis of Membrane Proteins	25
1.2.2.1 Shotgun Proteomics	25
1.2.2.2 Shotgun Proteomic Analysis of Membrane Proteins	27
1.2.2.3 Transmembrane Proteins Identified by Shotgun Proteomics	30
1.2.2.4 Quantitative Shotgun Proteomics	32
1.2.3 Summary of Membrane Proteomics	35
1.3 Shotgun Proteomics using Immobilised pH Gradient Isoelectric Focusing	36
1.3.1 Isoelectric Focusing	36
1.3.2 IPG-IEF as a Shotgun Proteomics Dimension	37
1.3.3 Theoretical Peptide Isoelectric Point and Peptide Separation	40
1.3.4 Peptide pI Filtering for Assigning High Confident Protein Identifications	42
1.3.5 Quantitative Shotgun Proteomics and IPG-IEF Separation of Peptides	44
1.3.6 Summary of Isoelectric Focusing based Shotgun Proteomics	45
1.4 Liver Integral Membrane Proteins and Tumor-induced Inflammation	46
1.4.1 Introduction	46
1.4.2 Liver Structure and Organisation	47
1.4.3 Proteomic Analysis of the Liver	50
1.4.4 Xenobiotic Metabolism and Transportation	51
1.4.5 Summary of Liver Proteomics and Tumor-Induced Inflammation	55
1.5 Thesis Aims and Scope of Research	57
Chapter 2: Shotgun Proteomic Analysis of Rat Liver Membrane Proteins	58
2.1 Introduction	59
2.2 Materials and Methods	62
2.2.1 Membrane protein isolation and preparation for peptide IPG-IEF	62
2.2.2 Peptide IPG-IEF	63
2.2.3 Liquid Chromatography Mass Spectrometry	64
2.2.4 Protein and Peptide Identification	64
2.3 Results	66
2.3.1 Resolution of Identified Peptides within IEF Experiments	66
2.3.2 Analysis of Peptide Outliers in the Basic End of the pH Gradient	70
2.3.3 Evaluation of Peptide IPG-IEF for Protein Identifications	73
2.3.4 Integral Membrane Protein Identifications	75
2.3.5 Cellular Location of Proteins Identified from Each Digest	78
2.3.6 Identified Membrane Protein Families	79
2.4 Discussion	85
2.4.1 Peptide Separation	85

2.4.2 Protein Identifications	87
2.4.3 Integral Membrane Proteins	89
2.4.4 Cellular Location Analysis	90
2.4.5 Identified Membrane Protein Families	91
2.4.5 Conclusion	92
Chapter 3: A Combination of Immobilised pH Gradients Improve Membrane Proteomics	93
3.1 Introduction	94
3.2 Materials and Methods	97
3.2.1 Membrane protein isolation and preparation for peptide IPG-IEF	97
3.2.2 Peptide IPG-IEF	98
3.2.3 Nanoflow liquid Chromatography – Tandem Mass Spectrometry	99
3.2.4 Protein and Peptide Identification	99
3.2.5 Calculation of Normalised Spectral Abundance Factors	100
3.2.6 Statistical Analysis	101
3.3 Results	102
3.3.1 Peptide Separation and Analysis	102
3.3.2 Comparison of BR and NR IPG Strips for Total Peptide and Protein Identifications	106
3.3.3 Analysis of the Relative Abundance of Peptides between BR and NR Range IPG Strips Using NSAF	110
3.3.4 Identified Membrane Protein Families	114
3.3.5 Gene Ontology Annotation Analysis	117
3.4 Discussion	120
3.4.1 Peptide Separation	120
3.4.2 Comparison of Total Peptide, Protein and IMP's Between Broad and Narrow Range IPG Strips	121
3.4.3 Label-Free Quantification Analysis	123
3.4.4 Identified Protein Families	124
3.4.5 Gene Ontology Annotations	125
3.4.6 Conclusion	126
Chapter 4: Affects of Tumor-Induced Inflammation on Membrane Proteins Abundance in the Mouse Liver	127
4.1 Introduction	128
4.2 Materials and Methods	131
4.2.1 Sample Preparation	131
4.2.2 Peptide IPG-IEF	132
4.2.3 Nanoflow liquid Chromatography – Tandem Mass Spectrometry	132
4.2.4 Protein and Peptide Identification	133
4.2.4 Calculation of Normalised Spectral Abundance Factors	134
4.2.5 Statistical Analysis	135
4.3 Results	136
4.3.1 Label-Free Quantification Analysis	136
4.3.2 Membrane Proteome Dynamic Range and Protein Abundance	138
4.3.3 Gene Ontology Analysis of Identified Proteins	140
4.3.3.1 Molecular Process Annotation	140
4.3.3.2 Molecular Function Annotation	143
4.3.3.3 Cellular Component Annotation	146
4.3.4 Transmembrane Protein Abundance	148
4.4 Discussion	152
4.4.1 Proteomic Analysis	152
4.4.2 Gene Ontology Annotations	155
4.4.3 Integral Membrane Protein Analysis	156
4.4.4 Conclusion	158

Chapter 5: Affects of Tumor-Induced Inflammation on Biochemical Pathways in the Mouse Liver	159
5.1 Introduction	160
5.2 Materials and Methods	162
5.2.1 Sample Preparation	162
5.2.2 Peptide IPG-IEF	163
5.2.3 Nanoflow liquid Chromatography – Tandem Mass Spectrometry	163
5.2.4 Protein and Peptide Identification	164
5.2.4 Calculation of Normalised Spectral Abundance Factors	165
5.2.5 Statistical Analysis	166
5.3 Results	167
5.3.1 Fatty Acid Metabolism	167
5.3.2 Xenobiotic Metabolism and Transportation	173
5.3.3 Electron Transport Chain	177
5.3.4 Glycosylation Enzymes and UDP-Sugar Transportation	179
5.4 Discussion	182
5.4.1 Interleukin-6 Receptor	182
5.4.2 Fatty Acid Metabolism and Cholesterol Biosynthesis	183
5.4.3 Xenobiotic Metabolism and Clearance	185
5.4.4 Electron Transport Chain	187
5.4.5 Protein Glycosylation	188
5.4.6 Conclusion	189
Chapter 6: General Discussion	191
6.1 Shotgun Proteomics Using IPG-IEF Separation of Peptides	192
6.1.1 Method Development	192
6.1.2 Improvements to the Methodology	193
6.1.3 Membrane Proteomics	194
6.1.4 Mouse Liver Membrane Profile	196
6.1.4 Tumor-Induced Inflammation and Liver Membrane Proteins	198
6.1.5 Final Conclusion	199
References	200
Appendices	214
Appendix 1	209
Appendix 2	216
Appendix 3	218
Appendix 4	226
Supplementary Tables	229
Table 1	230
Table 2	272
Table 3	310
Table 4	427
Table 5	458
Table 6	562

Abstract

The aim of this thesis was to develop a proteomics methodology that improves the identification of membrane proteomes from mammalian liver. Shotgun proteomics is a method that allows the analysis of proteins from cells, tissues and organs and provides comprehensive characterisation of proteomes of interest. The method developed in this thesis uses separation of peptides from trypsin digested membrane proteins by immobilised pH gradient isoelectric focusing (IPG-IEF) as the first dimension of two dimensional shotgun proteomics. In this thesis, peptide IPG-IEF was shown to be a highly reproducible, high resolution analytical separation that provided the identification of over 4,000 individual protein identifications from rat liver membrane samples. Furthermore, this shotgun proteomics strategy provided the identification of approximately 1,100 integral membrane proteins from the rat liver. The advantages of using peptide IPG-IEF as a shotgun proteomics separation dimension in conjunction with label-free quantification was applied to a biological question: namely, does the presence of a spatially unrelated benign tumor affect the abundance of mouse liver proteins. IPG-IEF shotgun proteomics provided comprehensive coverage of the mouse liver membrane proteome with 1,569 quantified proteins. In addition, the presence of an Englebreth-Holm-Swarm sarcoma induced changes in abundance of proteins in the mouse liver, including many integral membrane proteins. Changes in the abundance of liver proteins was observed in key liver metabolic processes such as fatty acid metabolism, fatty acid transport, xenobiotic metabolism and clearance. These results provide compelling evidence that the developed shotgun proteomics methodology allows for the comprehensive analysis of mammalian liver membrane proteins and detailed some of the underlying changes in liver metabolism induced by the presence of a tumor. This model may reflect changes that could occur in the livers of cancer patients and has implications for drug treatments.

Declaration

I certify that the work in this thesis entitled “Proteomic Analysis of Liver Membrane Proteins through an Alternative Shotgun Methodology” has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University. I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Joel Chick (40936228)

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Publications Contributing to the Production of the Thesis

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2. Chick, J. M.; Haynes, P. A.; Bjellqvist, B.; Baker, M. S., A Combination of pH Gradients Improves Membrane Proteomics, *J Proteome Res* **2008**, 7, (11), 4974-4981.
3. Chick, J. M.; Haynes, P. A.; Baker, M. S.; Robertson, G.; Affects of tumor-induced inflammation on mouse liver membrane protein abundance. Publication in preparation.

Abbreviations

2D	Two-dimensional
2D-GE	Two-dimensional gel electrophoresis
3D	Three Dimensional
ABC	ATP-binding Cassette
Acaa	Acetyl-CoA acetyltransferase
Acadm	acyl-CoA dehydrogenase medium chain
Adh	Alcohol Dehydrogenase
Aldh	Aldehyde Dehydrogenase
Apo	Apolipoprotein
B3galnt	Beta-1,3-Galactosyltransferase
Bcrp	Breast Cancer Resistance Protein
BR	Broad Range
Bsep	Bile Salt Export Pump
C1Galt	Core 1 Synthase, Glycoprotein-N-acetylgalactosamine 3- beta-Galactosyltransferase, 1
cIEF	Capillary Isoelectric Focusing
Cpt	Carnitine O-palmitoyltransferase
CRP	C-Reactive Protein
CSF1R	Colony Stimulating factor Receptor 1
Cyp	Cytochrome p450
E-Fabp	Epidermal Fatty Acid Binding Protein
Ehhadh	Enoyl-CoA, hydratase/3- hydroxyacyl-CoA dehydrogenas
EHS	Englebreth-Holm-Swarm
Fabp	Fatty Acid Binding Protein
FT MS	Fourier Transform Tandem Mass Spectrometry
GPI	Glycosyl phosphatidylinositol
GPM	Global Protein Machine
Gst	Glutathione S-Tranferase
ICAM	Intracellular adhesion molecule
ICAT	Isotope Coded Affinity Tags
IL-6	Interluekin-6
IMP	Integral Membrane Proteins
IPG-IEF	Immobilised pH Gradient Isoelectric Focusing
i-TRAQ	Isobaric Tag for Relative and Absolute Quantification
LACS	Acyl CoA Synthase Long Chain
LC	Liquid Chromatography
LC-MS/MS	Liquid Chromatography - Tandem Mass Spectrometry
Ldlr	Low-Density Lipoprotein Receptor
Lrp	Low-Density Lipoprotein Associated Protein
Man	Mannose
Manea	Glycoprotein Endo-Alpha-1,2- Mannosidase
Mdr2	Multidrug Resistannce Proten
Mgat	Mannoside Acetylglucosaminyltransferase
Mgst	Microsomal Glutathione S-Transferase
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MudPIT	Multidimensional Protein Identification Technology
NR	Narrow Range
NSAF	Normalised Spectral Abundance Factors
NTCP	Na+/Taurocholate cotransporting polypeptide
OAT	Organic Anion Transporter
Pcca	Propionyl-CoA carboxylase α

Pccb	Propionyl-CoA carboxylase β
pepFDR	Peptide False Discovery Rate
protFDR	Protein False Discovery Rate
PTM	Post-Translational Modification
RT	Room Temperature
SAP	Serum Acute Protein
SCX	Strong Cation Exchange
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
SILAC	Stable Isotope Labelling of Amin Acids in Cell Culture
Slc	Solute Carrier
St3Gal	ST3 beta-galactoside alpha-2,3-Sialyltransferase
TM	Transmembrane
TMHMM	Transmembrane Hidden Markov Model
VDAC	Voltage Dependent Anion Channel
(v/v)	Volume/volume