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**This thesis is dedicated to my darling mama and dada**

**BIOCHEMICAL STUDIES ON ANIMAL MODELS OF  
CEROID-LIPOFUSCINOSES**

**By  
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## ABSTRACT

The ceroid-lipofuscinoses are recessively inherited lysosomal storage diseases of children and animals, characterised by brain and retinal atrophy and the accumulation of lipopigment in a variety of cells. A systematic study of isolated lipopigment from an ovine form of the disease had shown the major stored components to be proteinaceous.

This thesis presents further characterisation and identification of the stored ovine lipopigment proteins. Separation of the lipopigment proteins by LDS-PAGE showed the presence of the 3.5 kDa and 14.8 kDa proteins noted in earlier studies, and an additional band at 24 kDa. The 14.8 and 24 kDa bands varied between preparations and from different gels of the same isolate. Radioiodination of lipopigment and silver staining of the proteins separated by LDS-PAGE indicated that the 3.5 kDa protein was the dominant protein component. As these proteins were unable to be separated from each other, exploitation of the molar dominance of the 3.5 kDa protein led to its identification by a non traditional sequencing approach. The major stored protein was shown to be the full proteolipid subunit *c* of the mitochondrial ATP synthase complex. The 14.8 and 24 kDa proteins were shown to be stable oligomers of subunit *c*. Quantitation of the sequence data showed that subunit *c* accounted for at least 50% of the lipopigment mass. No other mitochondrial protein was detected. Analyses of isolated mitochondria showed that they were functionally normal and did not contain excess amounts of subunit *c*.

Subunit *c* is classified as a proteolipid, due to its lipid-like solubility in chloroform/methanol mixtures. Its storage in lysosome derived lipopigment bodies explained many of the described physical characteristics of lipopigment in the ceroid-lipofuscinoses.

Application of the same methodology showed that a bovine, and two distinct canine forms of the ceroid-lipofuscinoses were also subunit *c* storage diseases.

It is postulated that the lesions in the ceroid-lipofuscinoses involve defects in the degradative pathway of subunit *c* at some point after its incorporation into the inner mitochondrial membrane.

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## TABLE OF CONTENTS

	Page
ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iii
PUBLICATIONS.....	iv
TABLE OF CONTENTS.....	vi
LIST OF FIGURES.....	xii
LIST OF TABLES.....	xvi
ABBREVIATIONS.....	xviii
CHAPTER 1 : GENERAL INTRODUCTION.....	1
CHAPTER 2 : GENERAL MATERIALS AND METHODS.....	13
2.1 ANIMALS AND TISSUES.....	13
2.2 ISOLATION OF LIPOPIGMENT.....	13
2.3 THIN SECTION ELECTRON MICROSCOPY.....	14
2.4 AMINO ACID ANALYSIS.....	14
2.5 LITHIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS (LDS-PAGE).....	15
2.6 SILVER STAINING OF POLYACRYLAMIDE GELS.....	15
2.7 CHEMICALS.....	16
CHAPTER 3 : LDS-PAGE BEHAVIOUR AND <sup>125</sup> I RADIOLABELLING OF PANCREATIC LIPOPIGMENT PROTEINS.....	17
3.1 INTRODUCTION.....	17
3.2 SPECIAL MATERIALS AND METHODS.....	18



	Page
3.2.1 $^{125}\text{I}$ radiolabelling of pancreatic lipopigment proteins.....	18
3.2.2 Detection of the radiolabel.....	18
3.3 RESULTS.....	19
3.3.1 LDS-PAGE behaviour of lipopigment proteins.....	19
3.3.2 Incorporation of $^{125}\text{I}$ into lipopigment proteins.....	20
3.4 DISCUSSION.....	23
<b>CHAPTER 4 : IDENTIFICATION OF THE 3.5 kDa LIPOPIGMENT BAND.....</b>	<b>25</b>
4.1 INTRODUCTION.....	25
4.2 SPECIAL MATERIALS AND METHODS.....	25
4.2.1 Size exclusion high pressure liquid chromatography (HPLC).....	25
4.2.2 Amino acid sequencing.....	26
4.2.3 Repetitive yield and initial yield calculations.....	26
4.3 RESULTS.....	26
4.3.1 Size exclusion HPLC of lipopigment proteins.....	26
4.3.2 Amino acid sequencing of the molar dominant lipopigment protein.....	29
4.4 DISCUSSION.....	34

	Page
<b>CHAPTER 5 : THE CARBOXYL-TERMINAL DETERMINATION OF THE MAJOR STORED PROTEIN AND CHARACTERISATION OF THE OTHER LIPOPIGMENT PROTEINS.....</b>	<b>37</b>
5.1 INTRODUCTION.....	37
5.2 SPECIAL MATERIALS AND METHODS.....	37
5.2.1 Extraction of proteolipids from lipopigment.....	37
5.2.2 Diffusion elution from polyacrylamide gels.....	38
5.2.3 Electro blotting of lipopigment proteins.....	38
5.2.4 Cyanogen bromide (CNBr) digestion of lipopigment proteolipids.....	39
5.2.5 Mass spectroscopy analysis of CNBr digests.....	39
5.3 RESULTS.....	40
5.3.1 Characterisation of the proteolipids extracted from lipopigment.....	40
5.3.2 Identification of the 14.8 and 24 kDa lipopigment proteins.....	43
5.3.3 CNBr cleavage of lipopigment proteolipids and analysis of the digest fragments.....	44
5.4 DISCUSSION.....	46
<b>CHAPTER 6 : STUDIES ON MITOCHONDRIA ISOLATED FROM CONTROL AND AFFECTED SHEEP.....</b>	<b>49</b>
6.1 INTRODUCTION.....	49

	Page
<b>6.2 SPECIAL MATERIALS AND METHODS.....</b>	<b>49</b>
<b>6.2.1 Isolation of mitochondria and Inner mitochondrial           membrane vesicles from affected and control sheep.....</b>	<b>49</b>
<b>6.2.2 Negative staining electron microscopy.....</b>	<b>50</b>
<b>6.2.3 Respiratory measurements.....</b>	<b>50</b>
<b>6.3 RESULTS.....</b>	<b>51</b>
<b>6.3.1 Electron microscopy and LDS-PAGE of lipopigment and           mitochondrial preparations.....</b>	<b>51</b>
<b>6.3.2 Respiratory measurements on Isolated mitochondrial           fractions.....</b>	<b>53</b>
<b>6.4 DISCUSSION.....</b>	<b>56</b>
 <b>CHAPTER 7 : CELL CULTURE OF OVINE KIDNEY EPITHELIAL           CELLS.....</b>	 <b>58</b>
<b>7.1 INTRODUCTION.....</b>	<b>58</b>
<b>7.2 SPECIAL MATERIALS AND METHODS.....</b>	<b>59</b>
<b>7.2.1 Composition of growth and maintenance media.....</b>	<b>59</b>
<b>7.2.2 Preparation of affected and control kidney epithelial           cells for primary cell culture.....</b>	<b>59</b>
<b>7.2.3 Growth and maintenance of cell cultures.....</b>	<b>59</b>
<b>7.2.4 Preparation of cells for light and thin section           electron microscopy.....</b>	<b>60</b>
<b>7.2.5 Radiolabelling of cultured cells.....</b>	<b>60</b>
<b>7.3 RESULTS.....</b>	<b>62</b>

	<b>Page</b>
<b>7.3.1 Morphology and growth characteristics of cultured kidney cells.....</b>	<b>62</b>
<b>7.3.2 Measuring the synthesis of subunit c In cultured kidney cells.....</b>	<b>65</b>
<b>7.4 DISCUSSION.....</b>	<b>68</b>
 <b>CHAPTER 8 : ISOLATION AND CHARACTERISATION OF LIPOPIGMENT FROM A CASE OF BOVINE CEROID-LIPOFUSCINOSIS.....</b>	 <b>70</b>
<b>8.1 INTRODUCTION.....</b>	<b>70</b>
<b>8.2 SPECIAL MATERIALS AND METHODS.....</b>	<b>70</b>
<b>8.2.1 Bovine tissue.....</b>	<b>70</b>
<b>8.2.2 Isolation of bovine lipopigment bodies.....</b>	<b>70</b>
<b>8.2.3 Cyanogen bromide digestion of isolated lipopigment.....</b>	<b>71</b>
<b>8.3 RESULTS.....</b>	<b>72</b>
<b>8.3.1 Characteristics of bovine lipopigments.....</b>	<b>72</b>
<b>8.3.2 Amino acid composition and LDS-PAGE of bovine lipopigment proteins.....</b>	<b>74</b>
<b>8.3.3 Amino acid sequencing of bovine lipopigment.....</b>	<b>77</b>
<b>8.3.4 Mass spectral analysis of CNBr digest of bovine lipopigment.....</b>	<b>78</b>
<b>8.4 DISCUSSION.....</b>	<b>79</b>
 <b>CHAPTER 9 : CANINE CEROID-LIPOFUSCINOSIS.....</b>	 <b>80</b>
<b>9.1 INTRODUCTION.....</b>	<b>80</b>

	<b>Page</b>
<b>9.2 SPECIAL MATERIALS AND METHODS.....</b>	<b>80</b>
<b>9.2.1 Canine tissues.....</b>	<b>80</b>
<b>9.2.2 Isolation of canine lipopigment bodies.....</b>	<b>81</b>
<b>9.3 RESULTS.....</b>	<b>81</b>
<b>9.3.1 Characteristics of canine lipopigment.....</b>	<b>81</b>
<b>9.3.2 Amino acid sequencing of canine lipopigment.....</b>	<b>82</b>
<b>9.4 DISCUSSION.....</b>	<b>83</b>
<b>CHAPTER 10 : GENERAL DISCUSSION.....</b>	<b>84</b>
<b>REFERENCES .....</b>	<b>95</b>

## LIST OF FIGURES

Figure		Page
3.1 A,B	LDS-20% PAGE of pancreatic lipopigment protein from the same isolate run on different gels (A) and lipopigment proteins separated by LDS-20% PAGE in the presence and absence of 2-mercaptoethanol (B).....	19
3.2 A,B	Incorporation of $^{125}\text{I}$ at various iodogen concentrations (A) and at various times (B).....	20
3.3	LDS-20% PAGE of $^{125}\text{I}$ labelled pancreatic lipopigment proteins.....	21
4.1 A,B & C	HPLC profile of lipopigment proteins eluted from a TSK G2000 SW column and a TSK G2000 SW and G4000 SW column connected in series (A & B). LDS-20% PAGE of fractions eluted from the columns connected in series (C).....	27
4.2	LDS-20% PAGE of lipopigment proteins after acetone precipitation and size exclusion HPLC.....	28
4.3	Cycles 1, 2, 3 and 10, 11 and 12 obtained when pancreatic lipopigment protein was sequenced.....	29
4.4	The PTH amino acid yields of the major sequence obtained from pancreas (A), brain (B) and kidney (C) lipopigment protein.....	31
5.1	LDS-20% PAGE of total pancreatic lipopigment proteins (A) nonextractable proteins (B) and extracted proteolipid (C).....	41

<b>Figure</b>	<b>Page</b>
5.2	LDS-15% PAGE of pancreatic lipopigment proteins that were diffusion eluted from an LDS-15% polyacrylamide gel.....43
5.3	Coomassie blue stained pancreatic lipopigment proteolipids electro blotted onto a PVDF membrane.....44
5.4	Linear mass spectral scan over the molecular ion region of the carboxyl-terminal CNBr cleavage fragment of subunit c extracted from pancreatic lipopigment.....45
5.5	Amino acid sequence of the major stored lipopigment protein.....46
6.1	Thin section electron micrographs of pancreatic lipopigment and isolated mitochondrial preparations (A & B) and negatively stained lipopigment and inner mitochondrial membrane vesicles (C & D).....51
6.2	LDS-PAGE of inner mitochondrial membrane vesicle proteins from control and affected sheep livers (A) and PAGE of bovine ATP synthase (B).....52
6.3	Respiratory activity of isolated mitochondrial preparations from affected (A) and control (B) kidney tissue.....53
7.1	Thin section electron micrographs of affected kidney epithelial cells in primary culture (A) and in third passage culture (B).....62
7.2	The growth of affected (A), and control (B), kidney epithelial primary cultures.....63

<b>Figure</b>	<b>Page</b>
7.3	Light microscopic appearance of affected (A) and control (B) primary cultured cells stained with H&E and luxol fast blue.....63
7.4	Thin section electron micrographs of membrane bound cytoplasmic bodies from affected cultured cells (A & B) and autophagic structures from control cells (C & D).....64
7.5	% of <sup>3</sup> H Incorporated Into TCA precipitable protein from labelled affected (A) and control (B) primary kidney cell cultures.....65
7.6	<sup>3</sup> H distribution amongst the proteolipid fractions from labelled affected and control cultured cells separated by LDS-15% PAGE.....67
8.1	Thin section electron micrographs of lipopigment Isolated from bovine pancreas, liver, kidney and brain (A, B, C & D).....72
8.2	Isolated bovine lipopigments on CsCl isopycnic gradients.....74
8.3	LDS-15% PAGE of lipopigment protein isolated from ovine pancreas (A) and bovine pancreas, kidney and liver (B,C & D)..76
8.4	The PTH amino acid yields of the first 25 residues of subunit c sequenced from bovine pancreatic lipopigment....77
8.5	Linear mass spectral scan over the molecular ion region of the carboxyl-terminal CNBr cleavage fragment of subunit c isolated from bovine pancreatic lipopigment.....78
9.1	Thin section electron micrographs of lipopigment Isolated from frozen Border Collie brain (A) and Tibetan Terrier brain (B & C).....81



Figure	Page
9.2	<b>The PTH amino acid yields of the first 16 amino-terminal residues of subunit <i>c</i> sequenced from the Border Collie (A), and Tibetan Terrier (B), brain lipopigment.....82</b>

## LIST OF TABLES

Table		Page
3.1	The distribution of $^{125}\text{I}$ amongst radiolabelled pancreatic lipopigment proteins.....	22
4.1	Estimates of the contribution of the sequenced peptide to the total lipopigment protein masses.....	32
4.2	The amino acid composition, in moles % of total lipopigment protein and the full subunit <i>c</i> of mitochondrial ATP synthase...	33
5.1	The proportion of protein recovered as proteolipid by ether precipitation of chloroform/methanol/ammonium acetate solubilised lipopigment.....	40
5.2	The amino acid composition of the nonextracted lipopigment protein and the chloroform/methanol/ammonium acetate extracted proteolipid compared with the full subunit <i>c</i> of mitochondrial ATP synthase.....	42
6.1	Respiratory control ratios from control and affected kidney mitochondrial preparations using succinate and glutamate as respiratory substrates.....	54
6.2	ADP/O ratios from control and affected kidney mitochondrial preparations using succinate and glutamate as respiratory substrates.....	55
7.1	Specific activities of the proteolipid fractions extracted from affected and control cell cultures.....	66

<b>Table</b>		<b>Page</b>
<b>8.1</b>	<b>Characteristics of lipopigment isolated from bovine tissues.....</b>	<b>73</b>
<b>8.2</b>	<b>The amino acid composition of bovine lipopigments compared with the full bovine subunit <i>c</i> of mitochondrial ATP synthase.....</b>	<b>75</b>
<b>10.1</b>	<b>Ovine pancreatic lipopigment composition.....</b>	<b>86</b>

**COMMON ABBREVIATIONS USED**

ACR	Acceptor control ratio
ADP	Adenosine 5'-phosphate
ATP	Adenosine 5'-triphosphate
ATV	Antibiotic-trypsin-versene
Da	Dalton
DCCD	Dicyclohexylcarbodiimide
EDTA	Ethylenediaminetetra-acetate
FBS	Foetal bovine serum
H&E	Haematoxylin and eosin
HPLC	High pressure liquid chromatography
kDa	kilo Dalton
LDS	Lithium dodecyl sulphate
LDS-PAGE	Lithium dodecyl sulphate polyacrylamide gel electrophoresis
MEM	Minimum essential medium
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PSK	Penicillin, streptomycin and kanamycin
PTH	Phenylthiohydantoin
PVDF	Polyvinylidene difluoride
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
TCA	Trichloroacetic acid
TLC	Thin layer chromatography