This thesis is dedicated to my darling mama and dada

BIOCHEMICAL STUDIES ON ANIMAL MODELS OF CEROID-LIPOFUSCINOSES

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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*. Quantitaion 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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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