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Studies on Proteins Involved in Retinoid and Alcohol Metabolism

A thesis presented in partial fulfilment of the requirements for the
degree of Doctor of Philosophy in Biochemistry at Massey
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Abstract

The primary biological role of the aldehyde dehydrogenase enzymes has long been a contentious issue. It was initially thought that the main function of these enzymes could be acetaldehyde metabolism; however, it seems unlikely that a large family of proteins evolved for this purpose. It has been suggested that an important function of aldehyde dehydrogenase enzymes may be in the metabolism of the vitamin A derivative, retinal. This thesis describes an investigation into the ability of human and sheep cytosolic aldehyde dehydrogenases to oxidise all-*trans* retinal, 9-*cis* retinal and CRBP-bound retinal under physiologically relevant conditions. A fluorescence-based assay following the production of NADH was employed, allowing the accurate measurement of low K_m data.

Firstly the ability of ALDH 1 to oxidise its putative biological ligands, free all-*trans* and 9-*cis* retinal, was demonstrated. It has been proposed that retinoids occur naturally as a 1:1 complex with the lipocalins cellular retinol binding protein (CRBP) and cellular retinoic acid binding protein (CRABP). If the sheep and human class 1 enzymes play a role in retinoid metabolism *in vivo*, it is likely that they will accept CRBP-bound retinal as a substrate. To investigate this possibility, recombinant CRBP was produced using an *E.coli* expression system. Using a spectrophotometric method, the purified recombinant CRBP was shown to bind all-*trans* but not 9-*cis* retinal, and using the same fluorescence-based assay as mentioned above, it was shown that both sheep and human ALDH 1 could accept CRBP-bound retinal as a substrate at physiologically relevant levels. *In vivo* studies into retinal oxidation were initiated using the retinoid-responsive human neuroblastoma cell-line SH-SY5Y. It was shown that ALDH 1 was expressed in this cell line by Western blotting, and that the cells were responsive to retinal in addition to retinoic acid, indicating that retinal was being converted to retinoic acid.

In addition, a novel, putative alcohol dehydrogenase was isolated, purified and partially characterised. The protein was purified using the techniques of subcellular fractionation by centrifugation, PEG precipitation, ion-exchange chromatography, preparative isoelectric focusing, hydrophobic interaction chromatography and gel purification. Elucidated characteristics of this protein include: subunit molecular weight 42-45 kDa, native molecular weight 42-45 kDa, isoelectric point 8.3-8.5, and activity with ethanol and other longer chain alcohols, but not with glucose, sorbitol or methanol. The protein

was blocked at the N-terminus, and cleavage and internal sequencing attempts yielded some sequence information. However, this information did not appear to match closely with any known protein sequence when submitted to a protein database, suggesting that the protein is novel.

From all available information, we propose that in sheep and humans, the enzyme responsible for retinal oxidation is the major cytosolic class 1 aldehyde dehydrogenase, as opposed to the situation in rats and mice, where specific retinal-oxidising aldehyde dehydrogenases exist and the major class 1 enzymes play a more important role in acetaldehyde metabolism.

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List of Abbreviations

A ₂₈₀	Absorbance at 280 nm
ALDH	Aldehyde dehydrogenase
ADH	Alcohol dehydrogenase
AMP	Ampicillin
1,8-ANS	1-Anilinonaphthalene 8-sulfonic acid
ARAT	AcylCoA-retinol acyltransferase
Bis-Tris	Bis[2-hydroxyethyl]iminotris[hydroxymethyl]methane
BSA	Bovine serum albumin
CAPS	3-[Cyclohexylamino]-1-propane sulfonic acid
cDNA	Complementary DNA
CNBr	Cyanogen Bromide
CRABP	Cellular retinoic acid-binding protein
CRBP	Cellular retinol-binding protein
C-terminal	Carboxy-terminal
DEAE	Diethylaminoethyl
DMF	Dimethylformamide
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DNA-BD	DNA-binding domain
DR	Direct repeat
DTT	Dithiothreitol
EDTA	Ethylenediamine tetra-acetic acid
FAE	Fetal alcohol effects
FAS	Fetal alcohol syndrome
FPLC	Fast protein liquid chromatography
HEPES	N-2-hydroxyethylpiperazine-N'-ethanesulphonic acid
HPLC	High performance liquid chromatography
IEF	Isoelectric focusing
iLBP	Intracellular lipid binding protein family
IPTG	Isopropyl- γ -D-thiogalactopyranoside
kDa	kiloDalton
K _m	Substrate concentration at half maximum reaction rate
LB	Luria Broth

LBD.....	Ligand-binding domain
LRAT	Lecithin-retinol acyltransferase
λ_{max}	Wavelength of maximum absorbance
mRNA	Messenger RNA
NAD ⁺	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced form)
N-terminal.....	Amino-terminal
PBS	Phosphate buffered saline
PEG.....	Polyethylene glycol
pI.....	Isoelectric point
PMSF	Phenylmethanesulphonyl fluoride
PPAR.....	Peroxisome proliferator-activated receptor
PVDF	Polyvinylidene difluoride
RA.....	Retinoic acid
RALDH (or RalDH)	Retinal-specific aldehyde dehydrogenase
RAR	Retinoic acid receptor
RARE	Retinoic acid response element
RBP.....	Retinol-binding protein
RoDH	Retinol-specific alcohol dehydrogenase
RXR	Retinoid X receptor
RXRE	Retinoid X response element
[S]	Substrate concentration
SDR.....	Short-chain dehydrogenase-reductase family
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
TR	Thyroid hormone receptor
Tris	Tris(hydroxymethyl)aminoethane
UV-vis	Ultraviolet-visible
VDR.....	Vitamin D receptor
V	Rate of reaction
V_{max}	Maximum rate of reaction
v/v	volume/volume
w/v	weight/volume
w/w.....	weight/weight

Amino Acid Abbreviations

Amino Acid	Abbreviation	One-letter Abbreviation
Alanine	Ala	A
Asparagine or aspartic acid	Asx	B
Cysteine	Cys	C
Aspartic acid	Asp	D
Glutamic acid	Glu	E
Phenylalanine	Phe	F
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Lysine	Lys	K
Leucine	Leu	L
Methionine	Met	M
Asparagine	Asn	N
Proline	Pro	P
Glutamine	Gln	Q
Arginine	Arg	R
Serine	Ser	S
Threonine	Thr	T
Valine	Val	V
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Glutamine or glutamic acid	Glx	Z

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