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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 University

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Department of Biochemistry
December 1997
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 AlDH1 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 AlDH1 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 AlDH1 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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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>A&lt;sub&gt;280&lt;/sub&gt;</td>
<td>Absorbance at 280 nm</td>
</tr>
<tr>
<td>ALDH</td>
<td>Aldehyde dehydrogenase</td>
</tr>
<tr>
<td>ADH</td>
<td>Alcohol dehydrogenase</td>
</tr>
<tr>
<td>AMP</td>
<td>Ampicillin</td>
</tr>
<tr>
<td>1,8-ANS</td>
<td>1-Anilinonaphthalene 8-sulfonic acid</td>
</tr>
<tr>
<td>ARAT</td>
<td>AcylCoA-retinol acyltransferase</td>
</tr>
<tr>
<td>Bis-Tris</td>
<td>Bis[2-hydroxyethyl]iminotris[hydroxymethyl]methane</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>CAPS</td>
<td>3-[Cyclohexylamino]-1-propane sulfonic acid</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>CNBr</td>
<td>Cyanogen Bromide</td>
</tr>
<tr>
<td>CRABP</td>
<td>Cellular retinoic acid-binding protein</td>
</tr>
<tr>
<td>CRBP</td>
<td>Cellular retinol-binding protein</td>
</tr>
<tr>
<td>C-terminal</td>
<td>Carboxy-terminal</td>
</tr>
<tr>
<td>DEAE</td>
<td>Diethylaminoethyl</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
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<td>Dimethyl sulphoxide</td>
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<td>Deoxyribonucleic acid</td>
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<tr>
<td>DNA-BD</td>
<td>DNA-binding domain</td>
</tr>
<tr>
<td>DR</td>
<td>Direct repeat</td>
</tr>
<tr>
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<td>Dithiothreitol</td>
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<td>EDTA</td>
<td>Ethylenediamine tetra-acetic acid</td>
</tr>
<tr>
<td>FAE</td>
<td>Fetal alcohol effects</td>
</tr>
<tr>
<td>FAS</td>
<td>Fetal alcohol syndrome</td>
</tr>
<tr>
<td>FPLC</td>
<td>Fast protein liquid chromatography</td>
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<td>HEPES</td>
<td>N-2-hydroxyethylpiperazine-N'-ethanesulphonic acid</td>
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<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>IEF</td>
<td>Isoelectric focusing</td>
</tr>
<tr>
<td>iLBP</td>
<td>Intracellular lipid binding protein family</td>
</tr>
<tr>
<td>IPTG</td>
<td>Isopropyl-γ-D-thiogalactopyranoside</td>
</tr>
<tr>
<td>kDa</td>
<td>kiloDalton</td>
</tr>
<tr>
<td>K&lt;sub&gt;m&lt;/sub&gt;</td>
<td>Substrate concentration at half maximum reaction rate</td>
</tr>
<tr>
<td>LB</td>
<td>Luria Broth</td>
</tr>
</tbody>
</table>
LBD ......................... Ligand-binding domain
LRAT ........................ Lecithin-retinol acyltransferase
λmax .......................... 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 .......................... Phenylmethylsulphonyl fluoride
PPAR ........................... Peroxisome proliferator-activated receptor
PVDF .......................... Polyvinylidifluoride
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
Vmax ............................ Maximum rate of reaction
v/v ............................. volume/volume
w/v ............................. weight/volume
w/w ............................. weight/weight
# Amino Acid Abbreviations

<table>
<thead>
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<th>Amino Acid</th>
<th>Abbreviation</th>
<th>One-letter Abbreviation</th>
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<td>Alanine</td>
<td>Ala</td>
<td>A</td>
</tr>
<tr>
<td>Asparagine or aspartic acid</td>
<td>Asx</td>
<td>B</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Cys</td>
<td>C</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>Asp</td>
<td>D</td>
</tr>
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<td>Tryptophan</td>
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<td>Glutamine or glutamic acid</td>
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