STRUCTURAL AND FUNCTIONAL STUDIES ON HUMAN OXYGENASES

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..all the praises be to the Almighty, the Cherisher and Sustainer of the worlds.

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Abstract

The work described in this thesis focused on 2-oxoacid-dependent oxygenase enzymes. Most of the work focused on iron- and 2-oxoglutarate (2OG)-dependent oxygenases including JMJD6 and JMJD5, which are human enzymes of unassigned or controversial functions. Work was carried out also on human 4-hydroxyphenylpyruvate dioxygenase (HPPD), which is a structurally distinct 2-oxoacid oxygenase involved in tyrosine catabolism. The work involved protein purification, biophysical analysis, substrate screening, and inhibitor profiling. Chapter 1 gives an introduction to 2OG oxygenases summarizing their functions, structures, and mechanisms.

Chapters 2-6 describe functional assignment, structural, and inhibition studies on JMJD6, which has been previously reported as phosphatidylserine receptor (PTDSR), as an *N*-methyl arginine demethylase, and as a lysyl-hydroxylase. The overall results using peptide as substrate support the assignment of JMJD6 as lysyl C-5 hydroxylase. Crystallographic studies reveal JMJD6 to be more similar to the JmjC hydroxylases than demethylases. JMJD6 was found to act on sequences of substrates including splicing regulatory (SR) proteins, such as U2AF65 (U2 small nuclear ribonucleoprotein auxiliary factor 65 kDa subunit), LUC7L2 (putative RNA binding protein luc 7-like 2), and CROP (cisplatin resistance-associated overexpressed protein). It also acted on sequences of substrates including p53, estrogen receptor α (ERα), and von Hippel-Lindau (VHL). There was no evidence for JMJD6-catalysed demethylation activity either on *N*-methyl arginine- or *N*-methyl lysine-residues. Studies on the non-catalytic domain of JMJD6 reveal a role of the polyserine domain in regulating catalytic activity. Structural and kinetic studies reveal how JMJD6 is inhibited by the structural analogues of 2OG.

Chapters 7-8 describe crystallographic and other studies on JMJD5, which has been assigned as an arginine residue C-3 hydroxylase, and *N*-methyl lysine demethylase. The results support the assignment of JMJD5 as a hydroxylase rather than demethylase and will enable the development of selective JMJD5 inhibitors.

Chapter 9 describes studies on human HPPD from crystallographic and inhibition perspectives. The results highlight common features at the active sites of the two classes of human 2-oxoacid oxygenases.

Overall, the results inform on the functions of JMJD6 and JMJD5 as hydroxylases and will enable the development of selective inhibitors of them for use in target validation and the assignment of their biological roles.

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Author Contributions

All the experiments described in this thesis were carried out by me unless otherwise stated. At the start of each section, a description of contributions from collaborators is summarized (where applicable) and in addition, their names are included in parentheses in the main text anywhere their experiments are described.

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Abbreviations

2,4-PDCA	Pyridine-2,4-dicarboxylic acid
2OG	2-Oxoglutaric acid
2TY	2 x Tryptone/yeast extract
3D	Three-dimensional
ABHs	AlkB homologues
ACCO	1-Aminocyclopropane-1-carboxylate oxidase
Acinus S’	Apoptotic chromatin condensation inducer in the nucleus
AGC	Automatic gain control
AlkB	Alkylation Repair Protein B
ALPHA	Amplified luminescent proximity homogenous assay
APS	Ammonium persulphate
ARD	Ankyrin repeat domain
ASU	Asymmetric unit
AT	Adenine-thymine
BBOX	γ-butyrobetaine hydroxylase
Bio	Biotin
BSA	Bovine serum albumin
CAS	Clavaminic acid synthase
CAD	*C*-terminal transcriptional activation domain
CD	Circular dichroism
CHCA	α-cyano-4-hydroxycinnamic acid
CID	Collision-induced dissociation
CODD	*C*-terminal oxygen-dependent degradation domain
CPMG	Carr-Purcell-Meiboom-Gill
CROP	Cisplatin resistance-associated overexpressed protein
CSS	Complex formation significance score
C-terminus	Carboxy terminus
CV	Column volume
Da	Dalton
DACS	Deacetylcephalosporin C synthase
DAOCS	Deacetoxycephalosporin C synthase
DIC	Di-isopropylcarbodiimide
DLS	Diamond Light Source

DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide
DSBH	Double-stranded β-helix
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
EFE	Ethylene forming enzyme
EPO	Erythropoetin
EPR	Electron paramagnetic resonance
ERα	Estrogen receptor α
ESI-MS	Electrospray ionisation-mass spectrometry
ETD	Electron transfer dissociation
FAA	Fumarylacetoacetase
FBXL	Fbox, Leucine-rich repeat domain
FDA	Food and drug administration
FL	Full-length
FMD	Foot and mouth disease
Fmoc	9-Fluorenylmethyloxycarbonyl
FNS	Flavon synthase 1
FPLC	Fast protein liquid chromatography
FRET	Fluorescence resonance energy transfer
FTO	Fat, mass, and obesity associated protein
H1	Histone H1
H2A	Histone H2A
H2B	Histone H2B
H3	Histone H3
H4	Histone H4
HEK	Human embryonic kidney
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
HGD	Homogentisate 1,2-dioxygenase
HIF	Hypoxia inducible factor
His_6-tag	Hexa-histidine tag
HMGA	2,5-Dihydroxyphenylacetic acid or homogentisic acid
HOBt	1-Hydroxybenzotriazole

HPLC	High performance liquid chromatography
HPPA	4-Hydroxyphenylpyruvic acid
HPPD	4-Hydroxyphenylpyruvate dioxygenase
HSP70	Heat-shock protein 70 kDa
IDH	Isocitrate dehydrogenase
IMAC	Immobilized metal ion affinity chromatography
IOX1	5-Carboxy-8-hydroxyquinoline
IP	Immunoprecipitation
IPTG	Isopropyl β-D-1-thiogalactopyranoside
ITC	Isothermal titration calorimetry
JARID	Jumonji, AT rich interactive domain
JmjC	Jumonji-C
JMJD	Jumonji-C domain containing protein
K_D	Binding constant
K_m	Michaelis-Menten constant
KDM	Histone lysine demethylase
LC-MS	Liquid chromatography-mass spectrometry
LSD	Lysine specific demethylase
LUC7L2	Putative RNA binding protein luc 7-like 2
MAA	Maleylacetoacetate isomerase
MALDI	Matrix-assisted laser desorption/ionisation
Me	Methyl
MINA53	MYC-induced nuclear antigen 53
MS	Mass Spectrometry
NFATc1	Nuclear factor of activated T-cells calcineurin-dependent 1
NIH	National institute of health
Ni-TED	Ni-tris-carboxymethyl ethylene diamine
NLSs	Nuclear localisation sequences
NMR	Nuclear magnetic resonance
NODD	*N*-terminal oxygen-dependent degradation domain
NOG	N-Oxalylglycine
NO66	Nucleolar protein 66
NTBC	2-[2-Nitro-4-(trifluoromethyl)benzoyl]-1,3-cyclohexane dione
N-terminal	Amino terminal
OGFOD1	Human uS12 prolyl 3-hydroxylase

PAHX	Phytanoyl-CoA-hydroxylase
PAX3	Paired Box3
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PDB	Protein data bank
PHD	Plant homeodomain
PHD1-3	Prolyl hydroxylase 1-3
PHF	PHD finger protein
PISA	Protein interfaces surfaces and assemblies
PLOD	Procollagen lysine hydroxylase
ppm	Parts per million
PROJECT	Periodic refocusing of J evolution by coherence transfer
PSR/PTDSR	Phosphatidylserine receptor
PTM	Post-translational modification
PUF60	Poly(U)-binding-splicing factor 60
RCCD1	Regulator of chromosome condensation domain-containing protein 1
RHA	RNA helicase A
ROX	Ribosomal oxygenase
RPS6	40S ribosomal protein S6
RPS23	Ribosomal protein S23
RMSD	Root mean square deviation
RNA	Ribonucleic acid
RPM	Revolution per minute
RS	Arginine-Serine
SAR	Structure-activity-relationship
SAXS	Small angle X-ray scattering
SD	Standard deviation
SDM	Site-directed mutagenesis
SDS-PAGE	Sodium dodecylsulphate-polyacrylamide gel electrophoresis
SEC	Size-exclusion chromatography
SEC-MALLS	Size-exclusion chromatography-multiangle laser light scattering
SGC	Structural genomic consortium
SPR	Surface plasmon resonance
SPPS	Solid phase peptide synthesis
SR	Splicing regulatory

SRSF11	Serine/arginine-rich splicing factor 11
TAE	Tris-acetate-EDTA
TAT	Tyrosine aminotransferase
TauD	Taurine dioxygenase
TB	Terrific broth
TEM	Transmission electron microscopy
TEMED	*N,N,N',N'*-Tetramethylethylenediamine
TET	Ten-eleven translocation
TEV	Tobacco etch virus
TFA	Trifluoroacetic acid
TfdA	2,4-Dichlorophenoxyacetate/2OG oxygenase
TIPS	Tri-isopropyl silane
TMABA	Trimethylaminobutyraldehyde
TMLH	N^{ε}-trimethyllysine hydroxylase
TOF	Time-of-flight
TPR	Tetratricopeptide repeat
TRAF6	TNF receptor associated factor 6
Tris	*Tris*-(hydroxylmethyl)aminomethane
TYW5	tRNA wybutosine-synthesizing protein
U2AF65	U2 small nuclear ribonucleoprotein auxiliary protein 65 kDa subunit
UV	Ultra violet
v/v	Volume for volume
VEGF	Vascular endothelial growth factor
VHL	Von Hippel-Lindau
WH	Winged-helix
w/v	Weight for volume
wt	Wild-type