Investigation into Human Galectin-1
Structure and Function

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Statement of Originality

The work presented in this thesis was undertaken in the Institute for Glycomics at Griffith University (Gold Coast campus, Australia). The work was conducted between 2005 and 2009 under the supervision of Dr. Helen Blanchard and has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

Signed:

Stacy A. Scott
September 2009

Dr. Helen Blanchard
September 2009
Thesis Contributions

Approximately 95% of the work reported in thesis was performed directly by myself. Data supplied by others was conducted in collaboration.

Sections of the thesis where others contributed data:

Chapter 3: The testing of oxidized human galectin-1 activity on human leukemia MOLT-4 cells was conducted in collaboration with Emily Sullivan, a research student from Dr. Steve Ralph’s research group (Griffith University, Australia). I prepared the protein and planned the cell culture experimental design. Emily cultured and treated the MOLT-4 cells, stained the cells for analysis and recorded FACS measurements.

Chapter 3 and 6: The crystallisation trials that used Peak-3/Buffer_B (17 kDa) oxidized human galectin-1 in the absence and presence of farnesyl diphosphate were planned by myself, but set up with the help of Tatjana Seidens and Marie Destro, two visiting students to the Blanchard group, Institute for Glycomics.

Chapter 4: X-ray diffraction data on some galectin-1 crystals was collected by Xing Yu, a Ph.D student of Dr. Helen Blanchard’s research group, at the Stanford Synchrotron Radiation Laboratory (SSRL) in California, USA. All other synchrotron data on galectin-1 crystals was collected by myself at the Advanced Light Source (ALS) synchrotron.

Chapter 6: The STD NMR data of Peak-3/Buffer_B (17 kDa) oxidized human galectin-1 binding to farnesyl diphosphate was also provided by Xing Yu.

Editorial assistance was provided by Dr. Helen Blanchard, Dr. Steve Ralph and Dr. Darren Grice.
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Thank you to Emily Sullivan, and student’s of Helen’s group; Tatjana Seidens, Marie Destro and Xing Yu for providing some of the data presented in this thesis.

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Research Publications

Publications relevant to this thesis:


2) Stacy A. Scott, Andrea Bugarcic and Helen Blanchard, Characterisation of oxidized recombinant human galectin-1 (accepted for publication in *Protein and Peptide Letters*, 2009, 16(10). Impact factor 1.281.


4) Stacy A. Scott, Steve J. Ralph, Helen Blanchard, Evidence for a sulphenic acid intermediate between reduced and oxidized forms of human galectin-1 (Manuscript in preparation, to be submitted 2009).

Other publications:


Conference oral presentations

Stacy Scott. Investigation into Human Galectin-1 Function and Structure. East Coast Protein Meeting (Coffs Habour, Australia, 2007).

Conference poster presentations


Abstract

Human galectin-1 is a lectin protein that is ubiquitously expressed by most normal adult tissues, and is also over-expressed by many human cancers. When in a reducing environment, human galectin-1 exhibits specific binding affinity for β-galactosides and exists in a non-covalently bound homodimer conformation comprised of two 14.5 kDa subunits. Dimeric human galectin-1 exhibits cross-linking lectin activity because one carbohydrate-binding site per subunit is located at each end of the dimer. Mediated by cross-linking lectin activity, extracellular human galectin-1 is able to cluster specific glycoconjugate receptors on the T cell surface to initiate apoptosis. The apoptotic affects of extracellular galectin-1 upon T cells suggests that secretion of galectin-1 into the tumour stroma indirectly contributes to tumour survival and growth by essentially creating a “shield” from immune surveillance. Human galectin-1 cross-linking lectin activity also promotes cell migration and adhesion, two critical processes in angiogenesis and the invasion of metastatic cancer cells. Consequently, the production of β-galactoside derivatives as a means of specific inhibition of galectin-1 has become a focus in today’s fight against cancer.

For human galectin-1 to maintain cross-linking lectin activity, six cysteine residues within each subunit of the homodimer must be kept from oxidizing to form inter- and/or intramolecular disulphide bonds. Considering most cancers are associated with oxidative stress, it is intriguing to contemplate whether an oxidized form of human galectin-1 functions within a cancerous environment. An oxidized monomeric form of human galectin-1 (14.5 kDa) is known to interact with the cell surface of macrophages and stimulate their release of an axonal regeneration factor(s), however, the role oxidized human galectin-1 may play in tumourigenesis is currently unsubstantiated. Attempts to generate the oxidized monomeric form for further study during this Ph.D resulted in the generation of two forms of oxidized human galectin-1, an oligomer 68 kDa in size and a smaller protein species of apparent molecular weight 17 kDa. Extensive characterisation of these two previously unreported forms determined that the 68 kDa form contains inter- and intramolecular disulphide bonds, whereas the 17 kDa form contains only intramolecular disulphide bonds. Both forms were devoid of lectin activity as anticipated, but did exhibit an ability to protect a leukaemia cell line from hydrogen peroxide induced...
apoptosis. Protection from oxidative assault was not mediated via hydrogen peroxide consumption, rather, protection is suspected to be mediated via interaction with a receptor on the cancer cell surface. Attempts to obtain a crystal structure of the 17 kDa oxidized form, both apo and complexed with farnesyl diphosphate (the first reported ligand for oxidized human galectin-1) are ongoing, but given the observed increase in random coil structure within the 17 kDa oxidized form, crystallisation may never occur.

The 14.5 kDa form of oxidized human galectin-1 that exhibits the axonal regeneration activity is thought to be sourced from the extracellular oxidation of reduced dimeric human galectin-1 that is secreted from injured axons. This suggestion supports the existence of an intermediate state between the fully reduced and oxidized state of human galectin-1. Two X-ray crystallographic structures of homodimeric human galectin-1, determined from crystals grown in the presence of lactose (a ligand) during this Ph.D, exhibit oxidized features, and so may provide structural evidence for a putative intermediate state. Specifically, the putative intermediate structure within the asymmetric unit of both crystal structures is a homodimer that has one 14.5 kDa subunit not bound to lactose. Essentially, the homodimer has become partially in-active. Besides the loss of lectin activity, the “lactose-absent” subunit of the homodimer also exhibits loss of secondary structure to random coil, larger size, increased flexibility and weaker stabilizing salt bridge interactions between residues crucial to binding lactose. The loss of lectin activity and loss of secondary structure to random coil are characteristic features of oxidized human galectin-1, but the lactose-absent subunit does not contain disulphide bonds. Excessive crystal contacts, or a lack thereof, does not on its own provide an unequivocal explanation for these unique features either. An alternative finding that the lactose-absent subunit has lost all reducing agent molecules bound to cysteine residues, and exhibits a specific pattern of sulphenic acid formation (hydroxylated cysteines), is suspected to induce these features.

Sulphenic acid formation cannot be ignored as an anomaly within human galectin-1 crystal structures because human galectin-1 is a redox-reactive protein, and sulphenic acid is a physiologically relevant residue for many other redox-reactive proteins. To test the concept of an intermediate state of human galectin-1 that incorporates sulphenic acid, the buffers used to oxidize dimeric human galectin-1 to the 68 kDa and 17 kDa oxidized forms were supplemented with dimedone, a specific probe for sulphenic acid. The generation of
both oxidized protein species was disrupted because correct disulphide formation was inhibited in the presence of dimedone. Correct disulphide bond formation most likely did not occur because dimedone had trapped the protein in an intermediate state that incorporates sulphenic acid residues. Sulphenic acid formation is typically associated with hydrogen peroxide scavenging proteins, such as peroxiredoxins, and this thesis details the first report providing evidence that dimeric human galectin-1 also possesses the ability to consume hydrogen peroxide. Additionally, this thesis shows that human galectin-1 lectin activity is adversely affected prior to inducement of disulphide bridge formation when in the presence of mild concentrations of hydrogen peroxide, which is consistent with the lactose-absent subunit crystal structure phenomenon.

The data presented in this thesis, for the most part, provides evidence for the activity reduced and oxidized human galectin-1 exhibits in oxidizing environments. The reduced form of human galectin-1 has hydrogen peroxide scavenging activity, and the oxidized form stimulates cancer cells into survival mode via a cell surface interaction. The evidence presented in this thesis recommends future investigations test whether these affects are physiologically relevant in the context of solid tumour cancer. Hydrogen peroxide scavenging within tumours may be a role that reduced dimeric human galectin-1 performs in addition to lectin mediated tumourigenic roles such as T cell apoptosis and cell adhesion and migration. Additionally, the 68 kDa and 17 kDa oxidized species of oxidized human galectin-1 may enhance protection under oxidative stress via direct cell surface interaction with cancerous tumour cells.

More investigation into the structure and function of human galectin-1 within oxidizing environments is required, particularly in the context of cancer, because the oxidative stress associated with many cancers provides an opportunity to design treatment strategies that selectively target cancer cells based on their redox profile.
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>Å</td>
<td>angstrom</td>
</tr>
<tr>
<td>Amp</td>
<td>ampicillin</td>
</tr>
<tr>
<td>ASU</td>
<td>asymmetric unit</td>
</tr>
<tr>
<td>β-ME</td>
<td>β-mercaptoethanol</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>CD</td>
<td>circular dichroism</td>
</tr>
<tr>
<td>CRD</td>
<td>carbohydrate recognition domain</td>
</tr>
<tr>
<td>Cys-SOH/CSO</td>
<td>sulphenic acid</td>
</tr>
<tr>
<td>Cys-SO₂H</td>
<td>sulphinic acid</td>
</tr>
<tr>
<td>Cys-SO₃H</td>
<td>sulphonic acid</td>
</tr>
<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>E. coli</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>FPP</td>
<td>farnesyl diphosphate</td>
</tr>
<tr>
<td>Gal</td>
<td>galactose</td>
</tr>
<tr>
<td>Glc</td>
<td>glucose</td>
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<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>h-gal-1</td>
<td>hexahistidine tagged human galectin-1</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>hydrogen peroxide</td>
</tr>
<tr>
<td>HRP</td>
<td>horseradish peroxidase</td>
</tr>
<tr>
<td>IPTG</td>
<td>isopropyl-β-D-thiogalactopyranoside</td>
</tr>
<tr>
<td>K</td>
<td>kelvin</td>
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<tr>
<td>kDa</td>
<td>kilodalton</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>LacNAc</td>
<td>N-acetyllactosamine</td>
</tr>
<tr>
<td>LB</td>
<td>Luria-Bertani media</td>
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<tr>
<td>Man</td>
<td>mannose</td>
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<td>µg</td>
<td>microgram</td>
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<td>microlitre</td>
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</table>
µM micromolar
mM millimolar
M molar
msec millisecond
NAc N-acetyl group
ng nanogram
nm nanometre
nM nanomolar
OD optical density
PAGE polyacrylamide gel electrophoresis
PCR polymerase chain reaction
PBS phosphate buffered saline
pg picogram
PI propidium iodide
pmol picomole
PMSF phenyl methyl sulphonyl fluoride
ppm parts per million
Prx peroxiredoxin
PS phosphatidylserine
$R_g$ radius of gyration
$R_h$ hydrodynamic radius
ROS reactive oxygen species
rpm revolutions per minute
s second
S70 Sephacryl 100 High Resolution column
S100 Sephacryl S-100 High Resolution column
S200 Superdex 200 10/300 GL column
SD standard deviation
SDS sodium dodecyl sulphate
SEC size exclusion chromatography
STD NMR saturation transfer difference nuclear magnetic resonance
V voltage
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