# THE LYSOSOMAL DEGRADATION OF HEPARAN SULPHATE:

# A COMPARATIVE STUDY OF THE PHYSICAL AND CATALYTIC PROPERTIES OF THE HEPARAN SULPHATE DEGRADATIVE ENZYMES

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by

**CRAIG FREEMAN, B.Sc. (Hons.)**

Lysosomal Diseases Research Unit
Department of Chemical Pathology
Adelaide Children's Hospital
(A Division of the Adelaide Medical Centre for Women and Children)
North Adelaide, South Australia 5006.

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Department of Paediatrics
The University of Adelaide and
The Adelaide Medical Centre for Women and Children
(Adelaide Children's Hospital Campus)

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## ABSTRACT

The thesis area has been to study the enzymology of some of the nine lysosomal *exo*-enzyme activities which act together to degrade the more highly sulphated regions of the glycosaminoglycans heparin and heparan sulphate. A deficiency of any one of these enzyme activities can result in one of the lysosomal storage disorders collectively known as the Mucopolysaccharidoses (MPS). A comparative study was made of the physical and catalytic properties of purified human liver iduronate-2-sulphatase, α-L-iduronidase, sulphamidase, glucosamine-6-sulphatase and glucuronate-2-sulphatase activities and those activities present in normal control and MPS patient tissues. Glucosamine-6-sulphatase and glucuronate-2-sulphatase were purified 57,000-fold and 2 million-fold respectively for the first time from human liver.

For each enzyme studied, a series of heparin-derived substrates designed to match structural aspects of the physiological substrate were synthesised and characterised. Aglycone structures that influenced substrate binding ($K_m$) and turnover ($k_{cat}$) were observed to be similar for each of the activities which acted toward the uronic acid or the glucosamine residues. Iduronate-2-sulphatase and α-L-iduronidase both acted most efficiently toward substrates possessing a non-reducing terminal C-6 carboxyl group and a penultimate residue C-6 sulphate ester, while both glucosamine-6-sulphatase and sulphamidase preferred substrates possessing a penultimate residue with a C-6 carboxyl group in conjunction with a C-2 sulphate ester. More structurally complex substrates, where several aspects of the aglycone structure of heparan sulphate were maintained, were the most efficient substrates for hydrolysis. Sulphamidase and glucosamine-6-sulphatase respectively acted toward their most complex substrate evaluated 1,400,000-times and 3,900-times more efficiently than toward their simplest monosaccharide substrates. The most efficient

substrates were utilised to detect and characterise residual mutant enzyme present in MPS patient tissues. Enzyme activity in skin fibroblasts from patients deficient in sulphamidase and glucosamine-6-sulphatase activities had similar kinetic properties to the normal control enzyme, but were diminished in enzyme protein, suggesting a defective translation or post-translational stability of the mutant enzyme.

Despite differences in the physical characteristics (native protein and subunit $M_r$ and pI values) of enzyme from different sources (human liver, urine, skin fibroblasts and leucocytes), each particular enzyme was catalytically similar with respect to the observed $K_m$, pH optima and the influence of aglycone substrate structure upon enzyme activity. Enzyme activities were influenced by buffer composition and concentration, the addition of BSA, polycations, salts (NaCl, $Na_2HPO_4$, $Na_2SO_4$ and $CuCl_2$) and the presence of substrate analogues.

During the course of the investigation, several observations were made which suggested a requirement for interaction between sequentially acting enzymes *in vivo*, to alleviate the inhibition of activity by product, substrate and structurally related (but non-substrate) oligosaccharides. A model is proposed where the heparan sulphate-degradative enzymes act in juxtaposition towards their substrate, such as within a multienzyme complex in close proximity to the lysosomal membrane, in order to facilitate the efficient degradation of the *in vivo* substrate.