B-02

Invitro Profiling of Chondroitin/Dermatan Trisulphate Disaccharide Profiling as pharmacological chaperone for Mucopolysaccharidoses Type II

Affandi Omar, Fatimah Diana Amin Nordin & Julaina Abdul Jalil

Inborn Errors of Metabolism & Genetics Unit, Nutrition, Metabolism & Cardiovascular Research Centre, Institute for Medical Research, Block C, National Institutes of Health Complex, No 1, Jalan Setia Murni U13/52, Bandar Setia Alam, 40170 Shah Alam, Selangor, MALAYSIA

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Introduction

- Mucopolysaccharidosis type II (MPS II, OMIM 309900) also known as Hunter syndrome, is an X-linked recessive disorder caused by a deficiency of iduronate-2-sulphatase (IDS) (1).
- MPS II is characterized by progressive lysosomal accumulation of glycosaminoglycans (GAGs) comprised of dermatan sulphate and heparan sulphate, and presents systemic manifestations such as skeletal deformities, mental retardation, valvular heart disease, hepatosplenomegaly and skin abnormality (2).
- Enzyme replacement therapy (ERT) is currently the standard treatment for MPS II patients while hematopoietic stem cell transplantation (HSCT) is a potential option for MPS II treatment.
- However, these therapies have several limitations; no or little effects on brain, bone and heart valves, need of weekly intravenous administration of ERT, and risk of mortality due to the conditioning regiment using chemotherapy agents in HSCT.
- The use of small molecules as pharmacological chaperone in therapeutic alternative to restore the defective IDS has been extensively explored.
- Here, we demonstrate the profiling of chondroitin/dermatan trisulphate dissacharide (CD3S) using invitro recombinant human iduronate-2-sulphatase (rhIDS).

Material & Methods

21 small molecules (Iduron, Manchester, UK) with several concentrations each were screened using inhibition assay (3)

Any potential small molecules with lowest inhibition concentration (IC_{50}) and ~~highest~~ inhibition constant (K_i) were tested for thermal stability assay (4)

Inhibition Assay

- Small molecules were incubated with recombinant human IDS (rhIDS) for 10 minutes at 0°C.
- 50 µL of 2 mM ρ-nitrocatechol sulphate (ρNCS) was added into 50 µL of each concentration of the respective small molecules in the microplate.
- The plate was incubated at 37°C for 24 hours before the reaction was terminated with 100 µL of 0.2 M sodium hydroxide.
- Liberated product of ρ-nitrocatechol (ρNC) was measured using spectrophotometer at 515nm.

Thermal Stability Assay

- rhIDS was dissolved in phosphate citrate buffer supplemented with any potential small molecules.
- The mixture was heated at 37, 47, 57 and 67°C respectively, for 1 hour.
- 50 µL each of mixture was added into 50 µL of 2 mM ρNCS.
- The plates were then incubated at 37°C for 24 hours.
- The reaction was terminated with 100 µL of 0.2 M sodium hydroxide.
- Liberated product of ρNC was measured using spectrophotometer at 515nm.

Results

- Our study revealed that chondroitin dermatan trisulphate (CD3S), heparin tetrasaccharide (H4Sac), heparin octasaccharide (H8Sac) and heparin octadecasaccharide (H18Sac) showed low IC_{50} and high K_i (Table 1).
- After incubation with H4Sac, H8Sac and H18Sac, the activities of rhIDS were totally suppressed and denatured when reaching 67°C (Figure 1).
- However, there was still ~20% activity of rhIDS when incubated with CD3S at 67°C.

Discussion

- From the inhibition assay, only four small molecules showed the lowest IC_{50} with the highest K_i. IC_{50} referring to concentration required to produce 50% inhibition of enzyme activity while K_i is an equilibrium constant of a reversible inhibitor for complexation with its target enzyme.
- The thermal stability of rhIDS experiment was conducted to determine which small molecules have protective effect on the enzyme from heat-induced inactivation.
- A stable conformation of a protein resists denaturation as compared to fragile conformational structure which often intolerant to heat denaturation (5)
- It has been postulated that N-linked oligosaccharides played an important role in the folding, function and stability of glycoprotein (6).
- We hypothesized that the N-terminus in the structure of CD007 may interact with any of the eight N-linked glycosylation sites in the rhIDS to stabilise the folding of the enzyme during the heat-induced activation process. The importance of N-glycosylation for folding, catalytic activity and processing of IDS has been demonstrated (7).

Table 1: Inhibition assay results for potential small molecules

Small molecules of CD		IC_{50} (µM)	K_i	V_{max}
Heparin tetrasaccharide	H4Sac	19.5	59.3	892
Heparin octasaccharide	H8Sac	65.6	34.4	223
Heparin octadecasaccharide	H18Sac	8	11.6	47
Chondroitin dermatan trisulphate	CD3S	44	24.4	1429

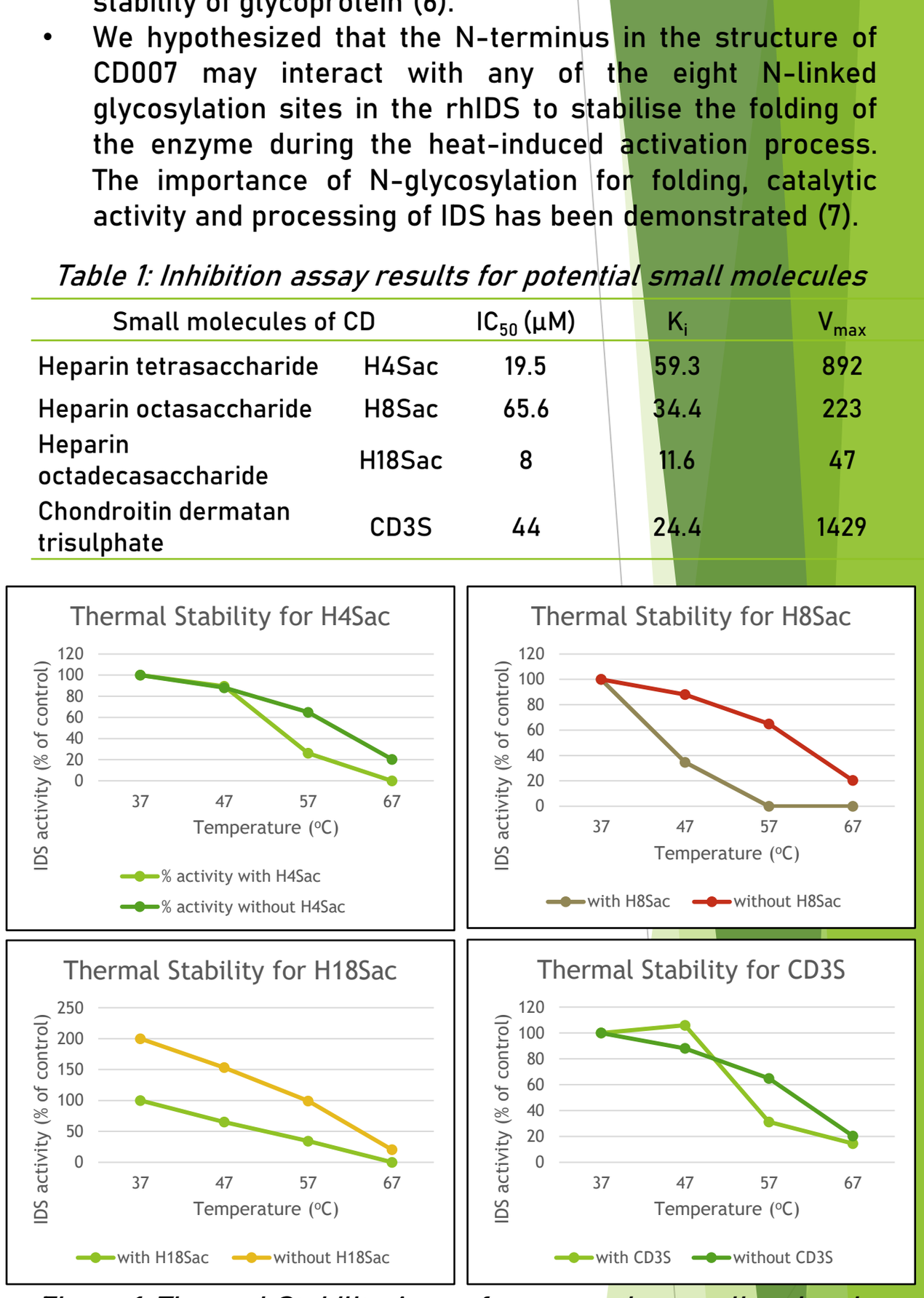

Figure 1: Thermal Stability Assay for respective small molecules

Conclusion

- Overall, our experiments discovered that CD3S was able to bind, inhibit and chaperone rhIDS and may serve as potential pharmacological chaperone for MPS II.

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Further information: fendi.omar@moh.gov.my