Optimization of enzymatic desialylation of human serum transferrin

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Introduction

Human serum transferrin (hTf) is one of the most important iron transport proteins in the human organism. It is a highly glycosylated bilobal protein that can bind up to two iron(III) ions. Glycosylation of transferrin is critical for its proper function in the body; various pathological conditions are closely associated with unusual sialylation patterns. In normal hTf, approximately 85% of carbohydrates are sialylated complex biantennary glycans and the remaining 15% are sialylated isomeric complex triantennary glycans. (Fu and van Halbeek, 1992) Different combinations of the two N-glycans result in a range of asialo to hexasialo variants of hTf, with asialo hTf occurring in trace amounts. *In vitro*, asialotransferrin is produced by the action of commercially available sialidase enzymes, which can be quite expensive. Therefore, we optimized the processes for two commercially available enzymes: **GlycoCleave®** and **SialEXO®**, to produce asialotransferrin at a lower cost. The pH gradient chromatofocusing method was used to monitor the efficiency of enzymatic desialylation. Moreover, the method can be used for additional chromatographic purification of the desialylated samples. (Friganović et al., 2021)

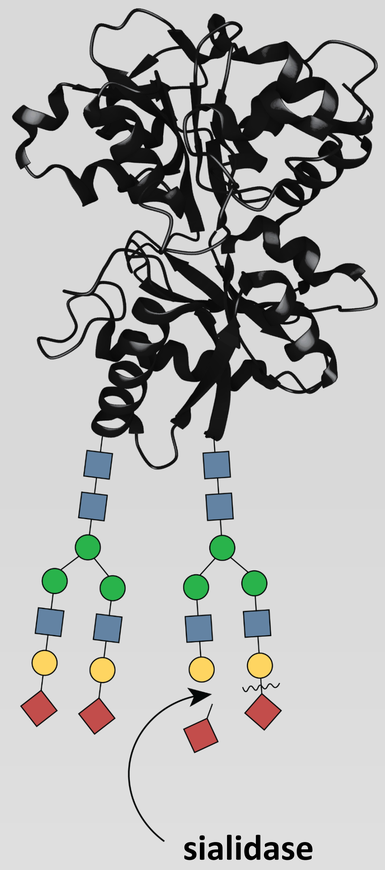

Figure 1. Schematic representation of the activity of the sialidase enzyme on human serum transferrin.

Optimization

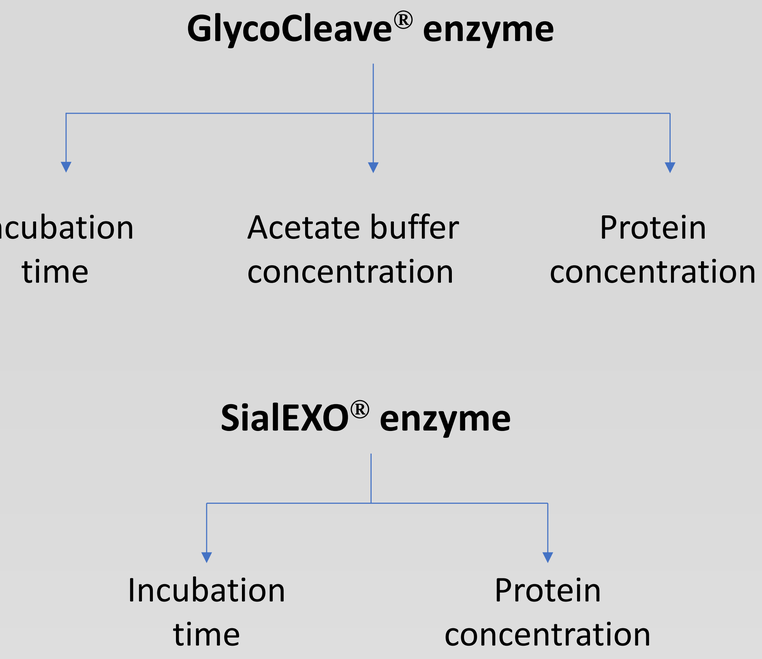

Figure 2. Schematic representation of the parameters involved in the optimization of enzymatic cleavage.

Results

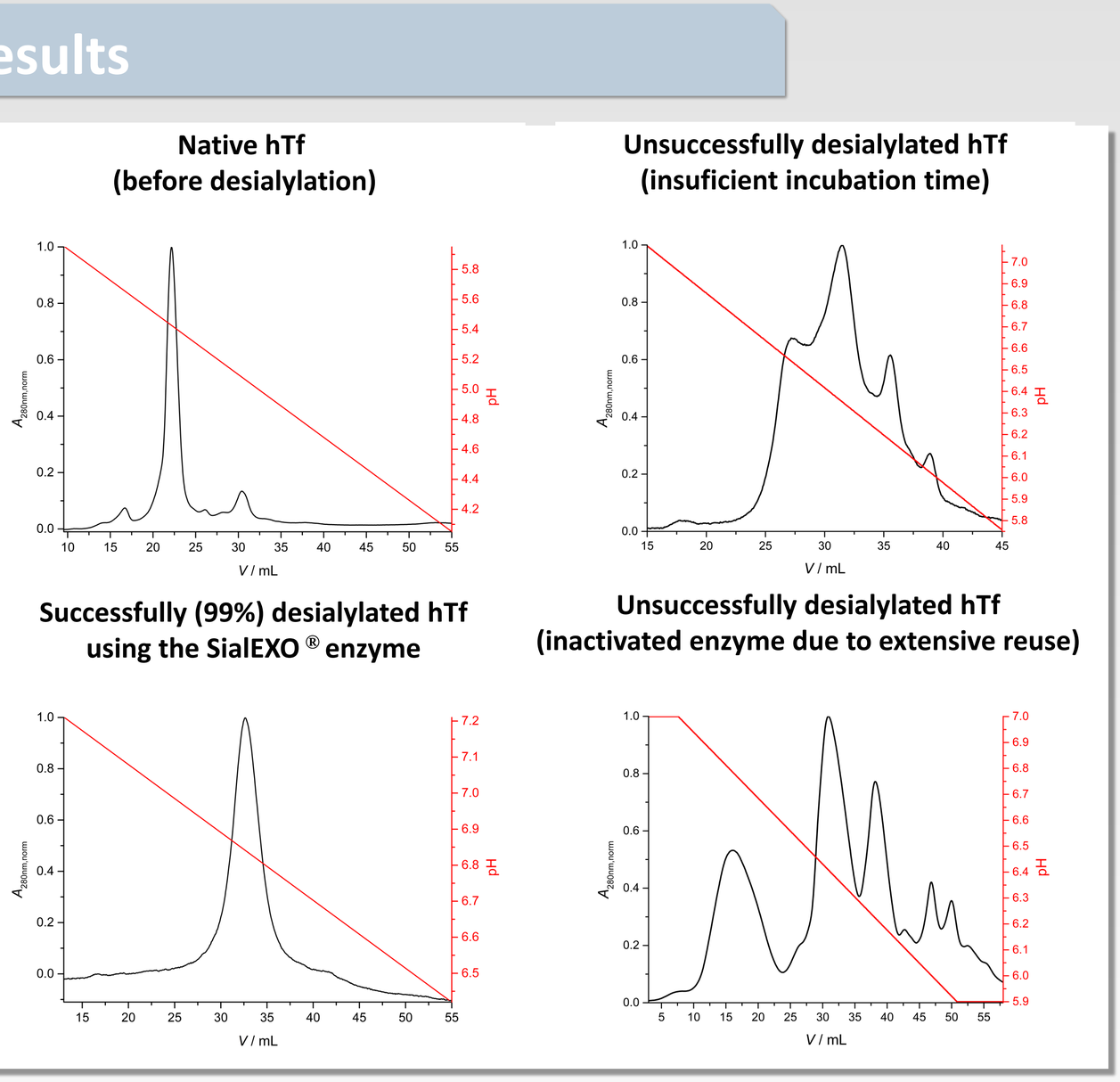

Figure 3. Results of pH gradient chromatofocusing used for monitoring the desialylation process.

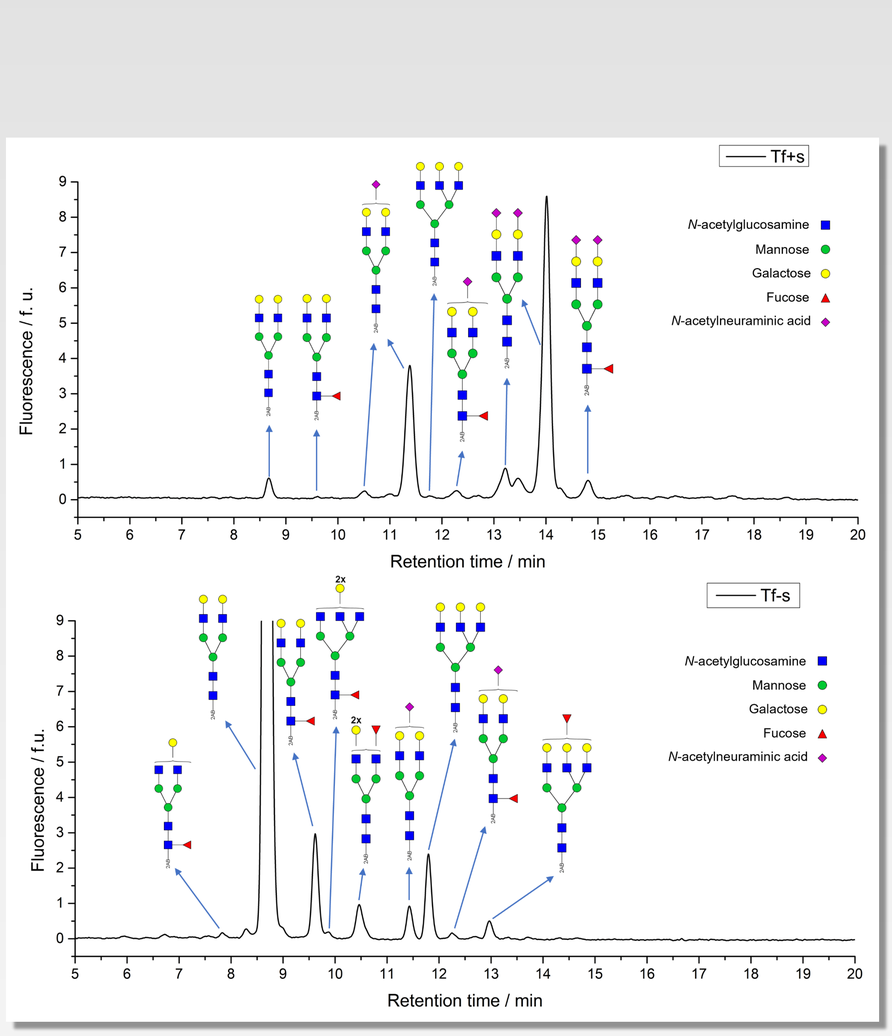

Figure 4. Structures of different N-glycan residues in native hTf (Tf+s, upper trace) and successfully desialylated hTf (Tf-s, lower trace) as determined by UPLC and assigned based on previously reported results. (Friganović et al., 2021)

GlycoCleave® enzyme: Increasing the incubation time and acetate buffer concentration allowed for a greater amount of desialylated transferrin per incubation cycle. The modified procedure resulted in removal of > 90% of the sialic acid content and one desialylation cycle was able to produce 5 mg of desialylated protein (instead of 2 mg as stated by the manufacturer).

SialEXO® enzyme: The modified method with increase in incubation time and protein concentration resulted in removal of ≈99% of the sialic acid content, and the amount of protein that can be desialylated with a single SialEXO® column was increased from the initial 0.5 mg (manufacturer's recommendation) to ≈50 mg.

References

Fu, D., van Halbeek, H., 1992. N-glycosylation site mapping of human serotransferrin by serial lectin affinity chromatography, fast atom bombardment-mass spectrometry, and 1H nuclear magnetic resonance spectroscopy. Anal. Biochem. 206, 53–63. DOI: 10.1016/S0003-2697(05)80010**.**

Friganović, T., Tomašić, A., Šeba, T., Biruš, I., Kerep, R., Borko, V., Šakić, D., Gabričević, M. and Weitner, T., 2021. Low-pressure chromatographic separation and UV/Vis spectrophotometric characterization of the native and desialylated human apo-transferrin. Heliyon, 7(9), p.e08030. DOI: 10.1016/j.heliyon.2021.e08030

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EUROPSKI STRUKTURNI I INVESTICIJSKI FONDOVI