Separation of the native and desialylated human apo-transferrin sialoforms using low-pressure pH gradient ion exchange chromatography

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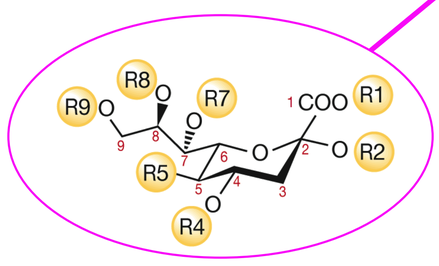
Introduction

Human transferrin is an iron binding plasma glycoprotein important for iron homeostasis. Each human transferrin molecule contains two Fe(III) binding sites [1]. The most abundant human transferrin glycoform contains two biantennary oligosaccharide chains with four terminal sialic acids [2]. Changes in the transferrin sialylation may significantly alter thermodynamic and kinetic properties of iron binding. The purpose of this study is to separate the native human apo-transferrin from the fully desialylated apo-transferrin using low-pressure pH gradient ion exchange chromatography.



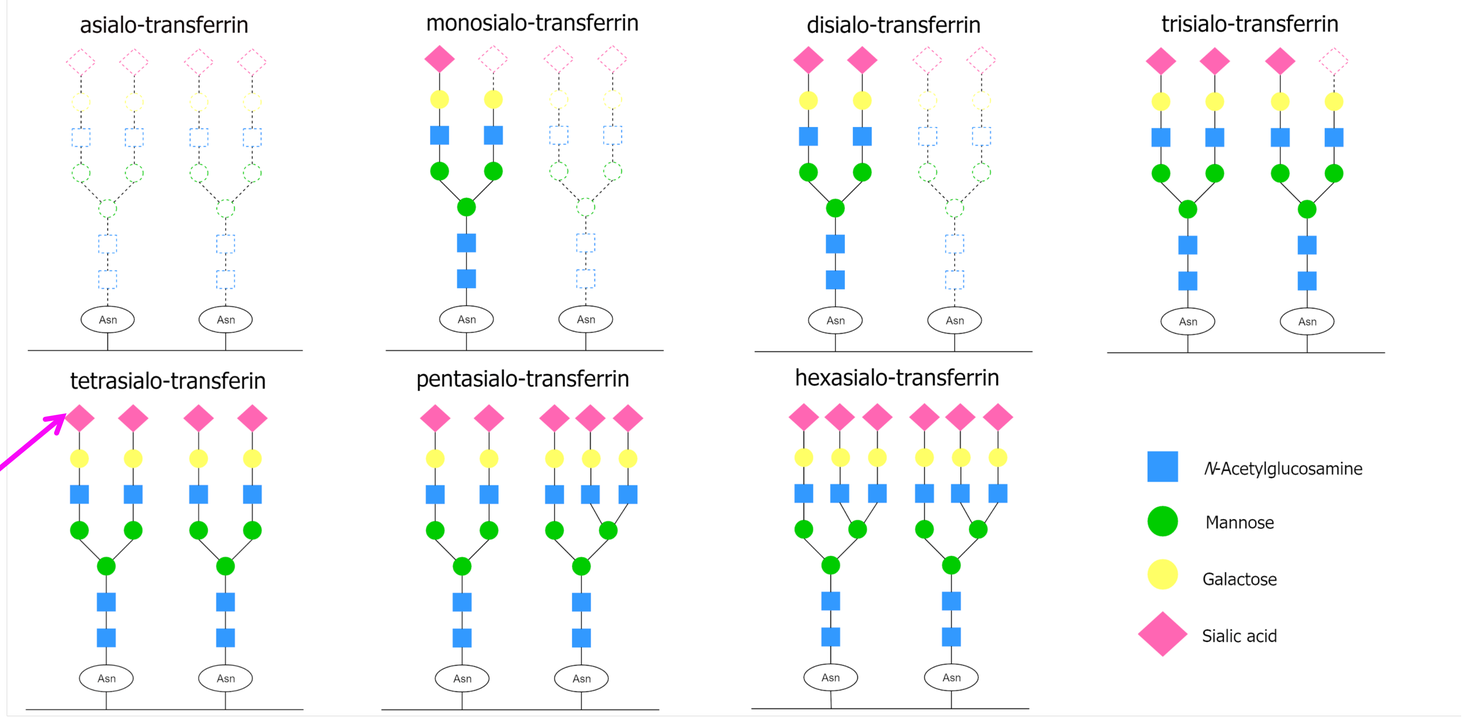


Figure 1. Schematic illustration of different human transferrin glycoforms.

Experimental

Desialylated apo-transferrin is prepared by incubation of immobilized neuraminidase enzyme beads suspension (Glycocleave, GALAB Technologies GmbH, Germany) in the native apo-transferrin buffered stock solution (pH = 5.5, t = 38 °C). After the incubation period of 48 hours, the desialylated sample is collected, washed out and concentrated by centrifugal filtration. Sialoform separation is performed by using specialized pH gradient ion exchange chromatography buffers (pIsep, CryoBioPhysica Inc., USA). The mixture of fully desialylated apo-transferrin and native apo-transferrin is dissolved in the start buffer pIsep A (pH = 8) and injected onto HiTrap Q HP anion exchange chromatography columns (GE Healthcare Bio-Sciences AB, Sweden). Two 1 mL columns are connected in a series for improved separation. Elution is done by single step linear gradient (0 – 100 % pIsep B, pH = 4) procedure using ÄKTA Start FPLC system (GE Healthcare). Protein concentration in the eluate is monitored by measuring absorbance at λ = 280 nm and protein fraction recovery can be calculated by integration over surface area (mL x mAU). After separation, pH values of each fraction are measured.

Results

The observed pI values for the native and desialylated apo-transferrin sialoforms differ significantly (**pI ≈ 4.8 for the native and pI ≈ 6 for the desialylated**) and hence can be fully separated. In order to confirm the results both elution fractions were analyzed by mass spectroscopy. FPLC pH gradient separation provides a fast, simple and cost-effective method for for purification and separation of different sialoforms of apo-transferrin. The method can easily be modified for other glycoproteins and is particularly appropriate for quick testing of protein sialic acid content prior to verification by mass spectrometry.

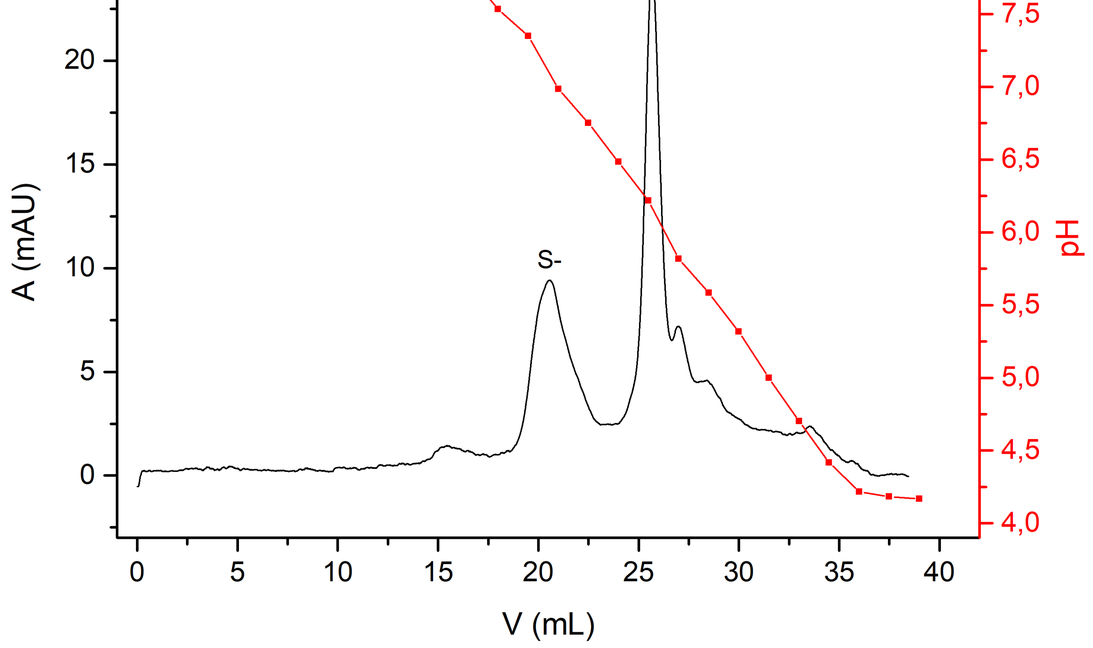


Figure 2. Low-pressure pH gradient ion exchange separation of native (S+) and desialylated (S-) glycoforms of human transferrin; 2 distinct signals match different glycoforms.

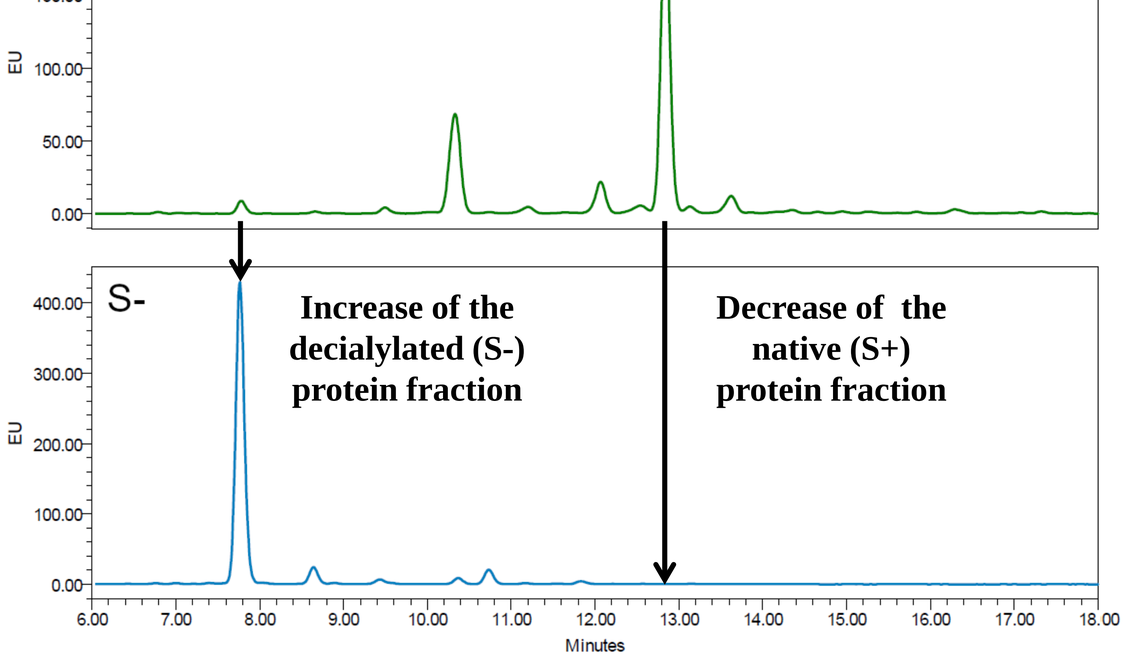


Figure 3. Mass spectroscopic *N*-glycan analysis for native (S+) and desialylated (S-) glycoforms of human transferrin.

Acknowledgements

This work was supported by funding from the Croatian Science Foundation grant **UIP-2017-05-9537 –** Glycosylation as a factor in the iron transport mechanism of human serum transferrin (GlyMech).

References

[1] H.G. van Eijk, W.L. van Noort, M.J. Kroos, C. van der Heul. Analysis of the iron-binding sites of transferrin by isoelectric focussing. *J. Clin. Chem. Clin. Biochem.* **16** (1978) 557-560.
[2] D. Fu, H. van Halbeek. 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** (1992) 53-63.