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

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Human transferrin is an iron binding plasma glycoprotein important for iron homeostasis. Each human transferrin molecule contains two Fe(III) binding sites. The most abundant human transferrin glycoform contains two biantennary oligosaccharide chains with four terminal sialic acids. Physiological of pathophysiological changes in 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. Desialylated apo-transferrin is prepared by incubation of immobilized neuraminidase enzyme (Glycocleave, GALAB Technologies GmbH, Germany) in the buffered stock solution of native apo-transferrin (2 mg/mL, pH = 5.5, t = 38 °C). After incubation for 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) using ÄKTA Start FPLC system (GE Healthcare). Protein concentration in the eluate is monitored by measuring absorbance at 280 nm and protein fraction recovery can be calculated by integration over chromatogram surface area. After separation, pH value of each fraction containing eluted protein is measured. The measured pH value corresponds to approximate protein pI value. 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. The 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.

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