UNIVERSITÄT ZU LÜBECK

NMR proteo-metabolomics: A rising technology for the quantification of acute-phase inflammation proteins from human serum/plasma

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Introduction

Nuclear Magnetic Resonance (NMR) spectra of human serum and plasma show, besides metabolites and lipoproteins, two characteristic signals termed GlycA and B, arising from the acetyl groups of glycans on the surface of acute phase proteins, which constitute good markers for inflammatory processes. Here, we report a comprehensive assignment of glycoprotein glycan NMR signals observed in human serum, showing that GlycA and GlycB signals originate from Neu5Ac and GlcNAc moieties from *N*-glycans, respectively. Conventionally determined concentrations of acute phase glycoproteins correlate well with distinct features in NMR spectra (R^2 up to 0.9422, p-value <0.001), allowing the simultaneous quantification of several acute phase inflammation proteins within 10 – 20 min acquisition time (Fig. 1).[1] This is exemplified in serum samples from COVID-19 and cardiogenic shock patients showing significant changes in several acute phase proteins compared to healthy controls.

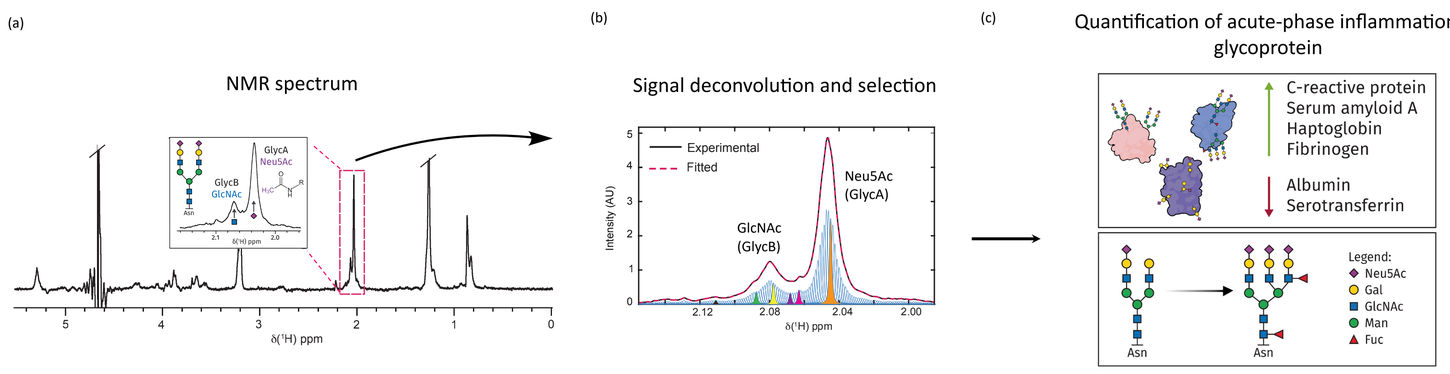

Figure 1: Strategy used for the quantification of acute-phase inflammation glycoproteins from human serum: a) A single diffusion- and T_2-filtered, *J*-edited spectrum is acquired from a serum/plasma sample in 5 min. b) Protein glycosylation profiles are extracted from the *N*-glycan signals GlycA (Neu5Ac) and GlycB (GlcNAc) *via* line shape fitting. c) Correlation of line shape integral and frequency with NMR spectra obtained from isolated *N*-glycans and serum glycoproteins provides the concentration of most abundant serum glycoproteins.

Assignment of GlycA and GlycB signals

As a first step we unequivocally assigned GlycA and GlycB signals. For this purpose, we removed metabolites and lipoproteins (Fig. 2a) using a combination of gradient ultracentrifugation and size exclusion chromatography (Fig. 2b). NMR spectra of isolated glycoproteins showed exclusively signals from glycoprotein glycans, allowing the assignment of all glycan signals under protein native, folded conditions (Fig. 2c).

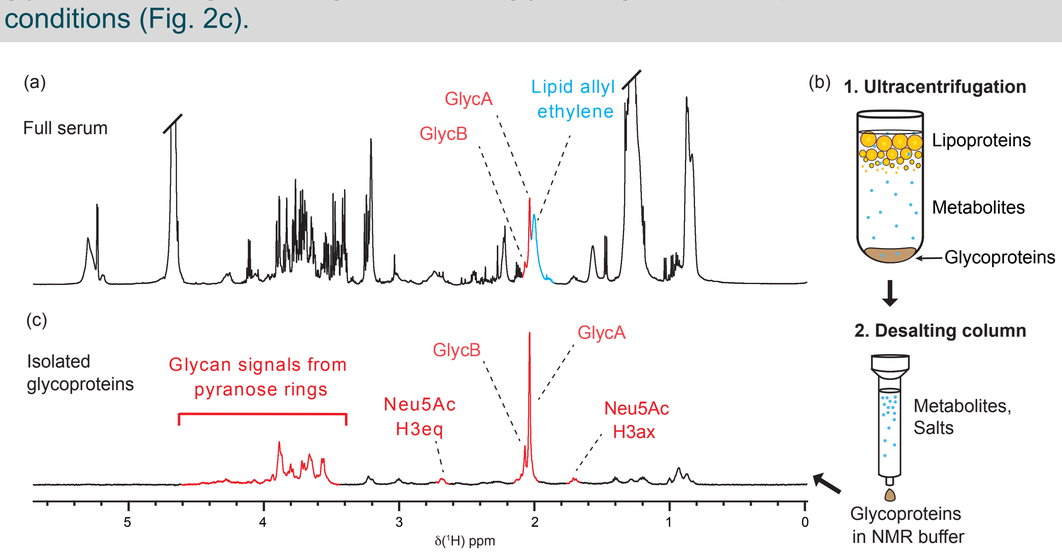

Figure 2: Isolation of glycoproteins from serum a) ^{1}H-CPMG spectrum of human serum. b) Strategy followed for the purification of serum glycoproteins. c) ^{1}H-CPMG spectrum of a metabolite- and lipoprotein-free serum glycoprotein.

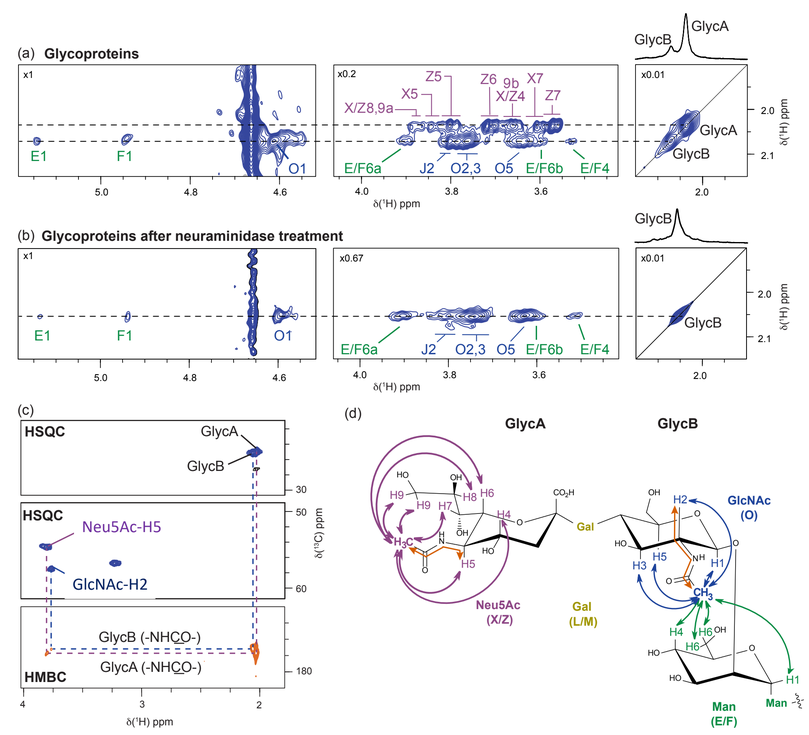

Figure 3: Assignment of GlycA/B signals. a,b) ^{1}H,^{1}H-NOESY experiments. c) ^{1}H,^{13}C-HMBC experiment. d) Cartoon summarizing experimental observations

Assignment of GlycA/B signals was achieved by using through-space correlations in ^{1}H,^{1}H-NOESY spectra. In such spectra GlycA signal shows correlations exclusively to Neu5Ac, while GlycB correlates with GlcNAc and Man (Fig. 3a). These assignments were validated by treating a sample of glycoprotein glycans with neuraminidase followed by removal of free sialic acid. Fig. 3b shows the ^{1}H,^{1}H-NOESY spectrum after the enzymatic digestion, where all cross peaks to sialic acid are missing. This assignment was further confirmed by an ^{1}H,^{13}C-HMBC spectrum showing a through-bound correlation between GlycA and Neu5Ac-H5; and between GlycB and GlcNAc-H2 (Fig. 3c). The observed NOESY cross peaks are summarized in Fig. 3d.

Quantification of Acute-phase Glycoproteins from a COVID-19 and Cardiogenic Shock Patients

As a proof of concept, we acquired JEDI-PGPE[2] NMR experiments from a set of 24 samples from COVID-19 and cardiogenic shock (CS) patients along with healthy controls (Fig. 4a). GlycA/B regions were fitted using a series of Lorentzian-Gaussian line shapes (Fig. 4b). Areas under the curve (AUC) from selected fitted line shapes showed good to excellent correlation coefficients with glycoprotein concentrations determined independently by standard laboratory methods, with *p*-values <0.001. For example, haptoglobin, α-1-antitrypsin, ceruloplasmin, complement factor C3, H and transferrin showed very good correlations with coefficients of determination R^2 >0.70 (Fig. 4c) Interestingly, identical glycoproteins could be identified as significantly up- or down-regulated in COVID-19 and CS patients using either standard laboratory methods or NMR quantification (Fig. 4d). Best correlations were observed for α-1-antitrypsin, haptoglobin, complement factor C3, and transferrin (Fig. 4e).

In COVID-19 we see significant changes for albumin, α-1-antitrypsin, ceruloplasmin, haptoglobin, and serotransferrin. **α-1-Antitrypsin** is known as an inhibitor of several proteases, especially elastase, which is linked to immune responses and neutrophil activity. **Ceruloplasmin** is a copper transport protein with ferroxidase activity, whose concentration is increased during infections and inflammatory processes. **Haptoglobin** is a hemoglobin scavenger which shows high concentrations during acute phase inflammatory reactions, thus preventing hemoglobin-driven oxidative damage. **Serotransferrin** is an iron transporter which decreases in inflammatory processes. Decreased **albumin** concentrations are also associated with inflammatory responses.

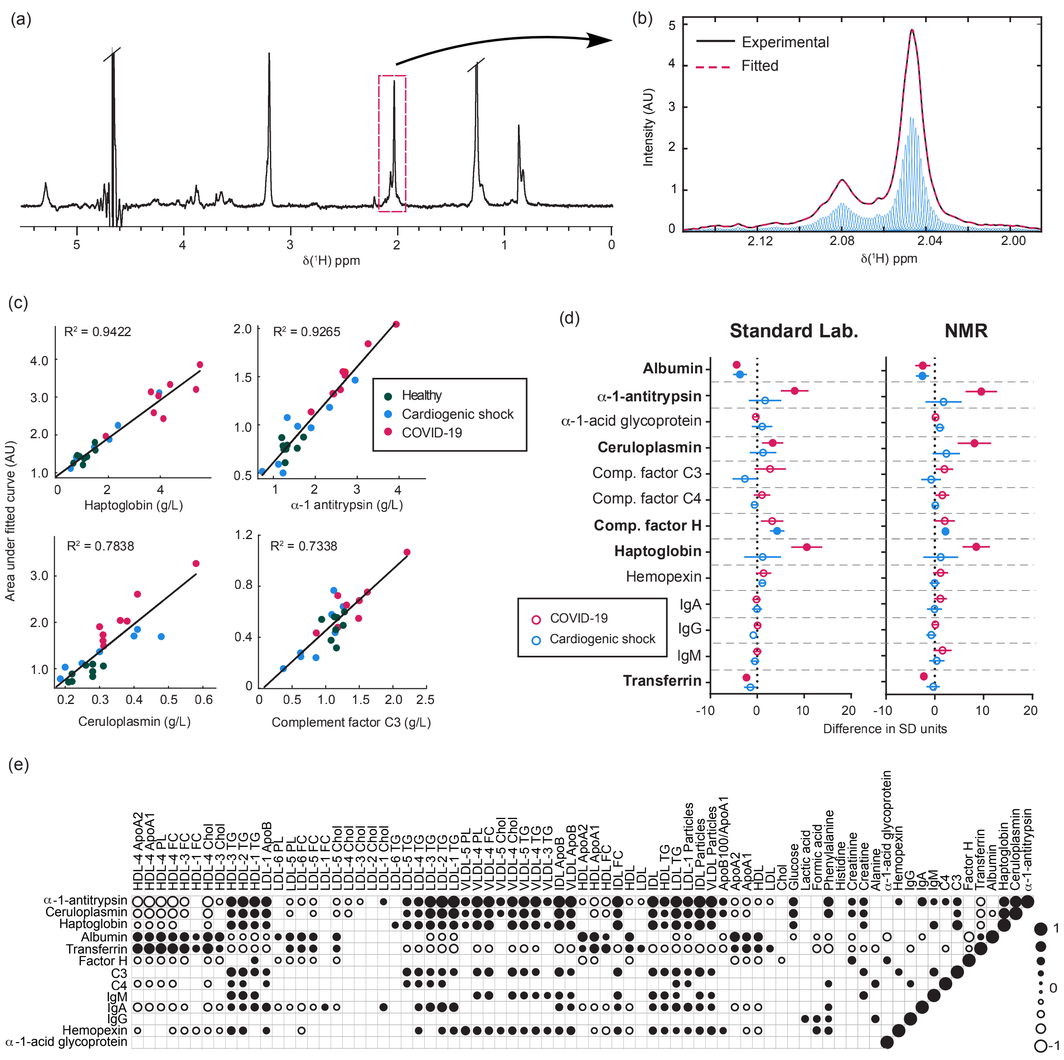

Figure 4: Analysis of COVID-19 and cardiogenic shock samples.

Conclusions and Outlook

We have reassigned the glycoprotein NMR signals from human serum and present a perspective how this information could be used for medical diagnostics of blood samples. This work demonstrates the overall potential of NMR proteo-metabolomics as a rising technology for complex diagnostic blood analysis. **For more details see Poster P-256.**

[1] Mallagaray A. *et al., Angew. Chem. Int. Ed.* **2023**, DOI: 10.1002/anie.202306154
[2] Nitschke, P. *et al. Anal. Chem.* **2022**, *94*, 1333-1341

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