Glycation Profile for Human Serum Albumin

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Abstract: The amino groups of lysine of plasma proteins are susceptible to a non-enzymatic reaction with glucose under physiological conditions. This glycation process ultimately can lead to diabetes complications, oxidative stress and aging process. Due to that diabetic patients have high glucose concentration, the amount of glycated proteins can be used as a marker of disease progression. Glycation degree for different lysine residues vary and depend from protein location, lysine reactivity and glucose concentration. We have studied glycation profile of most abundant plasma protein albumin. The degree of glycation for each site was defined as the percentage of glycated to sum of glycated + non-glycated lysines. To get most comprehensive profile two MS instruments were used: timsTOF-Pro and Q-trap. The whole method was used as an additional separation dimension; PASEF MS/MS scans for fragmentations in Tims-TOF. Q-trap instrument was used in two different modes: CID and neutral-loss. In vitro glycated HSA and plasma from diabetic and non-diabetic patients were reduced/alkylated and digested by Glu-C. The peptide mixture were separated in a nano-flow C18 system or HPLC C18 in a MeCN/H2O linear gradient depend from MS platform. Raw data was analyzed using PEAKS X or Proteome Discoverer. All discovered glycation peptides were manually validated. In summary, we created glycation profile for serum albumin that can be used in the future as hyperglycemia biomarker.

Introduction

In 2018, Centers for Disease Control (CDC) reported that from total US population - 327.2 million - around 26.8 million were diagnosed with type 1 or type 2 diabetes. As for the cost - in 2017 for diagnosis alone was spent around $327 billion. One patient need approximately $16,750 per year on spent on diagnosis and on prescription [1].

Diabetes mellitus (DM) occurs when immune system starts response against beta cells - responsible for production of insulin - and starts attacking them (type 1 DM) or from tissues increase resistance against insulin leading to decrease of insulin being used by the tissues (type 2 DM), both types lead to increase of glucose concentration in blood [2]. Because of high glucose concentration in blood - non-enzymatic reactions start to occur between glucose’s aldehydes group with amine groups of proteins, which are part of lysine amino acid [3].

Methods

In vitro analysis: in vivo glycated and in vivo glycated, both were digested with enzyme .

(1) In vitro glycation protocol: HSA was incubated with excess of glucose in the presence of NaN3 as bacteriostatic in PBS solution. The incubation was performed at 37°C for 24 hours. In vivo glycated samples were human plasma provided from patients with High and Low A1C scores.

(2) A digestion was done on fraction of glycated sample using general digestion protocol from Sigma-Aldrich using Glu-C endoprotease: For reduction and alkylation Dithiothreitol (DTT) and Iodoacetamide (IAM) were used. Also, one sample was enriched before boronate affinity, using dried powder - Boronate SPW - following resin protocol.

Two sets of samples were analyzed: in vitro glycated and in vivo glycated, both were digested with enzyme .

Separation

Before mass spectrum analysis of glycated peptides, the mixture was dissolved in aqueous 0.4% formic acid used (A) then pushed through HPLC to separate small polar from big nonpolar peptides. Mobile phase was Acetonitrile (MeCN) buffer 0.4% formic acid buffer (B).

PASEF

The PASEF method involves rapid switching of the quadrupole mass position to select multiple precursors at different m/z on the very same time scale. Which leads to all targeted ions to be fully used for fragmentation.

Results

The manual validation of found peptides were performed using PeaView®, PEAKS®, Proteomic toolkit website, and Excel (location of the site in HSA based on uniprot nomenclature, K-lysine)

Mass Spectrometers

Two different mass spectrometers were used for MS acquisition for enhancing Lysine’s sites coverage: Trapped Ion Mobility Spectrometry Times-of-flight (Tims-ToF) and Quadrupole ion trap mass spectrometry (Q-trap MS). Tims-ToF coupled to Bruker nano-Elute system and Q-trap MS connected to Shimadzu Prominance LC system: both systems used C-18 columns.

Conclusion

Several glycation sites were identified as being potential for developing as hyperglycemia biomarkers: K160, K569, K298, K286, K65, and K402. In the future we plan to develop precise quantitative method for each individual lysine site and to compare glycation profile between diabetic and non-diabetic patients.

Sources