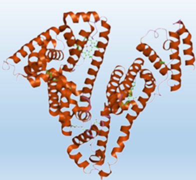

Glycation Profile for Human Serum Albumin

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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 fact 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. Ion mobility 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 spent on diagnosis and on prescription [1].

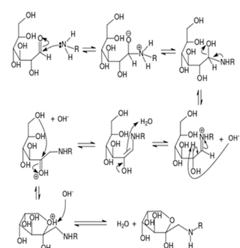
Diabetes mellitus (DM) occurs when immune system starts response against beta cells – responsible for production of insulin - and starts attacking them (type 1 DM) or from tissues increase resistance against insulin leading to decrease of insulin being used by the tissues (type 2 DM), both types lead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

Human Serum Albumin

For this project Human Serum Albumin (HSA) was chosen as a model to study, based on the following criteria:

- The most abundant protein in human plasma (35-50 g/L) with a variety of physiological functions.
- Large amount of lysine sites in the structure with a wide range of reactivity toward glycation.
- High concentration of glucose (diabetic patients) and HSA in plasma leads to easiness of glycation reaction.

Glycation Reaction



Each reaction is reversible and slow.

In case of DM, HSA's amine groups on lysine will react with aldehyde group of glucose.

First step is formation of Schiff base, through reaction between aldehyde group and amine group.

Second step is rearrangements into a N-substituted glycosylamine and losing one molecule of water in the overall process [3][5].

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 Prominence LC system: both systems used C-18 columns.

From left to right: proTims ToF Bruker , SCIEX QTRAP 6500+ Mass Spectrometer

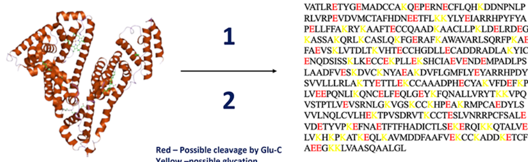
Methods

Two sets of samples were analyzed: in-*vitro* 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 NaN_3 as bacteriostate in PBS solution. The incubation was performed at 37 Cº for 24 hours. In-*vivo* glycated samples were human plasma provided from patients with High and Low A_1C scores.

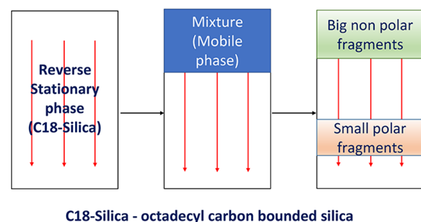
(2) A digestion was done on fraction of glycated sample using general digestion protocol from Sigma-Aldrich using Glu-C endoproteinase: For reduction and alkylation Dithiothreitol (DTT) and Iodoacetamide (IAM) were used.

Also, one sample was enriched through boronate affinity, using dried powder - Boronate SPW - following resin protocol.



Separation

Before mass spectrum analysis of glycated peptides, the mixture was dissolved in aqueous 0.4% formic acid buffer (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)



C18-Silica - octadecyl carbon bounded silica

Modes for analysis

Each Mass Spectrometer platform used different modes for identification of glycated peptides.

- Q-trap two acquisition modes were implemented: Collision Induced Dissociation (CID) full range 500-2000 m/z and CID triggered by neutral-loss (NL): when only ions that lost neutral mass chosen by user [4].
- Tims-ToF used CID full range 100-1700 m/z with unit resolution due to PASEF [4].

Tims – trapped Ion Mobility Spectrometry; Q-Quadropole; CC- Collision Chamber; ToF – time of flight

Q1 – First Quadrupole; CID – Collision Induced Dissociation; Q3-trap – A hybrid between third quadrupole and ion trap.

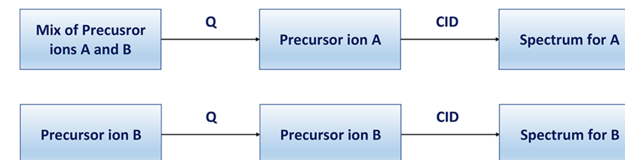
Q1 – First Quadrupole; NL – Neutral Loss; Q3-trap – A hybrid between third quadrupole and ion trap.

For neutral loss the mass of 84 was chosen [3], with +2 and +3 charges for precursor ions. The mass shift is based on loss of $3H_2O$ and CH_2O molecules from glucose residue during fragmentation.

PASEF provided separation in TIMS, which was shown in getting ten PASEF scans for each MS scan [3].

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.



If for simple Tims-ToF scan time spans for 50 ms, for one precursor, but if PASEF is synchronized with Tims-ToF each precursor will be separated on their cross section (geometrical structure of the molecule) and fragmented at the same time.

Results

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

Tims-ToF In vitro Glycated HSA	Tims-ToF Pure HSA	Tims-ToF In vivo – Low A_1C	Tims-ToF In vivo – High A_1C	Q-trap (28) z=3	Q-trap (42) z=2	Q-trap (42) z=2 longer run	Q-trap (28) z=3 longer run	Q-trap Boronate Enriched (CID)
K569	K569	K569	K569	K569	K524	K597	K543	K569
K524	K549	K548	K499	K337	K490	K413	K524	K468
K413	K548	K499	K468	K286	K396	K396	K468	K402
K402	K402	K413	K375	K257	K315	K310	K337	K298
K375	K383	K402	K300	K160	K310	K300	K310	K160
K347	K375	K347	K298	K65	K160	K75	K305	
K300	K310	K286	K286		K65	K65	K298	
K298	K286	K257	K229			K28	K160	
K286	K130	K205	K214				K88	
K198	K117	K160	K160				K75	
K161	K36	K117	K88					
K160		K88	K75					
K130		K75	K65					
K98		K65	K44					
K88		K36	K36					

- The most common sites of glycation: K160, K569, K298, K286, K65 and K402.
- The least common sites of glycation: K205, K161, K198, K249, K264, K205, K44, K214, K315, and K28.

Discussion

The reason why only several sites were detected among different works lays in three main factors:

- Instrumentational Limitations and Characteristics.
- Different methods of acquisitions.
- Location of lysine in Human Serum Albumin – inside or outside of the protein's globula.

From these factors several peptides are potential for developing as hyperglycemia biomarkers : **K160, K569, K298, K286** and **K402.**

While, sites: **K205, K161, K198, K249, K264, K205, K44, K214, K315,** and **K28** were only found in one out of 9 experiments that were run, indicating these sites being very hard to detect if not specific acquisition methods is being used, leading to decrease in confidence and reliability of these sites.

To increase validity of most common sites, each was confirmed with literature [6-8].

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.

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