Analysis of the Metabolic Effects of Diabetes on the Structure and Function of Glycated Human Serum Albumin

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Diabetes is a metabolic disease that affects 25.8 million people in the United States and 366 million people worldwide. This disease is related to a number of health disorders, which can result from insulin deficiency or a resistance to insulin. One of the major problems associated with diabetes is the elevated level of glucose that occurs in the bloodstream, which can lead to the non-enzymatic glycation of proteins. Recent studies have indicated that this modification can affect the structure and function of serum proteins and may transport diabetic serum albumin (HSA). During glycation, a nucleophilic reaction between a reducing sugar such as glucose and a free amine group on a protein can occur to form modified proteins such as glycated HSA (gHSA), as shown in Fig. 1.

**Introduction**

Sulfonfonylurea drugs are a class of drugs that are commonly used to treat type II diabetes (see Fig. 2). These drugs are known to bind tightly to serum proteins and particularly to HSA. High-performance affinity chromatography (HPAC) was used to examine the changes in drug-protein interactions with these drugs caused by glycation (as shown in Figs. 3-4). Zonal elution competition studies were used to profile the binding by each sulfonfonylurea to normal HSA and samples of in vitro or in vivo glycated HSA. In these experiments, R-warfarin was used as a site-selective probe for sulfonamide I of HSA and L-propylthiouracil was used as a site-selective probe for Sulfodine II. In zonal elution competition studies, a fixed concentration of a probe (e.g., R-warfarin or L-propylthiouracil) was injected onto a column in the presence of a competing agent (i.e., a sulfonfonylurea drug) in the mobile phase (see Fig. 4). Direct competition took place between the probe and the competing agent at a single site. Fig. 1 was then used to describe this process and to examine binding at the site of competition by using plots like those shown in Figs. 5(a-b). The results were then used to find the equilibrium association constants for these agents at the site of competing agent, as summarized in Tables 1 and 2.

**Drug Binding Studies by High-Performance Affinity Chromatography**

The results from the competition studies indicated that each sulfonfonylurea drug had direct competition with the specific sites for Sulfodine sites I and II on HSA and glycated HSA samples. The association equilibrium constants determined from HSA and glycated HSA samples of Acetohexamide were summarized in Table 1. Table 1 also summarizes the trends in binding affinity for these drugs at Sulfodine sites I and II for in vivo glycated HSA samples with various levels of glycation and for isolated samples of in vivo glycated HSA. The binding affinity ranged from a 0.6- to 6-fold change in comparison to normal HSA for the various sulfonfonylurea drugs during experiments involving the in vivo glycated HSA samples. The difference in binding affinity varied from one drug to the next and between the interaction sites. Similar results were seen for the in vivo glycated HSA samples, in which up to a 1.8-fold change in binding affinity was seen in some cases. In experiments involving gHSA, additional competition studies were performed with digoxin for normal HSA and the in vivo glycated HSA samples; these results may have shown a small decrease in affinity but the values were not significantly different.

**Results**

**Goals of Study**

The purpose of this study was to characterize the metabolic effects of diabetes on the structure and function of HSA. High-performance affinity chromatography (HPAC) was used to examine the binding of various sulfonfonylurea drugs to in vitro and in vivo glycated HSA samples. Mass spectrometry (MS) experiments were designed to matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS, nano-electrospray ionization time-of-flight MS, and ESI/MS were used to obtain qualitative and quantitative structural information on the glycation-related modifications that were occurring on the glycated HSA samples. The long-term goal is to use such information to better understand the metabolic effects of diabetes on drug binding with HSA and the effects of glycation on the function of this protein, which could be used in the future to develop improved treatment regimens for patients with diabetes.

**Mass Spectrometry**

Mass spectrometry (MS) was used to investigate the structural changes that occurred during the glycation of HSA. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS was used to provide qualitative and quantitative structural information about the glycation-related modifications that occurred on this protein. These results allowed for the identification of these modifications and determine which modifications are occurring near Sulfodine sites. MALDI-TOF MS analysis using isotopic labeling was also used to compare the extent to which these various modifications were occurring throughout the structure of HSA. This latter process (shown in Fig. 6) allowed for a comparison of all in vivo modifications by looking at the amount of non-modified peptides that remained versus a control sample. Sulfodine sites I and II were excised from gHSA and normal HSA, and then those samples were digested with endoproteinase Glu-C and Mass Spectra were obtained (as shown in Fig. 8). The results from the quantitative analysis showed that significant amounts of modifications were taking place at or near Sulfodine sites I and II. Arginine residues R299 and R328 (ASA) showed high or moderate susceptibility to glycation-related modifications. Residues K338, K439, and K428 (via AGC formation) showed high or moderate susceptibility to glycation-related modifications at Sulfodine site II. Nano-electrospray ionization time-of-flight (Nano-ESI-TOF) MS was then used to provide lower limits of detection and better sequence coverage for quantitative studies for glycated HSA. Preliminary studies using this approach with normal HSA has already provided a 89.2% sequence coverage for an unfunctionalized HSA sample that was digested with Lys-C, Glu-C, and trypsin, as shown in Fig. 8.

**Conclusion**

This study examined the metabolic effects of diabetes on the structure and function of glycated HSA. Binding studies by HPAC indicated that there can be significant changes in the site-specific affinities of sulfonfonylurea drugs with glycated HSA. These binding studies are now being expanded to include the use of free fraction analysis to see how new glycation may alter the effective dose of these drugs. Mass spectrometry studies through MALDI/TOF MS were used to investigate glycation-related modifications that may occur on HSA, which included an examination of different types and locations of these modifications. Nano-ESI-TOF MS was used to provide a more detailed analysis of these glycation-related modifications. Future work is planned with MS/MS methods to further investigate these glycation-related modifications.

**References**

R. Matsuda, et al., J Chromatogr B 1580
K.S. Joseph, et al., J Chromatogr B 1587
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**Drug & binding**

<table>
<thead>
<tr>
<th>Sulfonfonylurea Drug</th>
<th>Sulfodine Site I</th>
<th>Sulfodine Site II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetohexamide</td>
<td>1.4-fold</td>
<td>1.1-fold</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>1.3-fold</td>
<td>1.2-fold</td>
</tr>
<tr>
<td>Tolbutamide</td>
<td>1.2-fold</td>
<td>1.1-fold</td>
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</tbody>
</table>

**Table 2** Changes in the binding of various sulfonfonylurea drugs at Sulfodine sites I and II when competing with in vivo glycated HSA gHSA1-gHSA3 or in vivo glycated HSA (CS1-CS2) with normal HSA.