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Introduction

- In 2017, approximately 425 million adults worldwide were living with diabetes.[1] This number is rapidly increasing and is projected to affect 629 million individuals by 2045. [2]
- Diabetes is a disease that is associated with elevated levels of glucose in the bloodstream, resulting in nonenzymatic protein glycation, illustrated in **Figure 2**.[3]
- Human serum albumin (HSA), pictured in **Figure 1**, is the most abundant transport protein in serum with two major drug binding sites (Sudlow sites I and II) and is involved in several physiological processes. [4]
- Sulfonylurea drugs are orally administered drugs that are commonly used to treat type II diabetes and are known to bind tightly to serum proteins, especially HSA.[5]
- Individuals living with diabetes have approximately 20-30% more of their HSA in a glycated form. This can result in modification at both Sudlow sites, thus leading to possible changes in the binding of sulfonylurea drugs. [5]
- The goal of this study is to examine the overall binding of two sulfonylurea drugs with normal HSA vs methyl glyoxal modified-HSA.

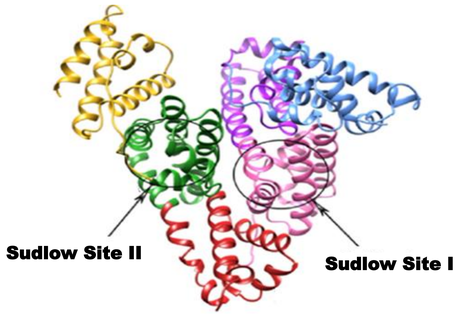


Figure 1. The structure of human serum albumin. The major binding sites on HSA bind bulky and heterocyclic drugs such as R-warfarin (Sudlow site I) and aromatic compounds such as L-tryptophan (Sudlow site II).

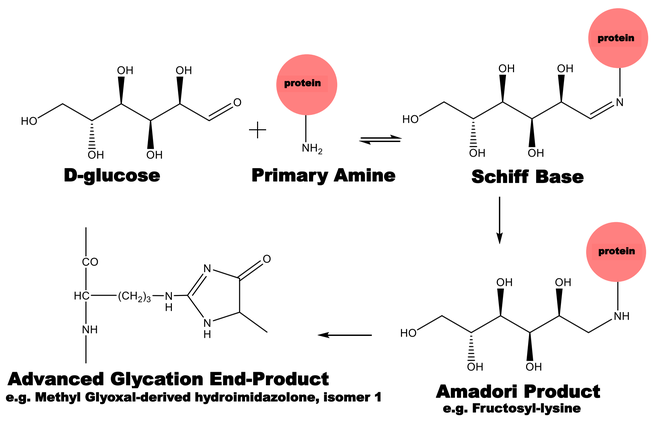


Figure 2. The process of early stage glycation and advanced glycation. Glucose reacts with a free amine group on a protein to form a reversible Schiff base which can then be rearranged to a more stable Amadori product, also known as glycated HSA. Through other pathways such as oxidation, dehydration, and degradation, glycated HSA yields advanced glycation end-products (AGEs).

Methods

- Drug solutions were injected onto 10 mm x 2.1 mm i.d. affinity microcolumns containing normal HSA and HSA modified with methylglyoxal (MGo), as can occur in controlled and advanced diabetes.
- Chromatographic experiments were carried out at a flow rate of 0.5 mL/min and at a temperature of 37 °C.
- The competition studies based on zonal elution were carried out using R-warfarin as a site-specific probe for Sudlow site I and L-tryptophan as a site-specific probe for Sudlow site II.
- These studies were performed with eight different concentrations of acetohexamide and tolbutamide in the mobile phase, spanning from 0 to 25 μM. The same solutions of the drugs were used to make samples of the desired probe at a concentration of 5 μM.

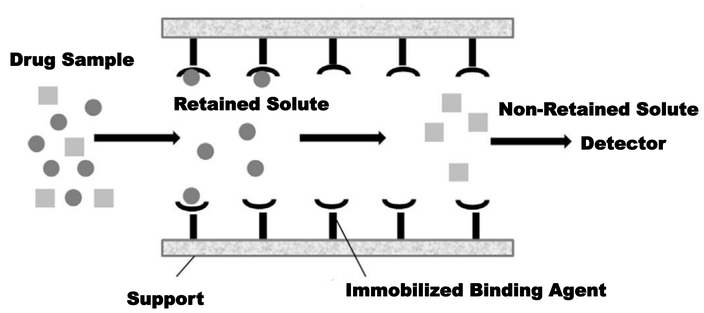


Figure 3. Method of separation for high-performance affinity chromatography (HPAC).

Sulfonylurea Drugs Examined

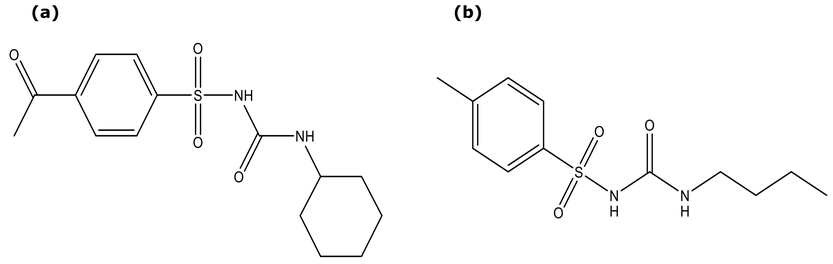
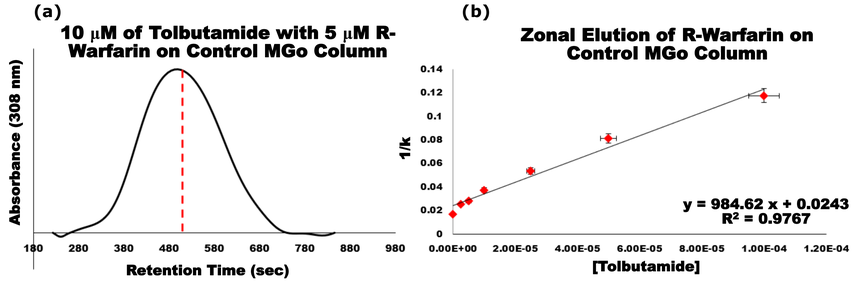


Figure 4. Structures of sulfonylurea drugs examined: acetohexamide (a) and tolbutamide (b).

Chromatographic Results



$$\frac{1}{k} = \frac{K_{aA}\, V_M\, [A]}{K_{aP}\, m_L} + \frac{V_M}{K_{aP}\, m_L}$$

Eq. 1. Equation used to calculate association equilibrium constant where k: retention factor, K_{aA} and K_{aP}: association equilibrium constant for the analyte and probe at the competition site, [A]: concentration of analyte, V_M: void volume, and m_L: moles of common binding sites

Figure 5. A typical chromatogram for injection of a single concentration of drug with a site-specific probe. This chromatogram represents 10 μM of tolbutamide with the R-warfarin probe where the dashed line represents the retention time of the probe in the absence of drug (a). The linear plot describing the direct competition between the probe and competition drug at a single site, as described by equation 1. This plot represents tolbutamide competition with the R-Warfarin probe (b).

Overall Binding Results

Table 1. Ka for Drugs at Sudlow Site I

	Acetohexamide (x 10^4 M^{-1})	Tolbutamide (x 10^4 M^{-1})
Normal HSA	4.20 (±0.1)	5.50 (±0.04)
Control Methyl Glyoxal HSA	1.62 (±0.2)	4.05 (±0.1)
Diabetic Methyl Glyoxal HSA	5.76 (±0.1)	3.81 (±0.1)

Table 1. The site-specific association equilibrium constants for normal HSA, control MGo-modified HSA, and diabetic MGo-modified HSA where the R-Warfarin probe was employed.

Table 2. Ka for Drugs at Sudlow Site II

	Acetohexamide (x 10^4 M^{-1})	Tolbutamide (x 10^4 M^{-1})
Normal HSA	13.0 (±0.1)	5.30 (±0.04)
Control Methyl Glyoxal HSA	7.53 (±0.1)	4.58 (±0.1)
Diabetic Methyl Glyoxal HSA	1.26 (±0.2)	3.92 (±0.06)

Table 2. The site-specific association equilibrium constants for normal HSA, control MGo-modified HSA, and diabetic MGo-modified HSA for the L-Tryptophan probe.

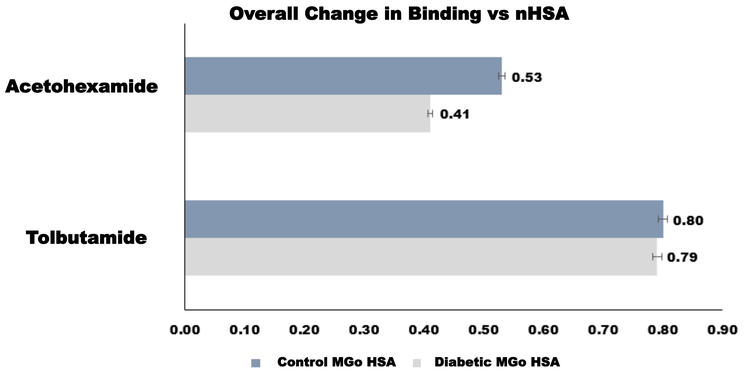


Figure 6. Changes in the binding of drugs when comparing control MGo-modified HSA vs normal HSA (dark grey) and diabetic MGo-modified HSA vs normal HSA (light grey).

Conclusions

- The site-specific association equilibrium constants for acetohexamide and tolbutamide were determined for normal HSA and control and diabetic MGo-modified HSA, **Tables 1 and 2**.
- An approximate 0.4-fold to 0.8-fold decrease in binding was found when comparing normal HSA vs control MGo-modified HSA and normal HSA vs diabetic MGo-modified HSA, respectively, shown in **Figure 6**.
- HPAC zonal competition method was demonstrated as a faster, alterative approach for studying drug-protein interactions over other techniques such as frontal analysis.

References

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Acknowledgements

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