

FRONTAL ANALYSIS STUDIES OF COUMARIN DERIVATIVES FOR A VIABLE ALTERNATIVE TO WARFARIN FOR DRUG-PROTEIN BINDING MEASUREMENTS

Krina S. Joseph and David S. Hage, Department of Chemistry, University of Nebraska, NE 68588

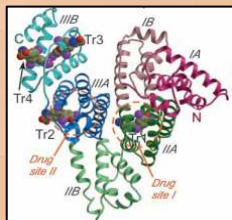
Abstract

Warfarin is often used as a competing agent in HPLC-based drug-protein displacement studies performed with columns containing the protein human serum albumin (HSA). Warfarin has proven useful in such studies due to its relatively high affinity for a single binding site on HSA (Sudlow site I). However, warfarin is a chiral compound, with each enantiomer having a different binding affinity for this protein. In this study, various related compounds (coumarin, 7-hydroxycoumarin, and 7-hydroxy-4-methylcoumarin) were studied as alternative competing agents for warfarin in HSA binding studies.

Frontal analysis was used with immobilized HSA columns to examine these binding processes. Association equilibrium constants of $4.6 \times 10^3 \text{ M}^{-1}$, $1.47 \times 10^4 \text{ M}^{-1}$, and $2.07 \times 10^4 \text{ M}^{-1}$ were obtained for coumarin, 7-hydroxycoumarin, and 7-hydroxy-4-methylcoumarin, respectively, with HSA at pH 7.4 and 37°C. All of these compounds had lower retention than warfarin, decreasing the time needed to run chromatographic experiments for HSA binding studies. All of the coumarins studied also had single-site binding with HSA, which is believed to occur at Sudlow site I. These properties should make these compounds useful alternatives to warfarin in studies of drug-protein binding with HSA.

Introduction

Human serum albumin (HSA) is the most abundant plasma protein in the circulatory system. HSA is an important transport protein in the body with a typical concentration of 50 g/L. It has a molecular mass of 66,438 Da and is a single polypeptide chain of 585 amino acids with 17 disulfide bonds.



The binding of HSA to drugs is important in determining drug activity, toxicity, excretion, and metabolism. This binding affects the pharmacological and pharmacokinetic properties of many drugs. To study this binding there are some well-characterized molecules known to bind to specific site on HSA. Warfarin is one of these molecules said to bind to a site known as Sudlow Site I. Our goal was to find a suitable replacement for warfarin that had similar affinity to HSA as well as being more cost-effective.

Method

Column preparation

HSA supports were prepared by covalently attaching HSA to diol-bonded silica through the Schiff base method with HSA column content determined by BCA analysis as $33.7 \pm 8 \mu\text{g HSA/mg silica}$. This support was packed into a stainless steel column (5 cm x 4.6 mm ID) and the column was stored and used with 0.067 M potassium phosphate buffer (KPB), pH 7.4. Frontal analysis was used to study solute-protein binding with these columns.

Frontal Analysis

Frontal analysis is performed when a solution with known concentration of analyte is continuously applied to a column with an immobilized ligand (HSA in this case). In our studies, the analytes were various coumarin compounds: coumarin, 7-hydroxycoumarin, and 7-hydroxy-4-methylcoumarin. As the analyte binds to ligand, the column becomes saturated and gives a characteristic breakthrough curve, as shown in the Results section.

If fast association/dissociation kinetics are present, the mean position of the breakthrough curve can be related to the:

- Concentration of analyte
- Amount of ligand in the column
- Association equilibrium constant for analyte-ligand binding

The following equation has been used to study the binding affinity of coumarin compounds to HSA. This equation is used in the case of single-site binding:

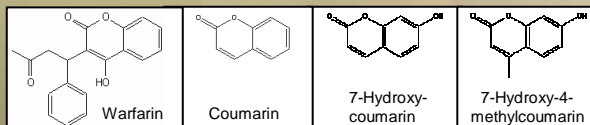
$$\frac{1}{m_{Lapp}} = \frac{1}{K_A m_L [A]} + \frac{1}{m_L}$$

m_{Lapp} : apparent moles of analyte required to saturate the column

m_L : total moles of binding sites in the column

K_A : equilibrium constant for A with ligand

[A]: analyte concentration

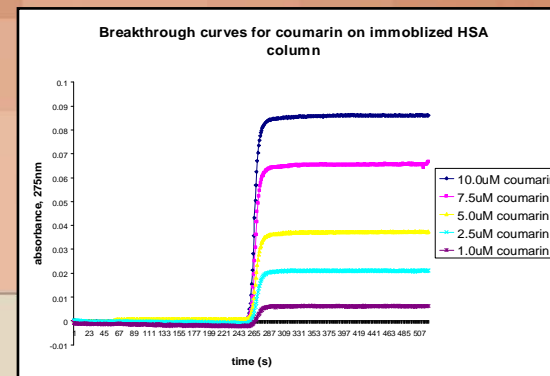


Results

The results indicate that all coumarin compounds studied had primary binding on HSA with the following association equilibrium constants (K_A) and moles of ligand (m_L) at 37°C:

Compound	$K_A \text{ (M}^{-1}\text{)}$	$m_L \text{ (moles)}$
Warfarin*	2.5×10^5	NA
Coumarin	4.6×10^3 ($\pm 1.8 \times 10^3$)	2.8×10^{-7} ($\pm 1.1 \times 10^{-7}$)
7-Hydroxy-coumarin	1.5×10^4 ($\pm 0.3 \times 10^4$)	7.0×10^{-7} ($\pm 1.6 \times 10^{-7}$)
7-Hydroxy-4-methylcoumarin	2.1×10^4 ($\pm 0.2 \times 10^4$)	5.5×10^{-7} ($\pm 1.0 \times 10^{-7}$)

*Reference values averaging the two warfarin enantiomers



Future Work

In the future I will run competition studies with warfarin to confirm that binding of these other compounds is occurring at the "warfarin site" and test other coumarin compounds.

Acknowledgments

This work was supported by NIH grant R01 GM044931