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Analysis of Hydroxychloroquine Interaction with Serum Proteins by High Performance Affinity Chromatography

Kyungah Suh
University of Nebraska-Lincoln, kyungah.suh@huskers.unl.edu

Sadia Sharmeen
University of Nebraska-Lincoln, sharmeen.sadia@huskers.unl.edu

David S. Hage
University of Nebraska-Lincoln, dhage1@unl.edu

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Kyungah Suh, Sadia Sharmeen, David S. Hage
Department of Chemistry, University of Nebraska-Lincoln, Lincoln, Nebraska 68588

Introduction

Personalized medicine or precision medicine has been an emerging market for better treatments of patients under varying physiological conditions. To improve the on growing development of personalized medicine, a higher understanding of drug-protein binding between serum transport proteins and the unbound form of pharmaceuticals is needed.

Human Serum Albumin (HSA) and Alpha1-acid glycoprotein (AGP) play a crucial role in pharmacokinetics as they are two most abundant transport serum proteins in body circulatory system. Inter-individual variabilities of serum proteins, such as the physiological condition of patients, have been believed to alter the drug-serum protein binding. Fig.1 shows an example of the cause of inter-individual variabilities of AGP by various degree of glycans.

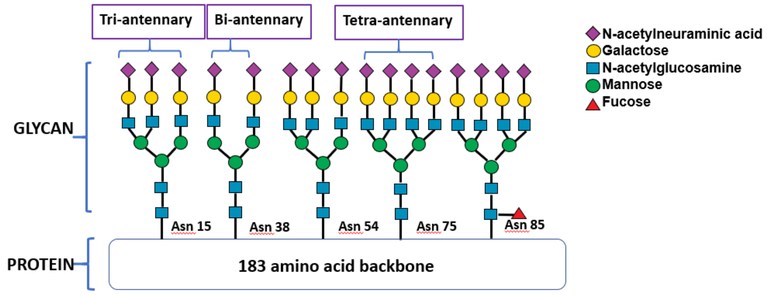

Figure 1. Five carbohydrate glycosylation sites of AGP

Hydroxychloroquine (HCQ) is an antimalarial drug and has been used to treat rheumatic disease such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) due to its immunomodulatory effects for rheumatic diseases.

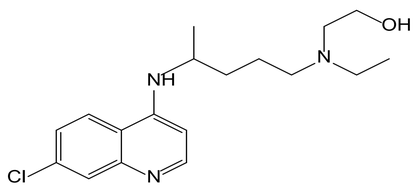

Figure 2. Structure of Hydroxychloroquine

In this study, a lectin affinity chromatography, a zonal elution, and ultrafast affinity extraction were performed to investigate hydroxychloroquine-serum protein interactions.

- **NIH R01 DK069629**

- S. Beeram, X. Zheng, K. Suh, D. S. Hage, Characterization of solution-phase drug-protein interactions by ultrafast affinity extraction. A review, Methods, 146 (2018), 46-57.
- T. Fournier, N. Medjoubi-N, D. Porquet, Biochim. Biophys. Acta. 1482 (2000) 157-171
- I. Ben-Zvi, S. Kivity, P. Langevitz, Y. Shoenfeld, Hydroxychloroquine: from malaria to autoimmunity. *Clin Rev Allergy Immunol*. 2012;42(2):145–153.

GOAL OF STUDY

Investigate hydroxychloroquine-serum protein interaction through HSA and AGP glycoforms immobilized affinity microcolumns.

METHODS

Lectin Affinity Chromatography

- Concanavalin A (Con A) can bind to complex type biantennary glycans (weak to moderate binding). A Con A immobilized column can be used to separate AGP with biantennary glycans from normal AGP.

Protein Immobilization

- Entrapment vs Schiff-base method

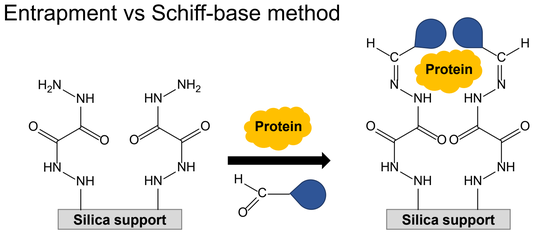

Figure 3. Entrapped serum protein by using mildly oxidized glycogen

Zonal Elution

- Retention factor analysis – determination of solute-protein interaction

$$k' = K_a \frac{m_L}{V_M} \qquad k' = \frac{t_{r,analyte} - t_{r,NaNO3}}{t_{r,NaNO3} - t_{void}}$$

k' : Retention factor
K_a : Association equilibrium constant
m_L : Protein content
V_M : Column void volume

Ultrafast Affinity Extraction

- Rapid extraction of free drug fractions by using affinity microcolumn

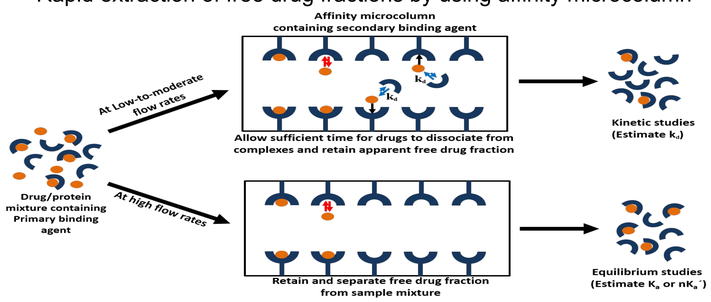

Figure 4. General scheme for ultrafast affinity extraction

$$K_a = \frac{1 - F_0}{F_0\,([P]_{total} - [D]_{total} + [D]_{total}F_0)} \qquad ln\frac{(1 - F_0)}{(1 - F_t)} = k_d t$$

D : Drug
F_0 : Original free fraction
F_t : Apparent free fraction
P : Protein

RESULTS

Lectin Affinity Chromatography

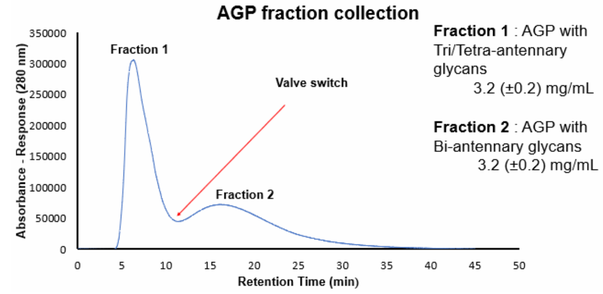

Figure 5. AGP fractions separation by Con A lectin affinity chromatography

Zonal Elution

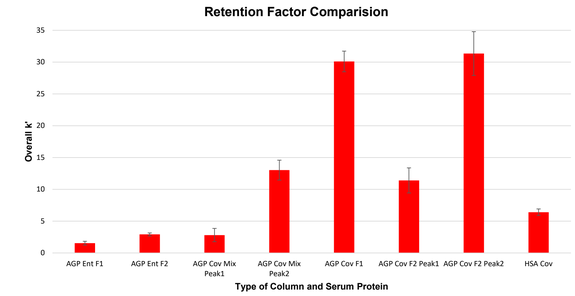

Figure 6. Comparison of retention factors of hydroxychloroquine

Ultrafast Affinity Extraction

HSA		AGP	
Free Fraction	nKa(M^{-1})	Free Fraction	nKa(M^{-1})
63%	1.65(±0.10)X10^4	84%	1.02(±0.16)X10^4

Conclusion

This study examined the interaction between hydroxychloroquine and serum proteins, HSA and AGP. AGP fractions with bi-antennary glycans and tri- tetra-antennary were separated by lectin affinity chromatography using Concanavalin A and were used for affinity microcolumns. Entrapment of serum proteins can result in the use of intact serum proteins.
Zonal elution results indicated the different contribution of the different glycoforms of AGP onto hydroxychloroquine binding. Ultrafast affinity extraction provided a relatively fast and precise binding information of hydroxychloroquine to serum proteins. The information obtained by this study can provide a better understanding for estimating hydroxychloroquine-serum protein interactions.