Application Note # 121

Interactions of small molecular weight drugs with human serum albumin

Human serum albumin (HSA) is immobilized on a sensor slide with amino coupling chemistry. Interaction of small molecule weight drugs with HSA was studied with MP-SPR instrument (Figure 1). Steady state affinity as well as kinetic of the binding was determined from measurements.

Introduction

Human serum albumin (HSA) is the most important protein in blood plasma, due to it´s abundant amount. HSA main functions are carry fatty acids and maintain blood colloidal osmotic pressure, among that it is an important carrier for many hormones and drugs, especially hydrophobic ones. Drug binding to HSA increases drug half-life and lowers the free drug concentration in blood, which makes it extremely important for clinical care. Early drug discovery, the plasma protein binding is important to determine because it is used to evaluate drug dosing needs and clearance from the body.

Materials and methods

Carboxymethyl dextran (CMD) coated sensor-slide was used to immobilize HSA with amine coupling chemistry (Figure 2). The immobilization was performed at 21 °C using 5 mM MES (2-(N-morpholino) ethanesulfonic acid) pH 5.0 as running buffer. Surface was activated with EDC/NHS and deactivated with 5mM ethanolamine pH 8.0.

Small molecule weight drugs indomethacin, quinidine and naproxen (357.8, 324.4 and 230.3 g/mol) interaction with immobilized HSA was determined with SPR Navi 220A instrument. Experiment was performed using pH 7.4 PBS (phosphate buffered saline) as running buffer at 21 °C. Five samples concentration serie (0.1-50 µM) was measured. Quinidine was found to be relatively weak binder so higher concentrations were measured (10-500 µM). Samples contained 3 % of DMSO (dimethylsulfoxide) to improve drugs solubility. The kinetics and affinity of the reaction were analyzed using TraceDrawer.

Results and discussion

Immobilization of HSA was successfully repeated several times following BioNavis immobilization protocol. Average amount of immobilized HSA was 2000 ng/cm² on a sensor-surface.

All three small molecule weight drugs steady state affinity to HSA as well as kinetic of the binding was successfully determined with MP-SPR measurement (Table 1). Indomethacin affinity plot and kinetic analyse is presented in Figure 3 and Figure 4. Quinidine binding was measured two times separately and equals binding values confirmed repeatability of the results.

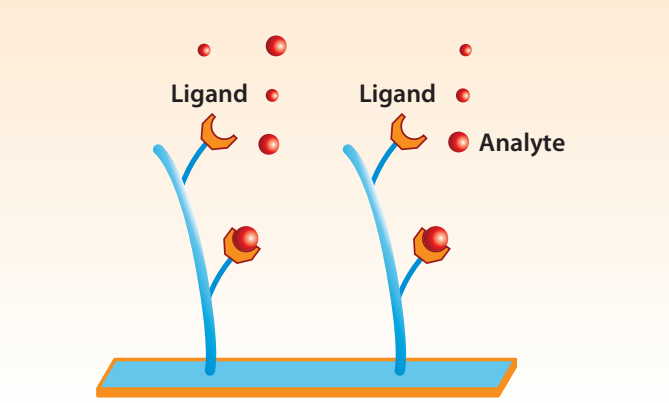

Figure 1. Ligand, HSA is immobilized in a 3D dextran matrix. Analyte, drug molecules bind to the HSA.

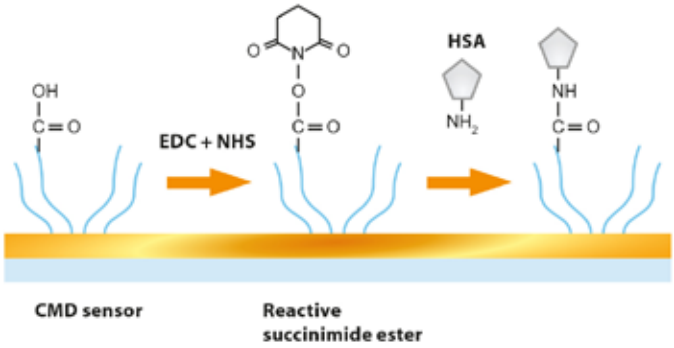

Figure 2. Human serum albumin binding to the carboxymethyl dextran sensor surface with amine coupling.

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		KD (M)	Bmax	ka (1/(M*s))	kd (1/s)
Indomethacin	Steady state analyse	2.25E-5	38.8		
	Kinetic analyse	2.13E-5	41.31	1.83E3	3.89E-2
Quinidine	Steady state analyse 1	1.53E-3	12.8		
	Steady state analyse 2	1.69E-3	46.5		
	Kinetic analyse	1.39E-3	11.71	3.11E3	4.32E0
Naproxen	Steady state analyse	4.25E-5	38.6		
	Kinetic analyse	3.28E-5	35.91	9.32E3	3.06E-1

Table 1. Indomethacine, quinidine and naproxen interaction with HSA. KD = steady state affinity, Bmax= maximum binding capacity, ka = association rate constant, kd = dissociation rate constant

Conclusions

MP-SPR instrument is excellent tool to measure direct affinities of small molecules. With instrument real-time and label free measurements not only steady state but also kinetic of the binding is effectively determined. These types of measurements can be utilized in pre-clinical drug screening and biochemical drug development.

Recommended instrumentation for reference assay experiments
SPR Navi™ 220A (contains autosampler for 96 samples)
Sensor surfaces: CMD-3D sensor-slide
Software: SPR Navi™ Control, DataViewer, TraceDrawer

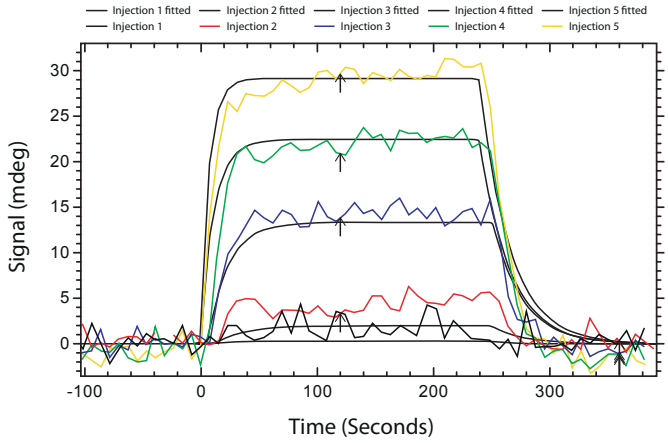

Figure 3. Indomethacin interaction with immobilized HSA

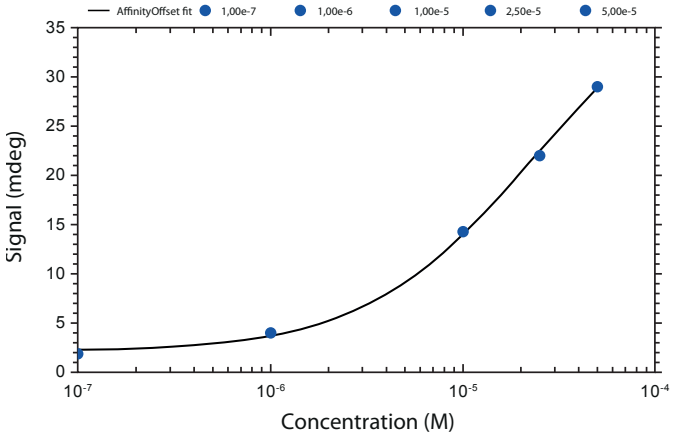

Figure 4. Affinity plot of Indomethacine

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