Instrumentation Science and Technology, 42:475–485, 2014

ISSN: 1073-9149 print/1525-6030 online
DOI: 10.1080/10739149.2014.880149

Taylor & Francis
Taylor & Francis Group

DETERMINATION OF BOVINE SERUM ALBUMIN CONJUGATED DRUGS BY IMMOBILIZATION AS MICROARRAYS AND OBLIQUE-INCIDENCE REFLECTIVITY DIFFERENCE MICROSCOPY

Yung-Shin Sun,[1,2] Kit S. Lam,[3,4] and X. D. Zhu[2]

[1]*Department of Physics, Fu-Jen Catholic University, New Taipei City, Taiwan*
[2]*Department of Physics, University of California at Davis, Davis, California, USA*
[3]*Department of Biochemistry and Molecular Medicine, School of Medicine, University of California at Davis, Sacramento, California, USA*
[4]*Division of Hematology and Oncology, Department of Internal Medicine, School of Medicine, University of California at Davis, Sacramento, California, USA*

□ *Human serum is a mixture of various proteins which may interact with drugs. Therefore, it is of interest to investigate the binding kinetics of pharmaceuticals with their corresponding antibodies in serum. In this article, microarrays and a label-free biosensor were used to study these interactions. Microarrays provide a high-throughput platform for characterizing biomolecular interactions, and the label-free oblique-incidence reflectivity difference biosensor avoids the drawbacks of fluorescence-based methods. The experimental results show that the binding affinities between most of the drugs and their antibodies were reduced in human serum because the bulky proteins block the access to or reduce the stability of the reaction complexes. Therefore, one should be mindful when in vitro or in vivo testing the efficiency of potential drugs and their antibodies.*

Keywords drug-antibody binding affinity, label-free, microarray, oblique-incidence reflectivity difference (OI-RD)

INTRODUCTION

Human serum is a mixture of blood proteins with a total concentration of approximately 70 g/L. The most abundant proteins in human serum are the albumins, having concentrations of 34–50 g/L (500–750 μM).[1] Human serum albumin creates osmotic pressure and helps transport lipids, hormones, vitamins, and metals. The second major class of blood proteins

Address correspondence to Yung-Shin Sun, Department of Physics, Fu-Jen Catholic Universit, No. 510, Zhongzheng Rd., Xinzhuang Dist., New Taipei City 24205, Taiwan, R.O.C.. E-mail: 089957@mail.fju.edu.tw

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/list.

are the immunoglobulins. Other blood proteins include regulatory proteins, α1-antitrypsins, α2-macroglobulins, and lipoproteins. Human serum proteins, especially albumins, are known to interact with drugs. These proteins serve as anchors for drugs with poor aqueous solubility, and their bindings affect both the activity and disposition of these drugs.[2] For example, human serum albumin has a specific binding site to flufenamic acids, ibuprofen, and benzodiazepine, and also several non-specific binding sites to other ligands.[3–5] Therefore, it is of interest to study the properties and functions of drugs in the presence of human serum.

A microarray is composed of hundreds to tens of thousands of biomolecules arranged in a regular pattern on a solid substrate. It provides a platform for screening the interaction between a large number of targets and a single probe in a high-throughput manner. These biomolecular targets may be DNA, proteins, sugars, small molecules, or cells, and usually they are immobilized on functionalized glass slides with a spot size of about 100–200 μm. Traditionally, microarrays are detected with fluorescence-based methods because of their sensitivity. However, it has been reported that labeling probes change the binding kinetics of probe-target interactions.[6,7] To avoid this drawback, we developed the oblique-incidence reflectivity difference (OI-RD) technique for real-time, label-free, and in-situ detection of biomolecular interactions in microarray format. OI-RD, the most sensitive form of optical elliposometry, measures the differential changes in the phase and magnitude of the reflectivities for *p*-polarized and *s*-polarized components of monochromatic light.[8] In the past, OI-RD-based microscopes have been used for studying various protein-protein,[9] sugar-protein,[6] cell-protein,[10] and small molecule-protein interactions,[11,12] as well as for drug screening[13–15] and micelle characterization.[16]

In this article, six bovine serum albumin-conjugated drugs were immobilized as microarrays and subsequently detected by an OI-RD microscope for studying their bindings to corresponding anti-drug antibodies in the presence of human serum. The first drug to be investigated is digoxin, which is widely used in the treatment of various heart conditions such as atrial fibrillation and atrial flutter. Anti-digoxin has been applied to treat potentially life-threatening digoxin and digoxin toxicity.[17] The second drug is methamphetamine, which is a psychostimulant and sympathomimetic drug. Mouse-derived anti-methamphetamine monoclonal antibodies were used as potential pharmacokinetic antagonists.[18] The third drug is morphine, which is a highly potent opiate analgesic drug. A number of antibodies with specificity to morphine have been purified and produced.[19,20] The fourth drug is phenobarbital, which is the most widely used anticonvulsant. An anti-phenobarbital, antibody-based immunoassay was used in serum.[21] The fifth drug is tetrahydrocannabinol, which binds to the cannabinoid receptor located in the central nervous system. An anti-tetrahydrocannabinol

antibody-based competitive immunoassay with fluorescence quenching was employed for the determination of tetrahydrocannabinol in saliva.[22] The last is theophylline, which is a methylxanthine drug used in therapy for respiratory diseases such as chronic obstructive pulmonary disease or asthma. The thermodynamic parameters of mouse anti-theophylline monoclonal antibody binding were studied using isothermal titration microcalorimetry.[23] All drug-bovine serum albumin conjugates were commercially available for immobilization on epoxy-coated glass slides. The anti-drug antibodies mixed with human serum at different dilutions reacted with the drug microarray to acquire the binding curves. The results show that the binding affinities were influenced by human serum. For that reason, the combination of microarrays and the oblique-incidence reflectivity difference microscope may be applicable to characterizing the binding of drugs and their antibodies in a convenient and high-throughput manner.

Materials and Methods

Drug Targets and Protein Probes

Digoxin-bovine serum albumin conjugate, the monoclonal antibody to digoxin (mouse), the monoclonal antibody to tetrahydrocannabinol (mouse), the theophylline-bovine serum albumin conjugate, and the monoclonal antibody to theophylline (mouse) were purchased from OEM Concepts (Saco, ME). Methamphetamine-N-bovine serum albumin conjugate, monoclonal antibody to methamphetamine (mouse), morphine-bovine serum albumin conjugate, phenobarbital-bovine serum albumin conjugate, monoclonal antibody to phenobarbital (raised in mouse), and Δ8-tetrahydrocannabinol-bovine serum albumin conjugate were purchased from Biodesign (Saco, ME). The monoclonal antibody to morphine (mouse) was purchased from Fitzgerald (Concord, MA). Figure 1 (A) shows the chemical structures of six drugs used in the present study. Antibody probes were dissolved in phosphate buffered saline in the presence of serum at different dilutions. Human serum was provided by Dr. Kit Lam (UC Davis Medical Center). All bovine serum albumin conjugates and proteins were used as received without further purification.

Preparation of Target Microarrays

The drug-bovine serum albumin conjugates were dissolved in phosphate buffered saline and further diluted into printing solutions with concentrations decreasing successively by a factor of 0.5 from 16 to 1 μM. Duplicates (in different columns) of each conjugate at each printing concentration were printed into microarrays on epoxy-coated glass slides (CEL Associates, Pearland, TX) with an OmniGrid 100 contact-printing arrayer (Digilab, Holliston, MA). Unmodified bovine serum albumin at a concentration of

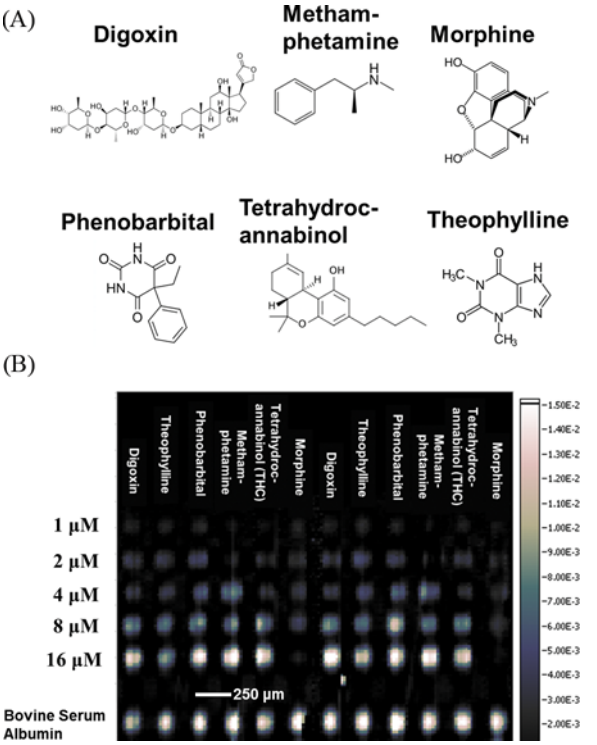

FIGURE 1 (A) Chemical structures of 6 drugs used in this study. (B) OI-RD image of a drug microarray immersed in phosphate buffered saline buffer. All drug-bovine serum albumin conjugates were printed at five concentrations as duplicates. Unmodified bovine serum albumin was printed as a negative control.

8.3 µM was also printed as a negative control. The microarray-bearing slides were stored for at least 24 hrs before further processing. Figure 1(B) shows the OI-RD image of a drug microarray immersed in phosphate buffered saline buffer. All drug-bovine serum albumin conjugates were printed at five concentrations with duplicates. The spot size was approximately 100 µm in diameter, and the center-to-center spacing was about 250 µm.

OI-RD Microscope

The working principles of the OI-RD microscope have been described in detail in earlier publications.[7,9] The reflectivities for light linearly polarized parallel to the plane of incidence (*p*-polarized) and perpendicular to the plane of incidence (*s*-polarized) are different. When the light has an oblique angle of incidence and is reflected from a surface, the reflectivities of its *s*- and *p*-polarized components will change disproportionately to the physical and chemical properties of this surface, such as thickness, mass density, and dielectric constant. By polarization-modulated nulling, OI-RD directly measures the difference in reflectivity change (both magnitude

and phase) between *p*- and *s*-polarized components of the laser beam. For real-time measurements, OI-RD signals from both spots (target pixels) and bare substrates (reference pixels) were monitored, and the difference was used as the binding curve.

Experimental Setup and Procedure

Before assembling into the fluidic chamber, a microarray-bearing slide was immersed in phosphate buffered saline overnight to remove excess unbound targets and buffer precipitates. After assembly, the slide was blocked with bovine serum albumin, reacted with blank serum solution (serum without antibody probes, 1:1000 diluted in phosphate buffered saline), and then reacted with desired protein probes. The flow rate was set to 30 mL/min for 10 s and then reduced to 0.01 mL/min for 20 min (association phase). Subsequently, the probe solution was replaced with buffer only at a flow rate of 30 mL/min for 10 s and then at 0.01 mL/min to observe the dissociation of captured probes (dissociation phase). Rate constants (association rate k_{on} and dissociation rate k_{off}) and equilibrium dissociation constants ($K_D \equiv k_{off}/k_{on}$) for probe-target interactions were deduced by globally fitting a set of binding curves, each of which corresponding to a concentration of the probe to the Langmuir two-site model.[7,11] A larger K_D value implied that the reaction had a weaker binding affinity, and a smaller value suggested that the reaction had a stronger binding affinity. In our experience, the binding between a solution-phase probe (e.g., antibody) and surface-immobilized target (e.g., drug-bovine serum albumin conjugates) was better described by treating each target as having two or more stereochemically different configurations. Therefore, the binding curve of an antibody-drug reaction was fitted to the Langmuir two-site model with fitting equations detailed in the literature.[7,11]

RESULTS AND DISCUSSION

The drug microarray was pre-treated with blank serum to avoid competitive binding to the targets between serum proteins and anti-drug antibodies. The microarray was then reacted with anti-drug antibodies in the presence of human serum. Figure 2 shows the association-dissociation curves for reactions of the drugs with their corresponding antibodies in the presence of serum at different dilutions (no serum, 1:10000, 1:5000, and 1:1000 diluted in phosphate buffered saline). Spots with a printing concentration of 16 μM were selected. At $t = 0$, the solution was changed from phosphate buffered saline or blank serum solution to an antibody solution at a desired concentration. At $t = 1200$ s, the ambient was restored back to phosphate buffered saline or blank serum solution for dissociation. The binding curves in each

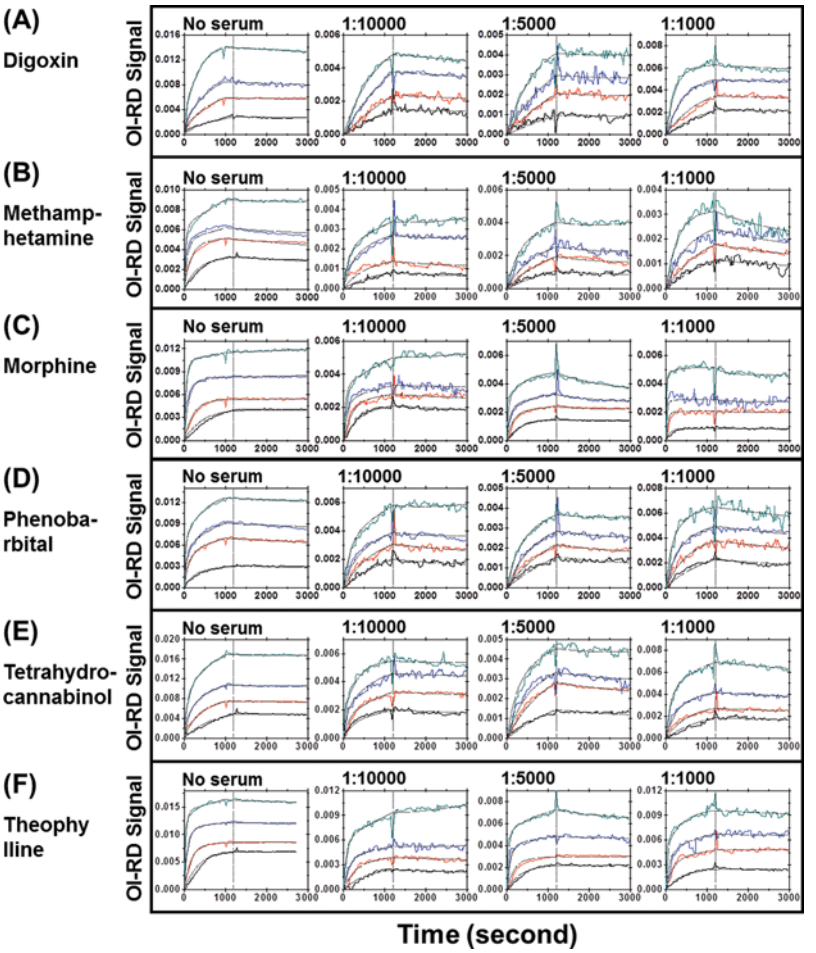

FIGURE 2 Association-dissociation curves of (A) digoxin, (B) methamphetamine, (C) morphine, (D) phenobarbital, (E) tetrahydrocannabinol, and (F) theophylline reactions with their corresponding anti-drug antibodies in the presence of serum at different dilutions (no serum, 1:10000, 1:5000, and 1:1000 diluted in phosphate buffered saline). Titrated concentrations of antibodies are 50 (black, bottom), 100 (red), 200 (blue), and 400 (green, top) nM. The curves were fitted globally to the two-site model (fits are in dashed lines) with fitting parameters listed in Table 1.

panel were fitted globally to the Langmuir two-site model with reaction rates ($k_{on}^{(1)}$, $k_{off}^{(1)}$ for the first site, and $k_{on}^{(2)}$, $k_{off}^{(2)}$ for the second site) and relative coverages ($\theta^{(1)}$ for the first site and $\theta^{(2)}$ for the second site) were the global fitting parameters. The fits are shown as dotted lines in Figure 2 and fitting parameters are listed in Table 1. The fitting parameters from duplicate target spots are the same within the standard deviations.

For anti-digoxin reacting with surface-immobilized digoxin, the K_D at the first site increased as the concentration of serum increased ($K_D = 4.9$ nM without serum, $K_D = 12$ nM with 1:10000 serum, $K_D = 18.4$ nM with 1:5000

TABLE 1 Fitting parameters and calculated K_D values to experimentally measured association-dissociation curves for anti-drug antibodies reacting with corresponding drug-bovine serum albumin conjugates in the presence of human serum. $k_{on}^{(1or2)}, k_{off}^{(1or2)}, K_D^{(1or2)}$, and $\theta^{(1or2)}$ are the association rate, dissociation rate, equilibrium dissociation constant, and relative coverage of the first or second site, respectively

Drug and serum dilution	$\theta^{(1)}$	$k_{on}^{(1)}$ $(\mathrm{Ms})^{-1}$	$k_{off}^{(1)}$ $(\mathrm{s})^{-1}$	$K_D^{(11)}$ (nM)	$k_{on}^{(2)}$ $(\mathrm{Ms})^{-1}$	$k_{off}^{(2)}$ $(\mathrm{s})^{-1}$	$K_D^{(2)}$ (nM)
Digoxin							
No Serum	0.82	6.78×10^{-6}	3.32×10^{-5}	4.9 ± 0.6	6.31×10^{-5}	1.53×10^{-5}	0.243 ± 0.02
1:10000	0.58	3.22×10^{-6}	3.87×10^{-5}	12.0 ± 1.1	3.22×10^{-6}	3.87×10^{-5}	12.0 ± 3.2
1:5000	0.41	4.32×10^{-6}	7.96×10^{-5}	18.4 ± 3.1	1.61×10^{-6}	$<1.2\times10^{-6}$	$<0.745\pm0.09$
1:1000	0.4	5.78×10^{-6}	1.95×10^{-4}	33.8 ± 5.2	1.42×10^{-5}	$<3.9\times10^{-6}$	$<0.276\pm0.02$
Methamphe-tamine							
No Serum	0.25	6.5×10^{-5}	5.57×10^{-5}	0.857 ± 0.1	4.72×10^{-7}	$<1.1\times10^{-6}$	$<2.3\pm0.5$
1:10000	0.69	2.99×10^{-6}	2.35×10^{-6}	0.793 ± 0.09	3.87×10^{-5}	$<4.1\times10^{-6}$	$<0.106\pm0.02$
1:5000	0.75	1.55×10^{-7}	9.54×10^{-7}	6.2 ± 0.8	5.8×10^{-6}	$<1.6\times10^{-6}$	$<0.276\pm0.03$
1:1000	0.77	5.47×10^{-6}	2.91×10^{-4}	50.7 ± 6.1	1.74×10^{-5}	$<3.7\times10^{-6}$	$<0.213\pm0.04$
Morphine							
No Serum	0.2	1.59×10^{-6}	$<1.1\times10^{-5}$	$<6.9\pm0.5$	4.81×10^{-5}	$<1.1\times10^{-6}$	$<0.023\pm0.01$
1:10000	0.31	6.67×10^{-6}	4.32×10^{-5}	4.3 ± 0.6	5.69×10^{-5}	$<2.1\times10^{-6}$	$<0.037\pm0.01$
1:5000	0.62	1.56×10^{-5}	1.29×10^{-4}	8.1 ± 0.7	1.55×10^{-4}	$<3.1\times10^{-6}$	$<0.02\pm0.01$
1:1000	0.66	1.39×10^{-4}	8.97×10^{-5}	0.62 ± 0.04	3.03×10^{-4}	$<3.9\times10^{-6}$	$<0.013\pm0.01$
Phenobarbital							
No Serum	0.42	1.16×10^{-4}	6.15×10^{-5}	0.53 ± 0.04	6.7×10^{-6}	$<2.8\times10^{-6}$	$<0.418\pm0.05$
1:10000	0.22	2.28×10^{-5}	1.01×10^{-4}	4.4 ± 0.5	9.58×10^{-7}	$<1.9\times10^{-6}$	$<2.0\pm0.3$
1:5000	0.62	8.23×10^{-6}	5.46×10^{-5}	6.6 ± 0.7	2.45×10^{-6}	$<3.8\times10^{-6}$	$<1.6\pm0.3$
1:1000	0.6	4.77×10^{-6}	2.14×10^{-4}	44.9 ± 6.2	2.84×10^{-5}	$<4.6\times10^{-6}$	$<0.162\pm0.02$

(Continued)

TABLE 1 Continued

Tetrahydroc-annabinol							
No Serum	0.59	2.65×10^{-6}	1.87×10^{-5}	7.1 ± 0.8	3.71×10^{-5}	$<2.3 \times 10^{-6}$	$<0.062 \pm 0.008$
1:10000	0.58	5.19×10^{-6}	7.35×10^{-5}	14.2 ± 1.2	5.87×10^{-5}	$<4.1 \times 10^{-6}$	$<0.07 \pm 0.007$
1:5000	0.89	3.65×10^{-6}	5.66×10^{-5}	15.5 ± 2.1	4.58×10^{-5}	$<2.2 \times 10^{-6}$	$<0.048 \pm 0.004$
1:1000	0.58	2.79×10^{-6}	1.16×10^{-4}	41.6 ± 5.1	3.12×10^{-5}	$<2.1 \times 10^{-6}$	$<0.067 \pm 0.008$
Theophyllin							
No Serum	0.38	2.01×10^{-5}	6.84×10^{-6}	0.335 ± 0.04	8.04×10^{-5}	$<3.9 \times 10^{-6}$	$<0.049 \pm 0.006$
1:10000	0.58	2.83×10^{-5}	1.26×10^{-5}	0.447 ± 0.05	3.61×10^{-6}	$<2.3 \times 10^{-6}$	$<0.637 \pm 0.04$
1:5000	0.45	1.21×10^{-5}	8.75×10^{-5}	7.2 ± 0.9	8.86×10^{-5}	$<3.6 \times 10^{-6}$	$<0.041 \pm 0.005$
1:1000	0.48	5.79×10^{-6}	8.44×10^{-5}	14.6 ± 2.1	6.72×10^{-5}	$<3.9 \times 10^{-6}$	$<0.058 \pm 0.007$

serum, and $K_D = 33.8$ nM with 1:1000 serum). It is clear that the presence of serum proteins slowed the binding between digoxin and its antibody. At the second site, the K_D did not have clear dependence on the concentration of serum, and its value was roughly in few hundreds of pM except in 1:10000 serum. For anti-methamphetamine reacting with surface-immobilized methamphetamine, the K_D at the first site increased by a factor of 8 from no serum ($K_D = 0.86$ nM) or 1:10000 serum ($K_D = 0.79$ nM) to 1:5000 serum ($K_D = 6.2$ nM), and by a factor of 63 to 1:1000 serum ($K_D = 50.7$ nM). This indicates that serum proteins slowed the binding of antibodies to methamphetamine targets at a dilutions between 1:10000 and 1:5000. Again, at the second site, the K_D was independent upon the concentration of serum, and it has values from a few hundreds of pM to few nM.

For anti-morphine reacting with surface-immobilized morphine, the K_D at the first site was in the order of nM for serum concentrations less than 1:5000 ($K_D < 6.9$ nM without serum, $K_D = 4.3$ nM with 1:10000 serum, and $K_D = 8.3$ nM with 1:5000 serum), but decreased to 0.63 nM in 1:1000 serum. Serum proteins accelerated the binding of antibodies to morphine targets at a dilutions between 1:5000 and 1:1000. At the second site, the K_D value was small (in few tens of pM) and independent of the concentration of serum. For anti-phenobarbital reacting with surface-immobilized phenobarbital, the K_D at the first site increased from 0.53 nM without serum to 4.4 nM with 1:10000 serum, 6.6 nM with 1:5000 serum, and even 44.9 nM with 1:1000 serum. Serum proteins again slowed the binding between phenobarbital and its antibody. At the second site, the K_D, independent of the concentration of serum, had values ranged from a few hundreds of pM to a few nM.

For anti-tetrahydrocannabinol reacting with surface-immobilized tetrahydrocannabinol, the K_D at the first site increased as the concentration of serum increased ($K_D = 7.1$ nM without serum, $K_D = 14.2$ nM with 1:10000 serum, $K_D = 15.5$ nM with 1:5000 serum, and $K_D = 41.6$ nM with 1:1000 serum). Similar as the in antibody-digoxin reactions, there was a clear increase in K_D from no serum to 1:10000 to 1:5000 serum, and a further increase with 1:1000 serum. At the second site, the K_D was small (few tens of pM) and showed no dependence on the concentration of serum. For anti-theophylline reacting with surface-immobilized theophylline, the K_D at the first site increased from roughly 0.4 nM with zero and 1:10000 serum, to 7.2 nM with 1:5000 serum, and to 14.6 nM with 1:1000 serum. At the second site, the K_D was less than a few tens of pM except in 1:10000 serum.

Overall, for all antibody-drug reactions except morphine, the binding affinities were reduced in the presence of human serum. Increasing the concentration of serum reduced the rates of the reactions. This phenomenon was likely caused by bulky proteins in the serum that blocked access

to or reduced the stability of the reaction complexes. In the case of morphine, the binding affinity increased when human serum was present, probably because certain proteins or molecules in the serum accelerated the reaction. The detailed mechanism remains unclear and thus further investigation is necessary.

CONCLUSIONS

The effects of human serum on the binding kinetics between anti-drug antibodies and their corresponding surface-immobilized drug-bovine serum albumin conjugates were studied. The results show that the binding affinities were affected when human serum was present. In the morphine-antibody reaction, the binding affinity increased, while in digoxin, methamphetamine, phenobarbital, tetrahydrocannabinol, and theophylline reactions, the binding affinities decreased. This effect should be considered when testing the efficiency of potential drugs and their antibodies.

FUNDING

The authors acknowledge financial support from NIH-R01-HG003827 (X.D. Zhu) and Taiwan NSC 101-2112-M-030-003-MY3 (Y.S. Sun).

REFERENCES

1. Talbert, A.M.; Tranter, G.E.; Holmes, E.; Francis, P.L.. Determination of Drug-Plasma Protein Binding Kinetics and Equilibria by Chromatographic Profiling: Exemplification of the Method using L-tryptophan and Albumin. *Anal. Chem.* **2002**, *74*(2), 446–452.
2. Parikh, H.H.; McElwain, K.; Balasubramanian, V.; Leung, W.; Wong, D.; Morris, M.E.; Ramanathan, M. A Rapid Spectrofluorimetric Technique for Determining Drug-Serum Protein Binding Suitable for High-Throughput Screening. *Pharm. Res.* **2000**, *17*(5), 632–637.
3. Menke, G.; Menke, G.; Worner, W.; Kratzer, W.; Rietbrock, N. Kinetics of Drug Binding to Human Serum Albumin: Allosteric and Competitive Inhibition at the Benzodiazepine Binding Site by Free Fatty Acids of Various Chain Lengths. *Naunyn-Schmiedebergs' Arch. Pharmacol.* **1989**, *339*(1–2), 42–47.
4. Kragh-Hansen, U. Molecular Aspects of Ligand Binding to Serum Albumin. *Pharmacol. Rev.* **1981**, *33*(1), 17–53.
5. Li, Y.F.; Zhang, X.Q.; Hu, W.Y.; Li, Z.; Liu, P.X.; Zhang, Z.Q. Rapid Screening of Drug-Protein Binding using High-Performance Affinity Chromatography with Columns Containing Immobilized Human Serum Albumin. *J. Anal. Meth. Chem.* **2013**, 439039.
6. Fei, Y.; Sun, Y.S.; Li, Y.; Lau, K.; Yu, H.; Chokhawala, H.A.; Huang, S.; Landry, J.P.; Chen, X.; Zhu, X. Fluorescent Labeling Agents Change Binding Profiles of Glycan-Binding Proteins. *Mol. Biosyst.* **2011**, *7*(12), 3343–3352.
7. Sun, Y.S.; Landry, J.P.; Fei, Y.Y.; Zhu, X.D.; Luo, J.T.; Wang, X.B.; Lam, K.S. Effect of Fluorescently Labeling Protein Probes on Kinetics of Protein-Ligand Reactions. *Langmuir.* **2008**, *24*(23), 13399–13405.
8. Zhu, X.D. Comparison of Two Optical Techniques for Label-Free Detection of Biomolecular Microarrays on Solids. *Opt. Commun.* **2006**, *259*(2), 751–753.

9. Landry, J.P.; Sun, Y.S.; Guo, X.W.; Zhu, X.D. Protein Reactions with Surface-Bound Molecular Targets Detected by Oblique-Incidence Reflectivity Difference Microscopes. *Appl. Opt.* **2008**, *47*(18), 3275–3288.
10. Lo, K.Y.; Sun, Y.S.; Landry, J.P.; Zhu, X.; Deng, W. Label-Free Detection of Surface Markers on Stem Cells by Oblique-Incidence Reflectivity Difference Microscopy. *Biotechniques* **2011**, *50*(6), 381–388.
11. Sun, Y.S.; Landry, J.P.; Fei, Y.Y.; Zhu, X.D.; Luo, J.T.; Wang, X.B.; Lam, K.S. Macromolecular Scaffolds for Immobilizing Small Molecule Microarrays in Label-Free Detection of Protein-Ligand Interactions on Solid Support. *Anal. Chem.* **2009**, *81*(13), 5373–5380.
12. Sun, Y.S.; Landry, J.P.; Fei, Y.Y.; Zhu, X.D. An Oblique-Incidence Reflectivity Difference Study of the Dependence of Probe-Target Reaction Constants on Surface Target Density Using Streptavidin-Biotin Reactions as a Model. *Instrum. Sci. Technol.* **2013**, *41*(5), 535–544.
13. Landry, J.P.; Fei, Y.; Zhu, X. Simultaneous Measurement of 10,000 Protein-Ligand Affinity Constants using Microarray-Based Kinetic Constant Assays. *Assay Drug. Dev. Technol.* **2012**, *10*(3), 250–259.
14. Landry, J.P.; Fei, Y.; Zhu, X. High Throughput, Label-free Screening Small Molecule Compound Libraries for Protein Ligands using Combination of Small Molecule Microarrays and a Special Ellipsometry-based Optical Scanner. *Int. Drug Discov.* **2011**, 8–13.
15. Landry, J.P.; Fei, Y.Y.; Zhu, X.D.; Ke, Y.H.; Yu, G.L.; Lee, P. Discovering Small Molecule Ligands of Vascular Endothelial Growth Factor That Block VEGF-KDR Binding Using Label-Free Microarray-Based Assays. *Assay Drug Develop. Technol.* **2013**, *11*(5), 326–332.
16. Sun, Y.S.; Luo, J.T.; Lam, K.S.; Zhu, X.D. Detection of Formation and Disintegration of Micelles by Oblique-Incidence Reflectivity Difference Microscopy. *Instrum. Sci. Technol.* **2013**, *41*(6), 545–555.
17. Ip, D.; Syed, H.; Cohen, M. Digoxin Specific Antibody Fragments (Digibind) in Digoxin Toxicity. *BMJ* **2009**, *339*, b2884.
18. McMillan, D.E.; Hardwick, W.C.; Li, M.; Gunnell, M.G.; Carroll, F.I.; Abraham, P.; Owens, S.M. Effects of Murine-Derived Anti-Methamphetamine Monoclonal Antibodies on (+)-methamphetamine Self-Administration in the Rat. *J. Pharmacol. Exp. Ther.* **2004**, *309*(3), 1248–1255.
19. Kim, H.; Oh, S.; Sung, B.; Tian, Y.; Yang, L.; Wang, S.; Mao, J. Anti-Morphine Antibody Contributes to the Development of Morphine Tolerance in Rats. *Neurosci. Lett.* **2010**, *480*(3), 196–200.
20. Pozharski, E.; Wilson, M.A.; Hewagama, A.; Shanafelt, A.B.; Petsko, G.; Ringe, D. Anchoring a Cationic Ligand: The Structure of the Fab Fragment of the Anti-Morphine Antibody 9B1 and its Complex with Morphine. *J. Mol. Biol.* **2004**, *337*(3), 691–697.
21. Bereczki, A.; Horvath, V.; Horvai, G. Immunoassay-based Determination of Phenobarbital using Size-Exclusion Chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* **2000**, *749*(2), 215–223.
22. Tan, C.; Gajovic-Eichelmann, N.; Stocklein, W.F.; Polzius, R.; Bier, F.F. Direct Detection of Delta9-tetrahydrocannabinol in Saliva using a Novel Homogeneous Competitive Immunoassay with Fluorescence Quenching. *Anal. Chim. Acta.* **2010**, *658*(2), 187–192.
23. Pokid'ko Iu, N.; Vasilov, R.G. Thermodynamic Parameters of Binding of Anti-Theophylline Mouse Monoclonal Antibodies 2G3 to Theophylline. *Biull. Eksp. Biol. Med.* **1997**, *124*(11), 570–573.