CHEMMEDCHEM

DOI: 10.1002/cmdc.200600252

Characterisation of the Binding of Cationic Amphiphilic Drugs to Phospholipid Bilayers Using Surface Plasmon Resonance

Matthew R. Nussio,[a] Matthew J. Sykes,[b] John O. Miners,[b] and Joseph G. Shapter*[a]

The interactions of three cationic amphiphilic drugs (CPZ, AMI, PROP) with phospholipid vesicles comprising DOPC, DMPC, or DSPC were investigated using surface plasmon resonance (SPR). Responses for CAD concentrations in the range 15.625 to 1500 μM were measured. The greatest uptake by each phospholipid bilayer occurred with CPZ. Inclusion of CAD concentrations between 750 and 1500 μM provided evidence for a second nonsaturable binding process, which may arise from intercalation of the drugs within the lipid bilayer. CAD binding was additionally shown to be dependent on membrane fluidity. Responses were initially fitted over a concentration range of 15.625 to 500 μM using a model which incorporated terms for a saturable binding site. This yielded very poor values of K_D and nonsensible values of saturation responses. Subsequently, responses were fit to the expression for a model which incorporated terms for both a saturable binding site and second nonsaturable site. Measurable binding affinities (K_D values ranged from 170 to 814 μM) were obtained for DOPC and DMPC bilayers which are similar to values reported previously. This work demonstrates that SPR studies with synthetic phospholipid bilayers provide a potentially useful approach for characterising drug–membrane binding interactions and for providing insight into the processes that contribute to drug–membrane binding.

Introduction

Previous approaches in drug design have typically focused on interactions of ligand molecules with proteins, such that the lipid environment has been considered to play a more passive role. However, it is now apparent that drug substrates may interact with membrane constituents, particularly through charged head groups.[1] It is recognised that such interactions can influence drug partitioning, orientation, and conformation within the membrane. Although there are intrinsic effects on drug substrates, complementary effects on membrane fluidity, curvature or phase separation may also potentially occur. Ultimately, these alterations can induce changes in the performance of cells, with the ability to affect the function of transmembrane receptor proteins and proteins responsible for signal transduction.[1]

Drug–membrane interactions also assume considerable importance in pharmacokinetics, including the prediction of pharmacokinetic parameters in vivo from kinetic data generated using in vitro models. For example, the absorption of an orally administered drug requires the compound to permeate membranes of the gastrointestinal tract. Hence, permeability is essential for drug absorption and bioavailability.[2] In vitro systems, typically hepatic microsomes and hepatocytes, are employed to calculate kinetic constants that may be extrapolated to the in vivo situation to predict drug clearance.[3] The nonspecific binding of drugs to incubation constituents, predominantly membrane phospholipids, decreases the concentration of free drug present in the experimental system and this in turn leads to underestimation of pharmacokinetic parameters.[4] Thus, understanding and ultimately predicting the extent of drug–membrane interactions assumes particular importance for experimental systems used to access the pharmacokinetic properties of newly discovered drugs.

Artificial membranes can be used as model systems for biological membranes and are finding increased application for the investigation of membrane-related processes. The ability of phospholipid molecules to readily form bilayers stems from their amphipathic molecular structure given they consist of both hydrophilic and hydrophobic regions. Results obtained from these models generally agree well with those obtained in vivo.[5] To further understand drug–membrane interactions, the research presented herein was aimed primarily at systematic investigations of the binding of three cationic amphiphilic drugs (CADs): chlorpromazine hydrochloride (CPZ), amitriptyline hydrochloride (AMI), and propranolol hydrochloride (PROP) (Figure 1) to bilayers synthesised from three different phospholipids: 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-di-

[a] *M. R. Nussio, Assoc. Prof. J. G. Shapter*
School of Chemistry, Physics and Earth Sciences
Flinders University, Sturt Road, Bedford Park, Adelaide, SA 5001 (Australia)
Fax: (+61) 8-8201-2905
E-mail: joe.shapter@flinders.edu.au

[b] *Dr. M. J. Sykes, Prof. J. O. Miners*
Department of Clinical Pharmacology
Flinders University, Sturt Road, Bedford Park, Adelaide, SA 5001 (Australia)

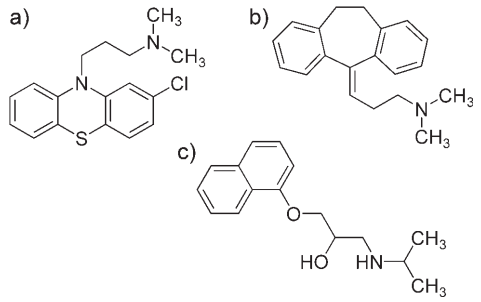

Figure 1. Structures of CADs: a) CPZ, b) AMI, and c) PROP.

myristoyl-*sn*-glycero-3-phosphocholine (DMPC), and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) using surface plasmon resonance (SPR). It has been demonstrated that SPR is a useful tool for the direct analysis of the binding of drugs to phospholipid vesicles immobilised on a commercially available L1 sensor chip.[6–8] Using SPR, we have extended previous studies of the effects of membrane fluidity[6] and demonstrated the ability of CADs to accumulate in various phospholipid bilayers.

Materials and Methods

Chemicals

1,2-Distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), amitriptyline hydrochloride $\geq$98% (AMI), chlorpromazine hydrochloride $\geq$98% (CPZ), propranolol hydrochloride 99% (PROP), DMSO ACS spectrophotometric grade $\geq$99.9%, octyl-β-D-glucopyranoside $\geq$98%, and HEPES $\geq$99% were purchased from Sigma–Aldrich (Sydney, Australia). 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was purchased from Avanti Polar Lipids (Birmingham, AL, USA). All other chemicals were of the highest commercial quality available and were used without further purification. All aqueous solutions were prepared with Milli-Q grade reagent water with resistance $\approx$18.2 MΩ. Aqueous solutions were also filtered through a membrane filter (0.2 μm); solutions comprising DMSO required a nylon membrane.

Vesicle preparation

Multilamellar vesicles (MLVs) were prepared by first dissolving aliquots of lipid in chloroform/methanol (3:1 *v/v*), followed by evaporation of the solvent under nitrogen. Lipid samples were further dried under vacuum for 3 h prior to being suspended in 10 mM HEPES buffer (pH 5.5) containing 150 mM NaCl. The final concentration of lipids was 1 mM. Samples were left to hydrate overnight, followed by sonication for 1 h. During sonication, periodic vortex mixing was carried out prior to their extrusion (Avanti Mini-Extruder, Avanti Polar Lipids, Birmingham, AL, USA) 30 times through a polycarbonate membrane filter of defined pore diameter, typically 100 nm. Extrusion was performed at temperatures higher than the transition temperature (T_M) of the component phospholipids, as gel-state lipids are difficult to extrude at lower temperatures.[9] Resultant small unilamellar vesicles (SUVs) yielded a homogenous size distribution and were used for all further experiments.

Surface plasmon resonance

SPR studies were performed at 25 °C using a Biacore 2000 (Biacore AB, Uppsala, Sweden) biosensor equipped with an L1 sensor chip (Biacore AB, Uppsala, Sweden). Figure 2 depicts a typical binding cycle of SUVs on an L1 sensor chip. Prior to each experiment, sensor chips were preconditioned with 100 μL of nonionic detergent (40 mM octyl-β-D-glucopyranoside). Phospholipid vesicles were subsequently attached to lipophilic groups of the L1 sensor chip at a flow rate of 2 μL min^{-1} in a running buffer of 10 mM HEPES, 150 mM NaCl, 1% DMSO, pH 5.5. Resulting phospholipid bilayers were washed with 20 mM NaOH prior to drug binding experiments. Drug was injected at a flow rate of 100 μL min^{-1}, and injections were performed in triplicate. Regeneration of the L1 sensor chip was accomplished by performing a second injection of nonionic detergent. To avoid carryover between experiments, a new lipid membrane surface was regenerated for each drug binding cycle. All data were double-referenced to account for bulk refractive index changes and systematic effects throughout the course of an experiment.

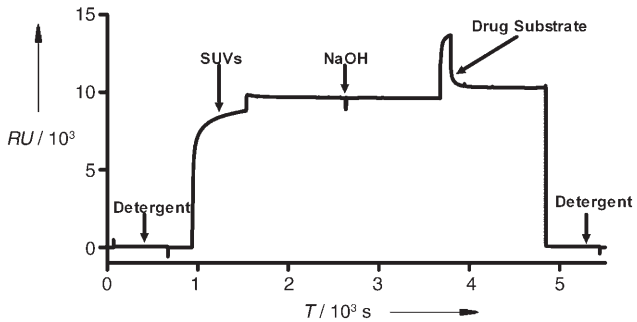

Figure 2. Sensorgram illustrating preconditioning, vesicle capture, NaOH wash, drug binding, and regeneration. DOPC vesicles and chlorpromazine (500 μM) were used for the sensorgram shown, which is representative of vesicle capture and drug binding, respectively. *RU* = response units for SPR.

Drug binding analysis

Drug binding to membrane surfaces was investigated for the three CADs; CPZ, AMI, and PROP (Figure 1). Drug concentrations ranged from 15.625 to 1500 μM. The drugs were initially dissolved in 100% DMSO prior to dilution to final assay concentrations. Final DMSO concentrations were identical to those of the running buffer. All results were normalised with respect to the amount of immobilised phospholipid[7] and further divided by their corresponding molecular weights. Normalised responses were further scaled with respect to the highest response observed, that for CPZ on a DOPC bilayer surface (see Results section below). For drug concentrations between 15.625 and 500 μM, binding affinities (K_D) were calculated by

fitting a single-site binding isotherm to a plot of equilibrium drug binding response (R_{eq}) versus concentration (C), according to the expression:

$$R_{eq} = R_{max}/((K_D/C) + 1) \quad (1)$$

for which R_{max} is equivalent to the maximum surface-binding capacity.

Results

Vesicle capture and drug binding

Vesicle capture was reproducible for all three phospholipids investigated. The average surface responses subsequent to injection of DOPC, DMPC, and DSPC were 9720, 7750, and 9400 *RU*, respectively. DOPC and DMPC exhibited excellent reproducibility with a standard deviation (SD) of 0.6%. For surfaces prepared with DSPC, the variability in response was approximately double (SD: 1.3%). Apart from CPZ, increasing the DMSO concentration from 0.1 to 3% did not significantly affect drug binding responses, as a standard deviation of 5% or less was observed between sensorgram responses at each drug concentration. However, CPZ concentrations greater than 500 μM resulted in spikes throughout the injection at a DMSO concentration of 0.1%. For this reason, all analysis were conducted in running buffer containing 1% DMSO.

Drug binding to DOPC, DMPC, and DSPC membrane surfaces

Binding responses of CPZ, AMI, and PROP were measured for three different phospholipids. Representative binding responses (CPZ–DOPC, AMI–DMPC, and PROP–DSPC) are shown in Figure 3. Drug binding responses to bilayer surfaces demonstrated a rapid association followed by a gradual increase to equilibrium. Subsequent to injection, stable drug–membrane complexes were observed. Drugs did not dissociate completely from the membrane as evidenced by the observation that response levels do not return to pre-injection levels after drug exposure was terminated.

Drug binding was measured as the maximum SPR response versus drug concentration. Although experiments were performed at concentrations between 15.625 and 1500 μM, initial analysis used data for drug concentrations up to 500 μM. Consistent with approaches adopted in a previous SPR study,[7] these relationships were analysed using a single-site equilibrium-binding isotherm [Eq. (1)]. Concentration-dependent responses for all three drugs are shown for each phospholipid membrane in Figure 4. Greatest uptake by each phospholipid bilayer surface occurred with CPZ. Responses for the DOPC and DMPC phospholipid bilayers provided measurable binding affinities (Table 1), whereas interactions with DSPC demonstrated very weak responses which could not be modelled by Equation (1). CPZ exhibited the highest binding affinity. AMI displayed greater uptake than PROP for all phospholipid bilayers examined herein, but binding affinities of PROP were greater than those of AMI. In comparison with concentration-dependent responses, it is apparent that binding does not approach saturation and, as a result, there is a degree of uncertainty associated with these calculated binding affinities. The reason for the lack of saturation was investigated further.

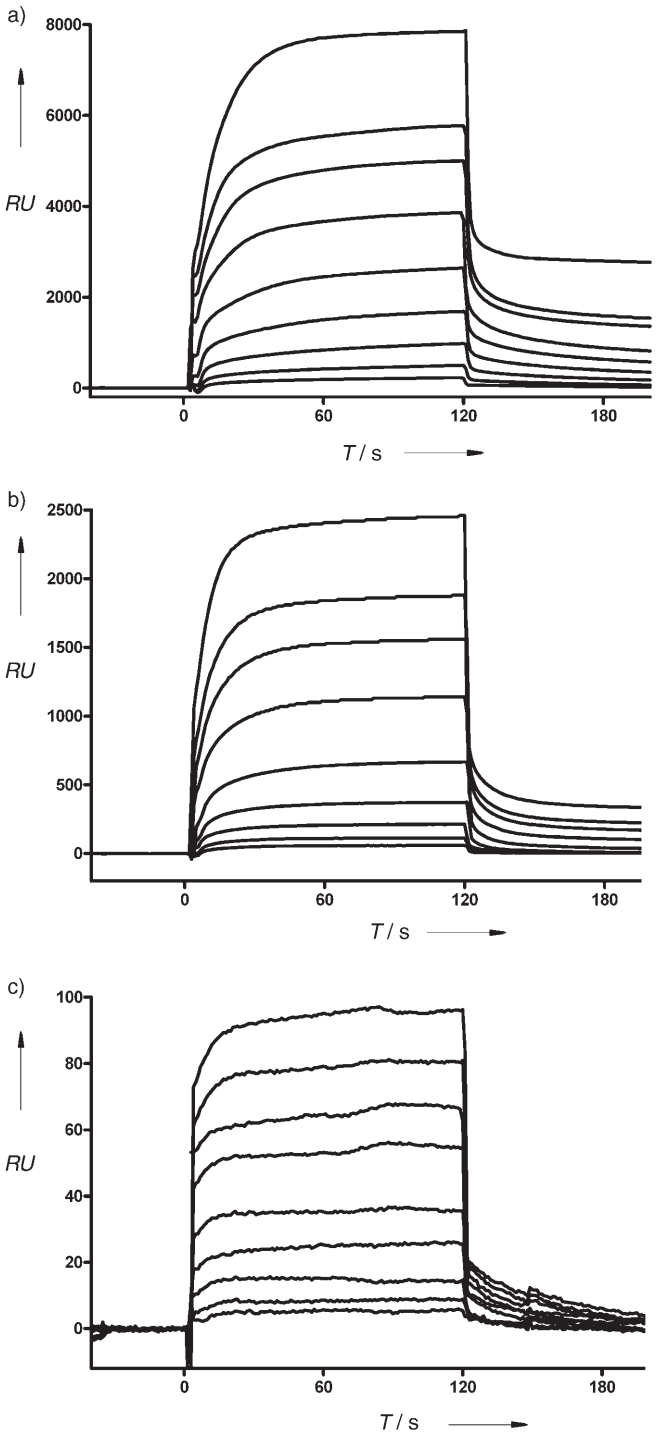

Figure 3. Concentration-dependent SPR responses of a) CPZ to DOPC, b) AMI to DMPC, and c) PROP to DSPC phospholipid bilayer surfaces. Experiments were performed in triplicate over freshly prepared surfaces.

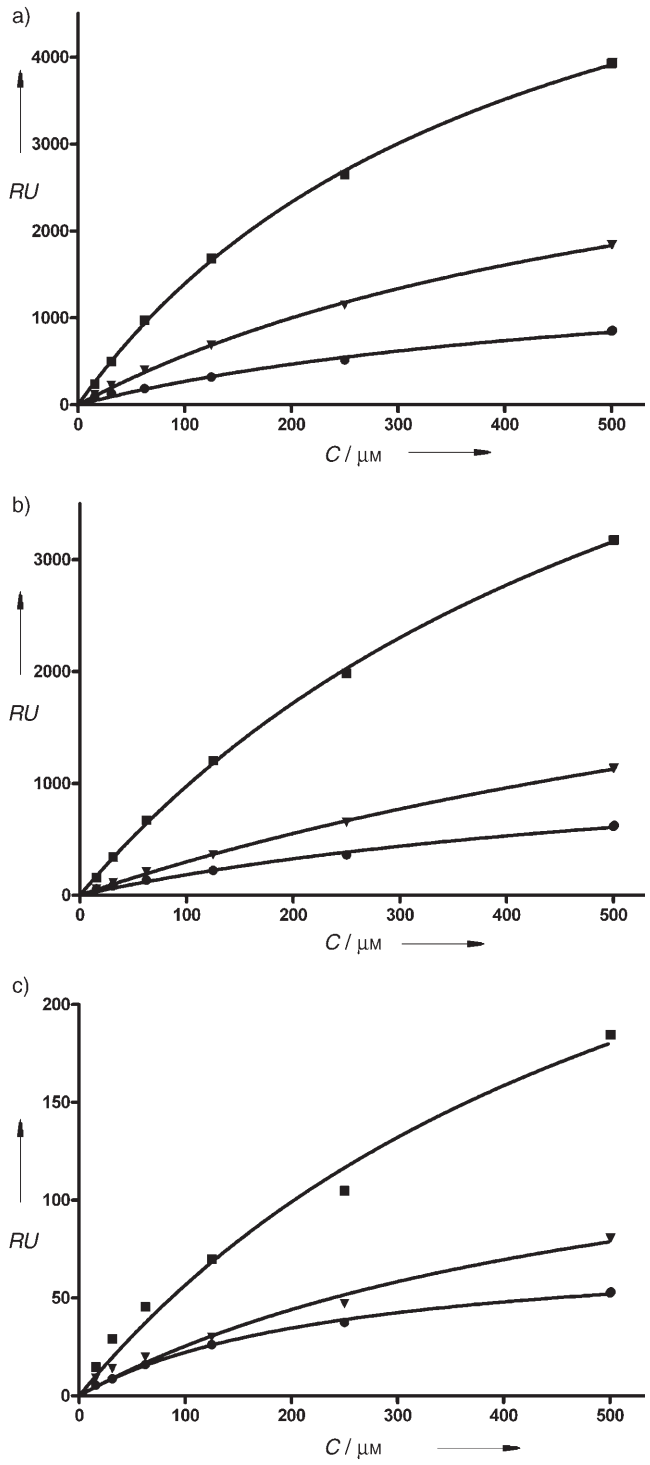

Figure 4. Concentration versus response plots for CPZ (■), AMI (▼), and PROP (●) binding to a) DOPC, b) DMPC, and c) DSPC membrane surfaces for CAD concentrations between 15.625 and 500 μM. Points are experimentally determined values, whereas curves are from model fitting with Equation (1).

Table 1. Calculated binding affinities.

CAD	K_D [μM][a]			
	Equation (1)		Equation (2)	
	DOPC	DMPC	DOPC	DMPC
CPZ	413 ± 14	636 ± 34	170 ± 33	510 ± 484
AMI	625 ± 39	1127 ± 111	479 ± 52	814 ± 147
PROP	551 ± 88	672 ± 101	234 ± 94	220 ± 94

[a] Values reflect the mean ± SE from triplicate measurements.

Further analysis of binding affinities

Results presented thus far examined experimental data for drug concentrations up to 500 μM, similar to previously published SPR data for drug–membrane interactions.[6,7] As preliminary experiments in the lower concentration range did not demonstrate a plateau in response versus concentration plots, a wider concentration range was investigated. Response relationships observed over the concentration range 15.625 to 1500 μM for the interaction of CPZ, AMI, and PROP with DOPC, DMPC, and DSPC phospholipid bilayers are depicted in Figure 5. Two processes are evident for each data set.

Based on data for concentrations between 15.625 and 500 μM, fitted single-site equilibrium-binding isotherms were extrapolated to 1500 μM. When the experimentally determined single-site equilibrium-binding isotherm between 750 and 1500 μM was overlaid as shown in Figure 5, it was evident a second process was taking place. Scatchard analysis also revealed two sites for the binding of each compound to DOPC and DMPC. The lower binding to DSPC bilayers precluded meaningful analysis using this approach. Representative Scatchard plots for the binding of CPZ to DOPC and DMPC are shown in Figure 6.

Effects of phospholipid alkyl chain on drug binding

Each phospholipid studied herein contains a phosphatidylcholine head group, but a different alkyl chain. This permits the investigation of the effects of alkyl chain length and structure on drug binding. Figure 7 shows the structure of each phospholipid. In contrast to DMPC, which has a 14-carbon alkyl chain, DOPC and DSPC have 18-carbon chains. Although it shares the same chain length as DSPC, DOPC possesses a double bond on each alkyl chain. These differences are reflected in each phospholipid's transition temperature (T_M), such that DOPC, DMPC, and DSPC exhibit T_M values of −20, 23, and 55 °C, respectively. As the surface temperature was set at a constant 25 °C, drug binding is also a function of membrane fluidity; in particular, interactions were measured between membranes that potentially exhibit liquid crystalline, gel-liquid crystalline, and gel phases.

Interactions of CPZ, AMI, and PROP with DOPC, DMPC, and DSPC phospholipid bilayers demonstrated a consistent trend with regard to drug binding. CAD binding data shown in Figure 4 are re-plotted in Figure 8 to clearly show differences for each phospholipid bilayer. Drug binding responses between 15.625 and 500 μM indicate that DOPC bilayers have the greatest uptake, followed by DMPC and then DSPC. This decrease in binding response for all three drugs is consistent with changes in membrane fluidity associated with the T_M

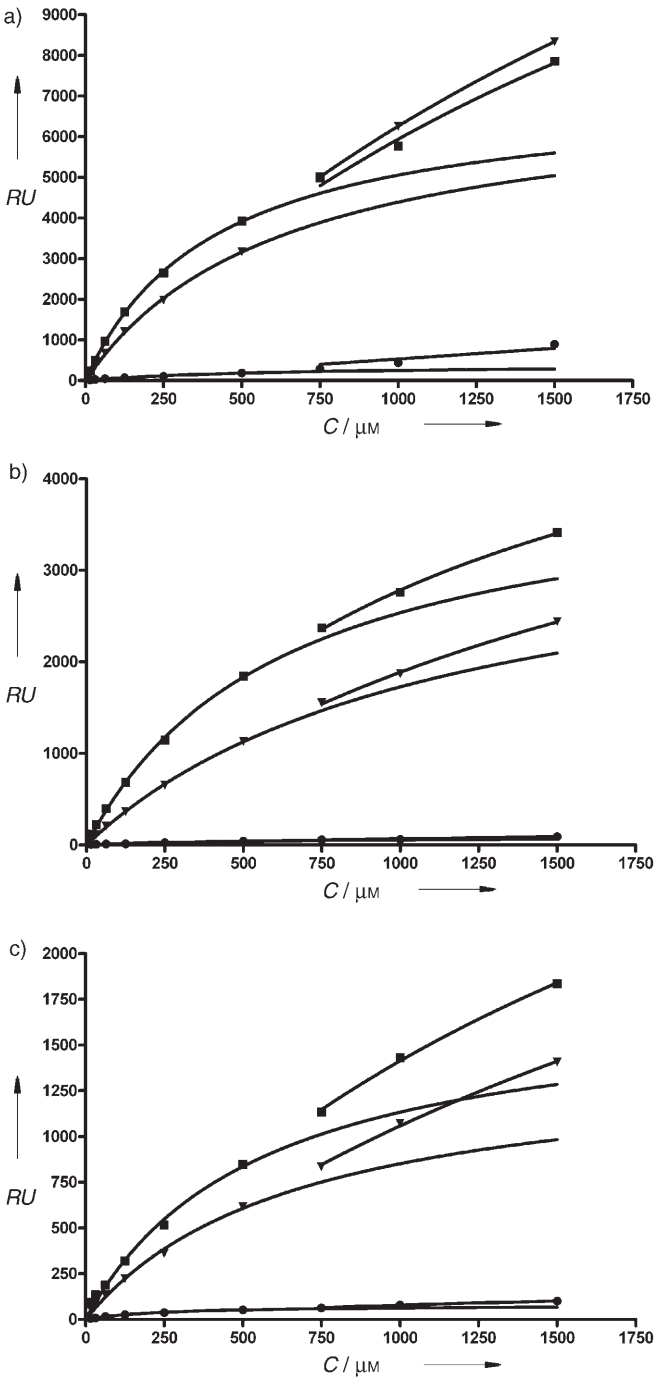

Figure 5. Concentration versus response plots for a) CPZ, b) AMI, and c) PROP binding to DOPC (■), DMPC (▼), and DSPC (●) membrane surfaces for CAD concentrations between 15.625 and 1500 μM. Points are experimentally determined values, whereas the continuous curves from 0–1500 μM are from model fitting with Equation (1).

value for each phospholipid, such that each drug exhibited a decrease in bilayer uptake. As previously stated, DOPC contains a double bond per alkyl chain in contrast to the fully saturated alkyl tails of DMPC and DSPC. This leads to an increase in membrane fluidity and hence drug partitioning.[6] This is evident from the stronger SPR response measured for DOPC with CADs.

Discussion

The results presented herein are based on SPR analysis of the interaction of CPZ, AMI, and PROP to a series of phospholipid bilayers. Of these CADs, CPZ exhibited the greatest uptake with respect to phospholipid interactions, whereas the largest interactions were observed for bilayers comprising DOPC. Previous studies have also demonstrated CPZ to have a greater interaction and thus uptake relative to AMI and PROP,[6,7,10–12] presumably as CPZ is more hydrophobic.[12] Importantly, however, the boundaries of previous work have been extended, as two discrete binding processes were identified. SPR responses were measured as a function of CAD concentration up to 1500 μM. The first process, which was observed for concentrations between 15.625 and 500 μM, exhibits saturable binding. This process, also reported by others, presumably arises from electrostatic and hydrophobic interactions whereby the negatively charged phosphate head group interacts with the positively charged amine group of the drug, whereas the lipophilic groups align with the hydrophobic carbon chains of the bilayer.[13–15] Recent studies have also yielded results consistent with the electrostatic interactions being of paramount importance.[16,17] The second process may be considered a high-capacity, low-affinity process. It has only been referred to previously in relation to SPR sensorgrams for which a heterogeneous interaction was established.[6,7] Previous studies using 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) phospholipid vesicles and lamellar bodies have also reported two binding affinities for CPZ over the concentration range employed in the current study.[10,18]

As noted previously, binding data to 500 μM were modelled with Equation (1). K_D values generated ranged from 413 to 1127 μM. The K_D values were approximately 5-fold higher than those reported by Abdiche and Myszka.[7] The reasons for these differences are not clear, but may arise from the liposome preparation, as the hydrating buffer used in the previous study contained 3% DMSO. Data from 15.625 to 1500 μM were additionally fitted to the expression for a model that incorporates terms for a saturable binding site and second nonsaturable site:

$$R_{eq} = R_{max}/((K_D/C) + 1) + N_s * C \quad (2)$$

Trends in K_D values are similar to those generated with Equation (1) using data up to 500 μM. However, the actual values (Table 1) are closer to those observed previously,[7] and the equilibrium response values, R_{max}, are more realistic than those generated with Equation (1) over the entire concentration range (data not shown). Overall, this provides further support for a second binding process.

Based on electron cryomicroscopy studies with PROP it has been proposed that the drug disrupts the bilayer by perturbation of the local organisation of phospholipids.[19] This results in the formation of thread-like micelles, and finally formation of spherical micelles after approximately two hours.[19] It is postulated herein that the second process represents intercalation of each drug to form a drug–phospholipid complex,[11,20]

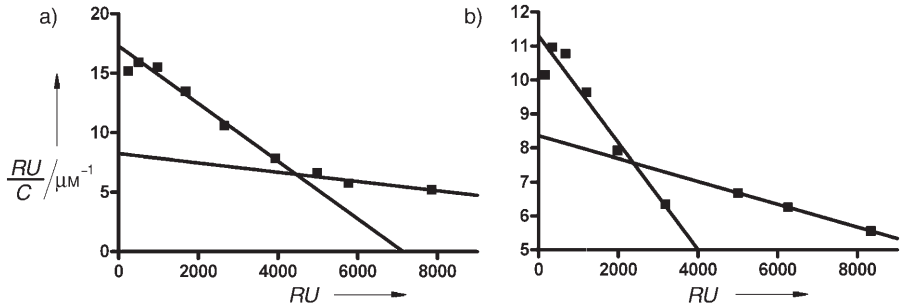

Figure 6. Scatchard plots for CPZ binding to a) DOPC and b) DMPC phospholipid bilayers.

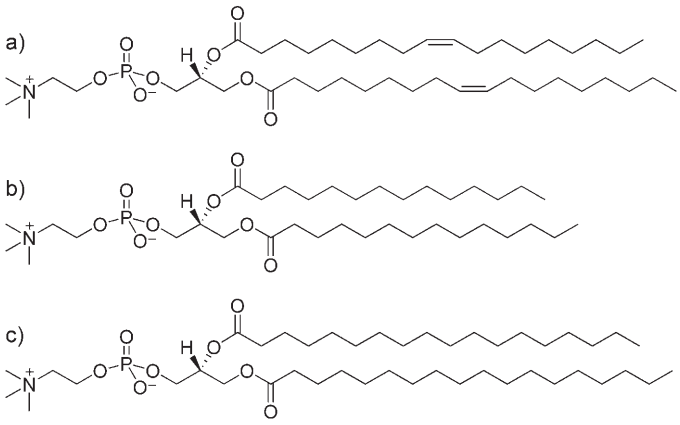

Figure 7. Molecular structures of a) DOPC, b) DMPC, and c) DSPC.

as drugs did not dissociate completely from the membrane as evidenced by the residual response in SPR following exposure to the drug. Earlier studies have also attributed further intracellular accumulation of drugs with these drug–phospholipid complexes.[11] It has been proposed that the formation of drug–phospholipid complexes mimics phospholipidosis,[7,18,20] a type of lipid storage disorder.[21] Essentially this phenomenon is related to the binding of CADs, whereby their hydrophilic and hydrophobic moieties inherently interact with the phosphate and alkyl chains of phospholipids to form a bulky lipophilic structure.[21]

A comparison between the alkyl chain lengths and molecular structure for three phospholipid molecules (DOPC, DSPC, and DSPC) on drug binding responses was investigated. It was demonstrated that each drug exhibited a much larger interaction to bilayers comprising DOPC. DOPC phospholipid molecules possess a T_M value of $-20\,^{\circ}C$ and therefore exhibit greater membrane fluidity than bilayer surfaces comprised of either DMPC ($T_M = 23\,^{\circ}C$) or DSPC ($T_M = 55\,^{\circ}C$). As experiments were performed at $25\,^{\circ}C$, the trend observed suggests binding is a function of membrane fluidity. Each CAD exhibited greater binding to DOPC phospholipid vesicles that were in their liquid crystalline state, followed by DMPC surfaces that displayed a gel-liquid crystalline phase[22] and, lastly, DSPC vesicles that were in their gel phase. Membranes that exhibit greater fluidity provide a more flexible structure for drug binding, and hence the ability of each drug molecule to partition into the bilayer is a more readily achievable process.

With respect to Figure 5, the uptake of CPZ to DMPC phospholipid bilayers increased markedly at concentrations greater than 750 μM. In particular, the increase in responses was greater than those observed for experiments with DOPC within the same concentration range. We believe this phenomenon is related to a lowering in phase transition of the phospholipid, an effect that has been associated with the partitioning of drugs into phospholipid hydrocarbon chains.[1,12,23,24] Although it has been demonstrated that PROP directly affects the T_M value of phospholipids,[12,13] the interaction is less pronounced than that of CPZ.[12] The greater effect of CPZ is attributable to its lower water solubility, which in turn promotes greater partitioning into the hydrophobic portion of the bilayer membrane.[12] In a previous study using DPPC ($T_M = 41\,^{\circ}C$),[12] a decrease in T_M of $5\,^{\circ}C$ was observed at CPZ concentrations around 200 μM. Although the current study employed DMPC, a similar effect on T_M may have occurred.[23] DMPC, which has a T_M value of $23\,^{\circ}C$, presumably has its upper leaflet in a fluid phase and its underside in a gel state at $25\,^{\circ}C$.[22] A lowering in the T_M value of DMPC would have potentially promoted the formation of a more fluid-phase bilayer. As drug injections were performed at 100 $\mu L\,min^{-1}$ for 2 min, it is difficult to comment on the magnitude of this effect during this time period. However, as there was a significant increase in response for concentrations between 750 and 1500 μM, this may be indicative of an increase in membrane fluidity. In addition, the standard error associated with the K_D value of CPZ to DMPC phospholipid bilayers using Equation (2) was close to 100%, which is much larger than the errors observed for the other systems modelled. It is postulated that the change in membrane fluidity of DMPC phospholipid bilayers contributed to the high standard error, as Equation (2) was unable to account for the phase change in the system. Thus, an increase in membrane fluidity of DMPC vesicles through the electrostatic and hydrophobic interactions of CPZ may have promoted a more flexible process for drug binding and, as a result, contributed to the observed increase in SPR response, consistent with our results showing greater binding in more fluid membranes. In contrast, effects of CPZ on the T_M value of DOPC surfaces are not expected to change the fluidity, as DOPC has a T_M value of $-20\,^{\circ}C$, and it is therefore evident that these bilayers were in a complete liquid phase at $25\,^{\circ}C$.

Previous studies[25,26] have suggested that there is uncertainty whether the membrane surface comprises intact vesicles or a planar bilayer. Using atomic force microscopy, the surface of the L1 chip after the loading of lipid vesicles was shown here to have intact vesicles (data not shown). Regardless of the sur-

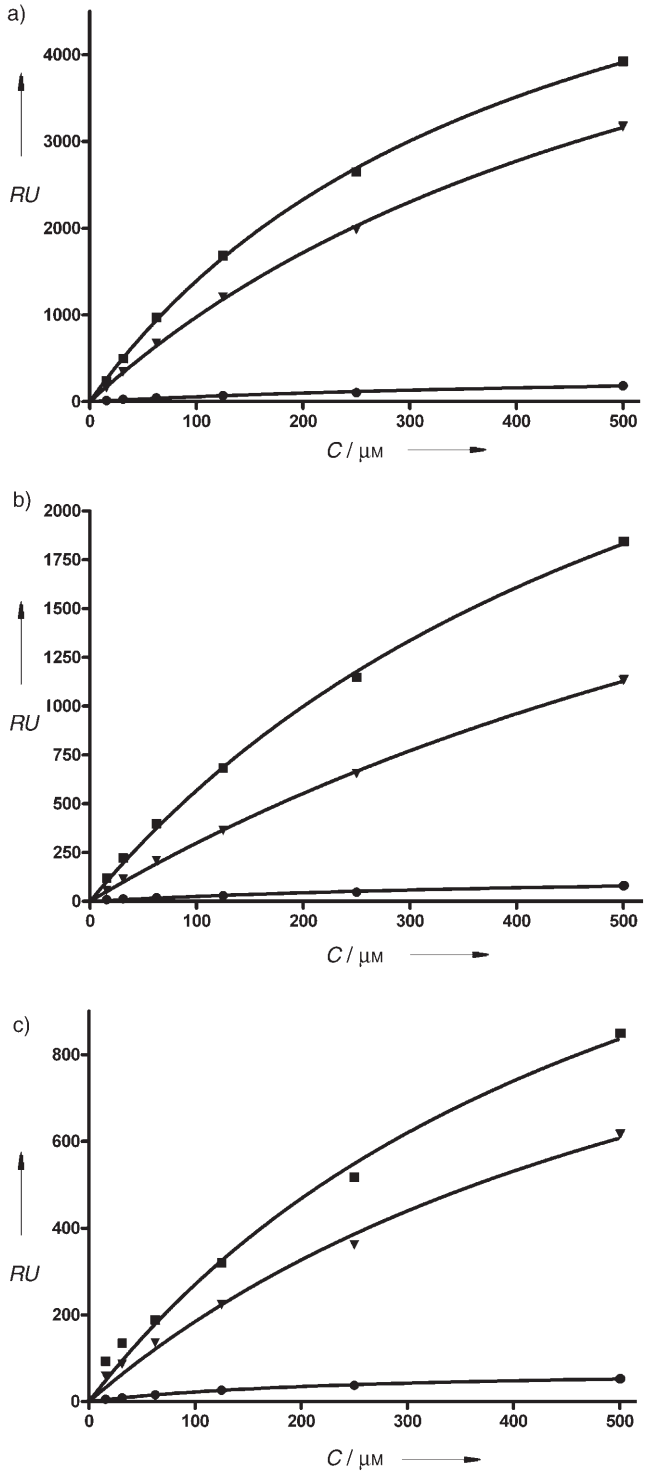

Figure 8. Concentration-dependent response plots for a) CPZ, b) AMI, and c) PROP binding to DOPC (■), DMPC (▼), and DSPC (●) membrane surfaces. Points are experimentally determined values while curves are from model fitting.

face structure, however, the interaction of each drug occurs at a single bilayer and hence provides an excellent model for investigating drug–membrane interactions.

SPR has proven to be an effective tool for the analysis of drug–phospholipid bilayer interactions. Extending previous research, it has been shown that SPR is capable of predicting the binding of CADs, and that binding involves two discrete processes. An effect of membrane fluidity on drug binding was also demonstrated such that phospholipid bilayers that exhibit a complete liquid-phase bilayer permit greater drug partitioning, and hence greater drug uptake. The effect of CPZ on the T_M of component phospholipids was also observed, indicative of the complementary effects associated with membrane partitioning of drugs. These experiments show that the methods used in the current study are potentially useful for the evaluation of drug–membrane binding interactions of new drug candidates and for providing insight into the processes that contribute to drug–membrane binding.

Acknowledgements

Financial support for this work was provided by the Flinders University program grant and Flinders University research scholarship.

Keywords: affinity · cationic amphiphilic drugs · drug–membrane interactions · phospholipids · surface plasmon resonance

[1] J. K. Seydel, M. Wiese, *Drug–Membrane Interactions: Analysis, Drug Distribution and Modeling*, Wiley-VCH, Weinheim, **2002**.
[2] P. S. Burton, J. T. Goodwin, T. J. Vidmar, B. M. Amore, *J. Pharmacol. Exp. Ther.* **2002**, *303*, 889 – 895.
[3] J. B. Houston, *Biochem. Pharmacol.* **1994**, *47*, 1469 – 1479.
[4] J. A. McLure, J. O. Miners, D. J. Birkett, *Br. J. Clin. Pharmacol.* **2000**, *49*, 453 – 461.
[5] Z. Fisar, *Gen. Physiol. Biophys.* **2005**, *24*, 161 – 180.
[6] C. L. Baird, E. S. Courtenay, D. G. Myszka, *Anal. Biochem.* **2002**, *310*, 93 – 99.
[7] Y. N. Abdiche, D. G. Myszka, *Anal. Biochem.* **2004**, *328*, 233 – 243.
[8] E. Danelian, A. Karlen, R. Karlsson, S. Winiwarter, A. Hansson, S. Lofas, H. Lennernas, M. D. Hamalainen, *J. Med. Chem.* **2000**, *43*, 2083 – 2086.
[9] R. C. MacDonald, *Biochim. Biophys. Acta* **1991**, *1061*, 297 – 303.
[10] U. M. Joshi, P. Rao, S. Kodavanti, V. G. Lockard, H. M. Mehendale, *Biochim. Biophys. Acta* **1989**, *1004*, 309 – 320.
[11] I. Scuntaro, U. Kientsch, U. N. Wiesmann, U. E. Honegger, *Br. J. Pharmacol.* **1996**, *119*, 829 – 834.
[12] A. G. Lee, *Mol. Pharmacol.* **1977**, *13*, 474 – 487.
[13] L. Herbette, A. M. Katz, J. M. Sturtevant, *Mol. Pharmacol.* **1983**, *24*, 259 – 269.
[14] A. Avdeef, K. J. Box, J. E. A. Comer, C. Hibbert, K. Y. Tam, *Pharm. Res.* **1998**, *15*, 209 – 215.
[15] R. P. Austin, P. Barton, A. M. Davis, R. E. Fessey, M. C. Wenlock, *Pharm. Res.* **2005**, *22*, 1649 – 1657.
[16] M. J. Sykes, M. J. Sorich, J. O. Miners, *J. Chem. Inf. Model.* **2006**, *46*, 2661 – 2673.
[17] J.-X. Guo, S. J. Wood, S. A. David, G. H. Lushington, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 714 – 717.
[18] U. M. Joshi, P. Rao, P. S. Kodavanti, B. Coudert, T. M. Dwyer, H. M. Mehendale, *J. Pharmacol. Exp. Ther.* **1988**, *246*, 150 – 157.
[19] S. De Carlo, H. Fiaux, C. A. Marca-Martinet, *J. Liposome Res.* **2004**, *12*, 61 – 76.
[20] H. Lullmann, M. Wehling, *Biochem. Pharmacol.* **1979**, *28*, 3409 – 3415.
[21] Z. Xia, G. Ying, A. L. Hansson, H. Karlsson, Y. Xie, A. Bergstrand, J. W. DePierre, L. Nassberger, *Prog. Neurobiol.* **2000**, *60*, 501 – 512.
[22] Z. V. Feng, T. A. Spurlin, A. A. Gewirth, *Biophys. J.* **2005**, *88*, 2154 – 2164.

[23] M. Luxnat, H.-J. Galla, *Biochim. Biophys. Acta* **1986**, *856*, 274 – 282.
[24] U. Seydel, K. Brandenburg, B. Lindner, H. Moll, *Thermochim. Acta* **1981**, *49*, 35 – 48.
[25] M. A. Cooper, A. Hansson, S. Lofas, D. H. Williams, *Anal. Biochem.* **2000**, *277*, 196 – 205.
[26] E.-M. Erb, X. Chen, S. Allen, C. J. Roberts, S. J. B. Tendler, M. C. Davies, S. Forsen, *Anal. Biochem.* **2000**, *280*, 23 – 35.

Received: October 27, 2006
Published online on December 27, 2006

