Available online at www.sciencedirect.com
SCIENCE DIRECT®
Chemistry and Physics of Lipids 141 (2006) 169–178
CPL
CHEMISTRY AND PHYSICS OF LIPIDS
www.elsevier.com/locate/chemphyslip

Review

Surface plasmon resonance in protein–membrane interactions

Mojca Beseničar [a], Peter Maček [a], Jeremy H. Lakey [b], Gregor Anderluh [a,*]

[a] *University of Ljubljana, Biotechnical Faculty, Večna pot 111, 1000 Ljubljana, Slovenia*
[b] *Institute for Cell and Molecular Biosciences, The Medical School, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne, Tyne and Wear, NE2 4HH, UK*

Received 21 December 2005; accepted 20 February 2006
Available online 20 March 2006

Abstract

Surface plasmon resonance (SPR) has become one of the most important techniques for studying macromolecular interactions. The most obvious advantages of SPR over other techniques are: direct and rapid determination of association and dissociation rates of binding process, no need for labelling of protein or lipids, and small amounts of sample used in the assay (often nM concentrations of proteins). In biochemistry, SPR is used mainly to study protein–protein interactions. On the other hand, protein–membrane interactions, although crucial for many cell processes, are less well studied. Recent advances in the preparation of stable membrane-like surfaces and the commercialisation of sensor chips has enabled widespread use of SPR in protein–membrane interactions. One of the most popular is Biacore's L1 sensor chip that allows capture of intact liposomes or even subcellular preparations. Lipid specificity of protein–membrane interactions can, therefore, be easily studied by manipulating the lipid composition of the immobilised membrane. The number of published papers has increased steadily in the last few years and the examples include domains or proteins that participate in cell signalling, pore-forming proteins, membrane-interacting peptides, coagulation factors, enzymes, amyloidogenic proteins, prions, etc. This paper gives a brief overview of different membrane-mimetic surfaces that can be prepared on the surface of SPR chips, properties of liposomes on the surface of L1 chips and some selected examples of protein–membrane interactions studied with such system.

Keywords: Protein–membrane interactions; Surface plasmon resonance; Sensor chip; L1

Contents

Abbreviations: DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DOPG, 1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)]; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; HBM, hybrid bilayer membrane; SAM, self assembled monolayer; SPR, surface plasmon resonance

* Corresponding author at: Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia. Tel.: +386 1 423 33 88; fax: +386 1 527 33 90.
E-mail address: gregor.anderluh@bf.uni-lj.si (G. Anderluh).

0009-3084/$ – see front matter
doi:10.1016/j.chemphyslip.2006.02.010

1. Introduction

Surface plasmon resonance (SPR) is now considered as one of the most important biochemical techniques for studying molecular interactions (Heyse et al., 1998b; Lakey and Raggett, 1998; Cho et al., 2001; Xenarios and Eisenberg, 2001). Since the first introduction of commercial apparatus in 1991 the number of publications has increased steadily and reached almost 1000 in 2003 alone (Rich and Myszka, 2005) (Fig. 1). More than 90% of experiments are done with Biacore apparatus and this platform is the most common device found in laboratories.

The bimolecular interactions are studied at the surface of so-called "sensor chips". These are glass slides coated with a very thin layer of gold, where the surface plasmon resonance occurs. The phenomenon of SPR was first described early in the 20th century and occurs at the condition of total internal reflection by thin layers of certain metals. A polarised laser light is directed through a medium with high refractive index (often a prism) to a thin layer of gold that lies on the border with a medium of low refractive index. At a critical angle of incident light surface plasmons are generated at the surface of the gold layer. This absorbs the light and is visible as a decrease in the intensity of reflected light. The critical angle is dependant upon the refractive index within a few hundred nanometers of the surface and this changes when molecules bind to the surface. Hence the fundamental unit of SPR is the degree of arc. In the Biacore system one substance, termed the *ligand*, is attached on the surface of a sensor chip. The second one, termed the *analyte*, is then pumped across the surface via a microfluidic system. If interaction between the ligand and analyte occurs, the refractive index at the surface of the chip changes and this is viewed as an increase in signal "on-line" (Fig. 2). The Biacore system uses "resonance units" (equal to a critical angle shift of 10^{-4} deg) to describe the increase of the signal. There is a linear relationship between the mass of the matter at the surface of the chip and the resonance unit (RU) such that 1 RU = 1 pg/mm^2 (Stenberg et al., 1990). As SPR measures the mass concentration at the surface of the chip, the binding between molecular partners is observed directly and there is no need to label molecules with fluorescent or radioactive tags. Furthermore, due to the high sensitivity of the system small amounts of sample are required, for strongly interacting proteins nanomolar concentrations may only be needed.

Several Biacore sensor chips have a dextran layer attached to the surface of the gold chip. This has several consequences. Firstly, functional groups for covalent immobilisation can be attached to the dextran. The original sensor chip is CM5, which is carboxymethylated.

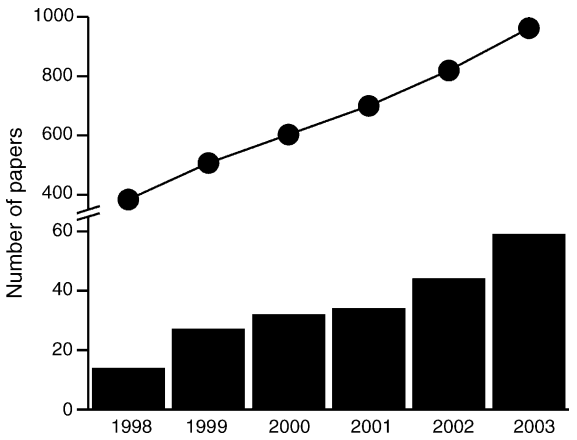

Fig. 1. The number of papers published in scientific literature describing experiments which use surface plasmon resonance. The total number of papers is represented by the line whilst the number of papers describing experiments involving various lipid and membrane systems are presented by columns. The data are taken from reviews of Rich and Myszka (Myszka, 1999; Rich and Myszka, 2000b, 2001, 2002, 2003, 2005).

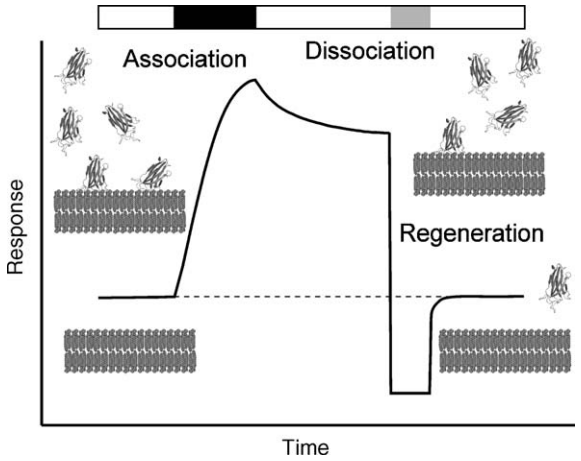

Fig. 2. The schematic representation of the sensorgram. A sensorgram is the curve that shows the response in time. The increase of response is dependant upon the mass of matter on the surface of the sensor chip. The sensor chip is equilibrated with buffer before the binding experiment and is washed with buffer between different phases of binding experiment (white bar). The analyte is injected across the chip at a fixed concentration for the desired association period (black bar). The concentration of the protein at the surface of the chip-immobilised membrane is increased due to protein binding to the membrane and this results in the increase of the response. After that, the sensor chip is washed with running buffer and dissociation of the protein from the membrane is followed as a decrease of response. The ligand surface must be regenerated if protein is stably attached to the membrane. This is usually achieved with high salt concentration or low or high pH (grey bar). If all of the bound protein is removed from the membrane surface, the next binding experiment can be performed.

Biological molecules are easily covalently attached to this chip via amino, thiol, aldehyde or carboxyl groups. Such covalent attachment of ligands to a dextran-layered sensor chip is probably the most common in Biacore applications. Secondly, the dextran layer enables high flexibility and, with little steric hindrance immobilised molecules behave similarly as in a solution (Day et al., 2002). Biacore offers a wide range of different sensor chips that allow many kinds of immobilisation methods. The most popular are protein–protein, protein–nucleic acid, protein–carbohydrate, protein–membrane and even protein-small molecule, such as drugs or other small molecular weight ligands, interactions. Many excellent general reviews are available describing SPR and its use in biochemistry (van der Merwe and Barclay, 1996; Silin and Plant, 1997; Salamon et al., 1999; Rich and Myszka, 2000a; Baird and Myszka, 2001; McDonnell, 2001; Cooper, 2003, 2004; Nedelkov and Nelson, 2003; Karlsson, 2004). Furthermore, there are reviews that describe the use of SPR in studies of function of membrane-associated proteins (Cho et al., 2001; Cooper, 2004), membrane-interacting peptides (Mozsolits and Aguilar, 2002; Mozsolits et al., 2003) or pore-forming toxins (Anderluh et al., 2003). We will provide status of SPR in protein–membrane interactions in the last few years and will briefly describe the preparation of various membrane-mimetic surfaces by using commercial Biacore sensor chips, with particular attention on its widely used L1 chip.

2. SPR and protein–membrane interactions

First reports of protein–membrane interaction studies by using home made SPR systems were published a few years before Biacore introduced the HPA and L1 chips dedicated for work with lipid systems. Much of the initial work on use of SPR spectroscopy in protein–membrane interactions was done by the groups of Vogel and Salomon and Tollin (Terrettaz et al., 1993; Lang et al., 1994; Salamon et al., 1994, 1996, 1999). The pre-requisite was the development of surfaces on thin gold layer that mimic natural membranes. The two main approaches for preparation of membrane-mimetic surfaces are the hybrid bilayer membrane (HBM) and immobilised membrane bilayers or liposomes (Table 1 and Fig. 3). HBM are formed on a hydrophobic surface that is generated by the deposition of an alkanethiol self assembled monolayer (SAM) on the gold surface. Polar lipids, usually in the form of small unilamellar vesicles, spontaneously adsorb to SAM so that acyl chains are in the contact with the hydrophobic surface and the polar headgroups are oriented towards solution (Fig. 3A) (Plant, 1993; Terrettaz et al., 1993; Plant et al., 1995; Cooper et al., 1998). The HBM are usually very stable

Table 1
Various membrane-mimetic surfaces that can be prepared by commercial Biacore sensor chips

	Membrane-mimetic surface	Description	Biacore chip	References
A	Supported monolayer	Liposomes (usually SUV) injected over the surface fuse with alkanethiol molecules on the HPA chip and form hybrid bilayer membrane	HPA	Plant (1993), Terrettaz et al. (1993), Plant et al. (1995) and Cooper et al. (1998)
B	Tethered membrane bilayer	Membrane bilayer is tethered on the gold chip by the use of thiolipids attached to a gold surface	Gold	Lang et al. (1994), Stora et al. (1999), Hong et al. (2002) and Terrettaz et al. (2002)
C	Immobilised liposomes	Liposomes with small amount of biotinylated lipids are retained on the chip by covalently attached avidin	CM5	Masson et al. (1994), Stachowiak et al. (1996) and Schlattner and Wallimann (2000a)
D	Immobilised liposomes	Liposomes with small amount of LPS are retained on the chip by covalently attached LPS-specific antibody	CM5	MacKenzie et al. (1997) and MacKenzie and Hirama (2000)
E	Immobilised liposomes	Liposomes injected over the surface of L1 chip are retained by lipophilic anchors attached to a dextran matrix	L1	Cooper et al. (2000), Erb et al. (2000) and Anderluh et al. (2005a)
F	Immobilised liposomes	Antisense DNA-derivatised liposomes are retained by the hybridisation to sense DNA tethers attached to a surface of the gold surface. DNA tethers were attached by avidin–biotin system that is not shown in this figure	Gold	Svedhem et al. (2003) and Graneli et al. (2005)

The surfaces are graphically presented in Fig. 3.

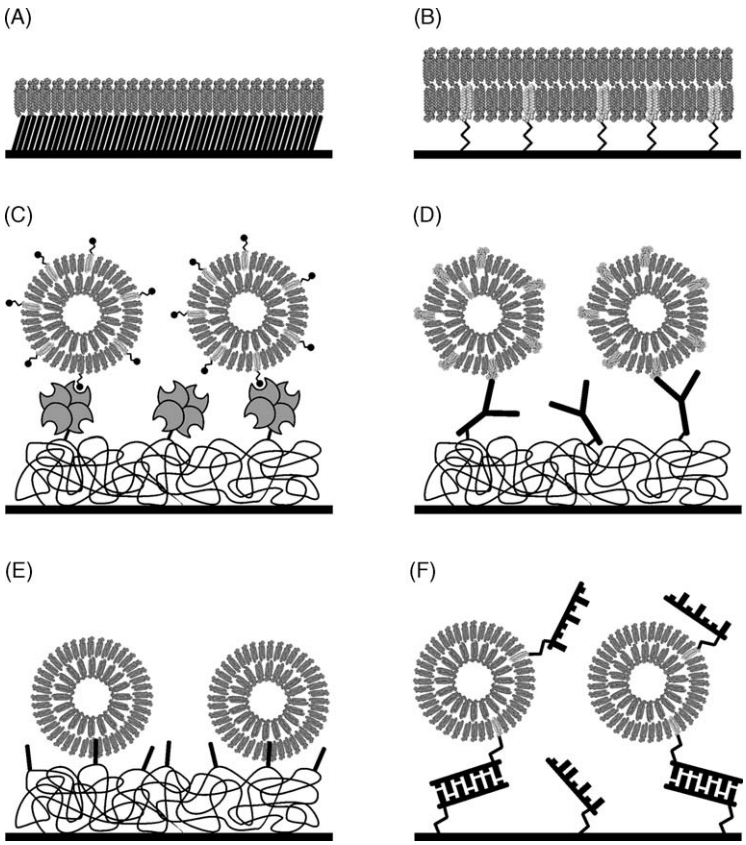

Fig. 3. Various procedures for preparation of membrane-mimetic surfaces. (A) Supported lipid monolayers created on HPA chip. (B) Lipid bilayers tethered by thiolipids ("light shade anchored to surface by zig-zag spacer") on a surface of a gold chip. (C) Immobilisation of liposomes, containing trace amounts of biotinylated lipid (light shade with attached biotin), by avidin covalently attached to a surface of CM5 chip. (D) Immobilisation of liposomes, containing trace amounts of lipopolysaccharide (light shade), by LPS-specific antibody covalently attached to a surface of CM5 chip. (E) Immobilised liposomes on the surface of L1 chip with protruding lipophilic groups. (F) Immobilisation of DNA-derivatised liposomes by hybridisation to DNA tethers attached on a gold chip. DNA tethers were bound to the chip by the avidin–biotin system that is not shown on this figure.

and resist many regeneration solutions used for release of adsorbed proteins from the monolayer. Biacore commercialised this hydrophobic sensor surface in 1995 when they introduced the HPA chip. Although this approach is very useful, it is not so widely used, because it is quite hard to work with its extremely hydrophobic surface and only half a fluid monolayer is formed. Other approaches with different advantages have been developed instead.

In the very rich SPR literature many different approaches have been reported, although general methods are few. A stable membrane bilayer can be *tethered* onto the surface of the gold chip with the aid of thiolipids (Fig. 3B) (Lang et al., 1994; Stora et al., 1999; Hong et al., 2002; Terrettaz et al., 2002). These have a thiol group, linked via a hydrophilic linker to the lipid headgroup, which enables attachment to the gold chip. When liposomes are passed over a sparse monolayer of thiolipids a continuous bilayer is formed with the thiolipids becoming part of the membrane bilayer. Another approach uses thio-peptides to tether the membrane. These have a flexible linker comprising a short peptide that possesses at one end a thiol group and on the other end a lipid (Bunjes et al., 1997; Knoll et al., 2000). Both systems provide a well-defined model membrane in which both upper and lower layers are fluid and with an additional aqueous layer between the chip and the membrane. Hence, one big advantage over HBM is that it is possible to include transmembrane proteins in an active form. Some examples of functional proteins reconstituted in tethered bilayer for SPR study include the G-protein coupled receptor rhodopsin (Heyse et al., 1998a), OmpF, an outer membrane protein channel from *Escherichia coli* (Stora et al., 1999) and the nicotinic acetylcholine receptor from *Torpedo californica* (Schmidt et al., 1998).

Finally, it is possible to retain intact liposomes on the surface of the chip by various means. First approaches

exploited biotin–avidin interaction. Liposomes containing trace amounts of biotinylated lipids are stably retained on a surface of the CM5 chip with covalently attached avidin (Fig. 3C) (Masson et al., 1994; Stachowiak et al., 1996; Schlattner and Wallimann, 2000a). Other surfaces can also be used for attaching biotinylated liposomes. Jung et al. (2000) tethered a mixed monolayer of biotin-terminated and hydroxyl-terminated poly(ethylene oxide) alkylthiolates on a gold surface. A flat streptavidin monolayer was formed on top of the monolayer and this enables a formation of high-density planar layer of intact liposomes. MacKenzie similarly exploited tight and stable interaction between lipopolysaccharide and LPS-specific antibody (MacKenzie et al., 1997; MacKenzie and Hirama, 2000) (Fig. 3D). Minor amounts of LPS were included in liposomes that were stably retained on the surface of anti-LPS antibody coated CM5 chip. Biacore introduced another approach in 2000. They offered an L1 chip that has lipophilic groups attached on the surface of carboxymethylated dextran layer. Here, intact liposomes are stably retained after the injection (Fig. 3E) (Cooper et al., 2000; Anderluh et al., 2005a). The introduction of this chip finally provided quick and reproducible method for the preparation of bilayer-mimetic system and the number of publications using this chip has increased steadily in the last few years (Fig. 1). Recently a DNA based approach, which may be used to assemble a 3D vesicle network, has been studied by SPR (Fig. 3F) (Svedhem et al., 2003; Graneli et al., 2005).

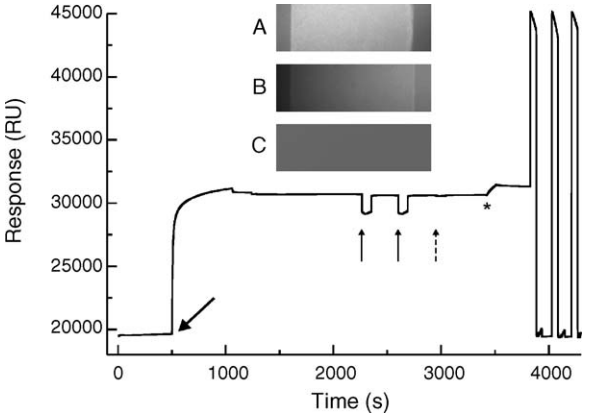

Fig. 4. Binding experiment on L1 chip. LUV were deposited on L1 chip for 10 min at 1 μl/min in running buffer (usually 140 mM NaCl, 1 mM EDTA, 20 mM Tris–HCl, pH 8.5; large arrow). The concentration of lipids is between 0.5 and 2 mM. The flow-rate was changed to 30 μl/min after the deposition and liposomes were washed with two 1 min consecutive injections of NaOH (thin arrow) and, finally 0.1 mg/ml bovine serum albumine (broken arrow). Liposomes remain stably attached in this way for several hours. Equinatoxin at a final 1 μM concentration was then injected across the immobilised liposomes (asterisk). The chip can be regenerated at the end of the binding assay by three consecutive washes with isopropanol:50 mM NaOH 2:3, mol:mol. Detergent solutions, such as 0.5% SDS or 40 mM octylglucopyranoside can also be used. The inset shows part of a flow-cell to show that intact vesicles are immobilised on the surface of the chip. Liposomes loaded with calcein were immobilised on the surface of L1 chip and immediately imaged with fluorescence microscope. (A) Deposited liposomes. (B) The same surface as in (A), only after immobilisation, 1 μM equinatoxin was injected over the surface for 90 s. An empty flow-cell is shown for comparison in (C) as a control. Adapted with permission from Anderluh et al. (2005a).

3. Retention of intact liposomes on the surface of an L1 chip

A typical experiment that involves L1 chip is as follows (Fig. 4): liposomes are deposited on the surface of the chip at 0.5–2 mM lipid concentration and at low flow-rate. Usually a few minutes are enough to saturate all lipophilic binding sites on the chip. Maximal response is in the range of 11,000–12,000 RU, but this differs with the lipids used. For example, if equimolar mixtures of zwitterionic and charged lipids are used, such as 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DOPG), lower amounts 7000–8000 RU are retained (Anderluh et al., 2005a). Negative charges on the liposome surface prevent tight packing on the surface of the chip. Two-three brief washes with 100 mM NaOH are then used at a higher flow-rate to remove losely bound vesicles. An injection of bovine serum albumin is used finally to cover all non-specific binding sites. This procedure yields homogeneous surfaces that are stable and resist treatment with many solutions, i.e. high salt, low or high pH, and can be directly used for protein binding studies. If proteins can be removed completely from the liposomes at the end of the binding experiment, then the same surface can be used for hours and different concentrations of protein can be probed.

The state of the lipids on a chip is an important determinant of the ability of a protein or a peptide to interact with the lipid layer (see below). Therefore it is important to know whether the liposomes are retained on the surface of L1 chip intact or if they fuse to form lipid bilayers. The very first characterisation by Cooper et al. (2000) gave some indications that intact vesicles could be immobilised. They observed discrete spots when they imaged, with confocal microscopy, sulforhodamine loaded liposomes immobilised on a chip. Sulforhodamine could only be released with detergent (CHAPS) treatment. They also observed that liposomes do not penetrate to a dextran hydrogel, but remained on the surface. We have recently confirmed their finding

when characterising calcein loaded immobilised liposomes. Calcein is a fluorescent dye that has low membrane permeability (Dalla Serra and Menestrina, 2003). It could only be released from the liposomes with treatment with solutions used for regeneration of L1 surface (in our case isopropanol:50 mM NaOH 2:3, mol:mol) or by high concentrations of a pore-forming toxin that bound to the liposome membrane and formed calcein-permeable pores. In addition, calcein release from the immobilised liposomes was compared to the release of calcein from liposomes free in solution, i.e. stirred in a cuvette setup, and was found to be very similar (Anderluh et al., 2005a). Other authors also failed to detect any fluorescence released from immobilised liposomes loaded with fluorescent probes, indicating that they remained intact (Stahelin and Cho, 2001a). In fact, this test was also used as a control to show that binding of particular protein is only to the outer leaflet and that it does not perturb the liposome structure (Stahelin and Cho, 2001a). And finally, just recently liposomes immobilised on a L1 chip were imaged by electron microscopy and found to remain intact (Honing et al., 2005). So in conclusion, in many cases liposomes stay intact on the surface of the chip. However, as always in biology, there are some exceptions, i.e. Erb et al. (2000) have concluded in their studies that liposomes fuse on the surface of the chip. There are indeed some cases when this can occur, especially when synthetic saturated lipids are used, i.e. 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) or conditions that differ from those described in the previous paragraph (Cooper, 2004). It is, therefore, advisable to test for the state of lipids on the surface of the chip, if lipids and procedures that differ from published examples are used.

4. Is it any good?

Most probably, as SPR related literature in protein–membrane interactions is vast. It is, therefore, not possible to cite everything here. We tried to encompass the most common examples of its applicability. SPR was used to monitor the binding of domains or proteins that participate in cell signalling (Bittova et al., 2001; Stahelin and Cho, 2001b; Stahelin et al., 2002, 2003). It gave very useful information on initial membrane attachment of pore-forming proteins (Puu, 2001; Yamaji et al., 1998; Chenal et al., 2002; Hong et al., 2002) and membrane-interacting peptides (Thomas et al., 1999; Mozsolits et al., 2001; Gaidukov et al., 2003; Papo and Shai, 2003). In addition, SPR was used to study binding of coagulation factors (Saenko et al., 2001), enzymes (Stachowiak et al., 1996; Schlattner and Wallimann, 2000b; Stahelin and Cho, 2001a), amyloidogenic proteins (Kremer and Murphy, 2003; Anderluh et al., 2005b), prions (Critchley et al., 2004), etc.

SPR studies, in general, give both qualitative and quantitative data on molecular interactions. In protein–membrane interactions the typical qualitative study is the description of lipid specificity of a membrane-binding protein. In many cases the recognition of particular lipid in the membrane is the key regulatory step of protein action, i.e. many pore-forming proteins in the first step of the intoxication mechanism bind to specific lipid or protein receptors in lipid membranes. For example, lysenin, a sphingomyelin-specific protein from earthworm, showed better binding to membranes when the proportion of sphingomyelin was increased (Yamaji et al., 1998). HBM were used to show that ostreolysin, a pore-forming protein from the edible oyster mushroom, binds to sphingomyelin/cholesterol mixtures and to some extent to mixtures of lipids with saturated side chains and cholesterol, but not if lipids with unsaturated side chains were used (Sepčić et al., 2004). As was shown in this study, the visual inspection of binding curves can be sufficient grounds upon which to base clear conclusions, e.g. protein binds to particular lipid but not at all to some other type. However, sometimes it is preferable to determine the apparent rate and affinity constants from sensorgrams, especially when the differences between different conditions, either different types of membranes or different variants of protein studied, are subtle. The affinity constants can be directly determined from the equilibrium binding responses over a range of protein concentrations by fitting the data to a Langmuir adsorption isotherm. In addition, binding constants can be determined directly from the kinetics of the binding data (sensorgrams) by numerical integration analysis (Myszka, 1997; Karlsson and Falt, 1997; Schuck, 1997). This is conveniently done by Biacore BIAevaluation software or other dedicated programs (Myszka and Morton, 1998) by the use of appropriate binding model.

Some of the most useful information that SPR can provide in protein–membrane interactions is the magnitude of effects that particular amino acid side chains of proteins have on membrane association and dissociation. Site-directed mutagenesis coupled with structural information can give detailed information of the protein binding process. A typical example is the study by Stahelin and Cho (2001a), where they discussed the role of ionic, aliphatic and aromatic amino acids in the binding of phospholipases A_2 to immobilised liposomes. Five different phospholipases, together with several mutants, were checked for their ability to bind to negatively

charged and zwitterionic liposomes. On the basis of their results they proposed a general mechanism for protein attachment to the membranes. In this model, proteins are initially brought down to the membranes by electrostatic interactions or complex interactions between aromatic residues and zwitterionic membranes. Subsequent penetration of aliphatic and aromatic residues results in firmly bound protein stabilised primarily by hydrophobic interactions. This model is extended by another step in certain cases to include additional membrane association. Many pore-forming proteins change conformation after initial contact with the membrane to expose parts of the polypeptide chain, which are transferred to a hydrophobic core, usually to form a conductive channel (Chenal et al., 2002; Hong et al., 2002). These final states of the protein are remarkably stable structures and it is often nearly impossible to remove them from the lipid membrane (Anderluh, unpublished observation).

Additional information about the mechanism of membrane association of protein or peptide can be obtained when both HPA and L1 chip are used. Due to structural differences between HBM and immobilised liposomes, it is possible to differentiate between the surface adsorption in the former and insertion into the hydrophobic core of the membrane in the latter. If protein binds only to the water–lipid interface and binding is not accompanied with deeper penetration then similar equilibrium constants should be observed when using both chips. This was indeed observed in the case of coagulation factor VIII (Saenko et al., 2001). On the other hand, if the protein inserts deeper in the membrane and needs a transmembrane compartment, then no binding is observed in HBM, as shown by Rossi et al. (2003) for adenylate cyclase from *Bordetella pertussis* which is able to translocate across the membranes in the presence of calcium.

Different binding affinities of certain peptides on L1 or HPA chips have also been observed. For example Papo and Shai (2003) studied binding of magainin and melittin to zwitterionic (phosphatidylcholine/cholesterol 10/1, w/w) or charged (phosphatidylethanolamine/phosphatidylglycerol 7/3, w/w) lipid mixtures. Binding affinities to different lipid systems corresponded to the differences in their membrane-disrupting capabilities. Magainin acts by a detergent-like mechanism, so the ratio of affinity constants between bilayers and monolayers for zwitterionic membranes was close to 1, indicating that magainin preferentially interacts with the surface of the membranes. In agreement with this it showed 100 times higher affinity for negatively charged vesicles over zwitterionic ones. In contrast, melittin forms transmembrane pores, so the ratio of affinity constants was 25, indicating that it clearly prefers hydrophobic core of the bilayer. Higher affinity of melittin for lipid preparations on L1 chip was observed also by Mozsolits and Aguilar (2002). In summary, the use of SPR and various lipid systems enabled differentiation between different steps in mechanism of action of membrane-active peptides.

5. Perspectives

Probably the trend of increase of SPR publications in protein–membrane interactions will increase also in future. There are however, many other aspects of membrane preparations on Biacore chips and their use in biochemistry and other related disciplines. For example, Biacore chips can be used not only to immobilise liposomes prepared from the synthetic lipids, but also other vesicular preparations from cells (Kim et al., 2004). Just recently Ferracci et al. (2004) immobilised pure intact synaptic vesicles on the surface of the Biacore sensor chip. Such preparations enable the study of intact membrane proteins, i.e. binding of ligands to proteins, protein profiling, etc. Artificial membranes may not always be the best system to use for membrane-binding studies and in such cases vesicular preparations of lipids isolated from cells or membrane ghosts can be particularly useful. It is possible to immobilise erythrocyte ghosts to the L1 chip in order to study the binding of various pore-forming toxins (Anderluh, unpublished observation). Recent reports on characterisation of drug–membrane interactions indicate that SPR will become an important tool in preclinical drug discovery (Danelian et al., 2000; Baird et al., 2002; Abdiche and Myszka, 2004; Kim et al., 2004; Frostell-Karlsson et al., 2005). And finally, encouraging reports of successful on-chip reconstitution of transmembrane proteins, such as G-protein coupled receptors, means that it will be possible to use these medically important proteins in biosensor applications (Karlsson and Lofas, 2002; Stenlund et al., 2003) and that it will probably be possible to prepare in the same way also other transmembrane proteins. For sure, we will hear more about SPR in protein–membrane interactions in the years to come.

Acknowledgements

We would like to apologise to all those working with SPR whose work was not cited here due to length constraints. The authors would like to thank to The Ministry of Higher Education, Science and Technology and The Wellcome Trust for the support.

References

Abdiche, Y.N., Myszka, D.G., 2004. Probing the mechanism of drug/lipid membrane interactions using Biacore. Anal. Biochem. 328, 233–243.

Anderluh, G., Maček, P., Lakey, J.H., 2003. Peeking into a secret world of pore-forming toxins: membrane binding processes studied by surface plasmon resonance. Toxicon 42, 225–228.

Anderluh, G., Beseničar, M., Kladnik, A., Lakey, J.H., Maček, P., 2005a. Properties of nonfused liposomes immobilized on an L1 Biacore chip and their permeabilization by a eukaryotic pore-forming toxin. Anal. Biochem. 344, 43–52.

Anderluh, G., Gutierrez-Aguirre, I., Rabzelj, S., Čeru, S., Kopitar-Jerala, N., Maček, P., Turk, V., Žerovnik, E., 2005b. Interaction of human stefin B in the prefibrillar oligomeric form with membranes. Correlation with cellular toxicity. FEBS J. 272, 3042–3051.

Baird, C.L., Courtenay, E.S., Myszka, D.G., 2002. Surface plasmon resonance characterization of drug/liposome interactions. Anal. Biochem. 310, 93–99.

Baird, C.L., Myszka, D.G., 2001. Current and emerging commercial optical biosensors. J. Mol. Recognit. 14, 261–268.

Bittova, L., Stahelin, R.V., Cho, W., 2001. Roles of ionic residues of the C1 domain in protein kinase C-alpha activation and the origin of phosphatidylserine specificity. J. Biol. Chem. 276, 4218–4226.

Bunjes, N., Schmidt, E.K., Jonczyk, A., Rippmann, F., Beyer, D., Ringsdorf, H., Graber, P., Knoll, W., Naumann, R., 1997. Thiopeptide-supported lipid layers on solid substrates. Langmuir 13, 6188–6194.

Chenal, A., Nizard, P., Forge, V., Pugniere, M., Roy, M.O., Mani, J.C., Guillain, F., Gillet, D., 2002. Does fusion of domains from unrelated proteins affect their folding pathways and the structural changes involved in their function? A case study with the diphtheria toxin T domain. Protein Eng. 15, 383–391.

Cho, W., Bittova, L., Stahelin, R.V., 2001. Membrane binding assays for peripheral proteins. Anal. Biochem. 296, 153–161.

Cooper, M.A., Try, A.C., Carroll, J., Ellar, D.J., Williams, D.H., 1998. Surface plasmon resonance analysis at a supported lipid monolayer. Biochim. Biophys. Acta 1373, 101–111.

Cooper, M.A., Hansson, A., Lofas, S., Williams, D.H., 2000. A vesicle capture sensor chip for kinetic analysis of interactions with membrane-bound receptors. Anal. Biochem. 277, 196–205.

Cooper, M.A., 2003. Biosensor profiling of molecular interactions in pharmacology. Curr. Opin. Pharmacol. 3, 557–562.

Cooper, M.A., 2004. Advances in membrane receptor screening and analysis. J. Mol. Recognit. 17, 286–315.

Critchley, P., Kazlauskaite, J., Eason, R., Pinheiro, T.J., 2004. Binding of prion proteins to lipid membranes. Biochem. Biophys. Res. Commun. 313, 559–567.

Dalla Serra, M., Menestrina, G., 2003. Liposomes in the study of pore-forming toxins. Methods Enzymol. 372, 99–124.

Danelian, E., Karlen, A., Karlsson, R., Winiwarter, S., Hansson, A., Lofas, S., Lennernas, H., Hamalainen, M.D., 2000. SPR biosensor studies of the direct interaction between 27 drugs and a liposome surface: correlation with fraction absorbed in humans. J. Med. Chem. 43, 2083–2086.

Day, Y.S., Baird, C.L., Rich, R.L., Myszka, D.G., 2002. Direct comparison of binding equilibrium, thermodynamic, and rate constants determined by surface- and solution-based biophysical methods. Protein Sci. 11, 1017–1025.

Erb, E.M., Chen, X., Allen, S., Roberts, C.J., Tendler, S.J., Davies, M.C., Forsen, S., 2000. Characterization of the surfaces generated by liposome binding to the modified dextran matrix of a surface plasmon resonance sensor chip. Anal. Biochem. 280, 29–35.

Ferracci, G., Seagar, M., Joel, C., Miquelis, R., Leveque, C., 2004. Real time analysis of intact organelles using surface plasmon resonance. Anal. Biochem. 334, 367–375.

Frostell-Karlsson, A., Widegren, H., Green, C.E., Hamalainen, M.D., Westerlund, L., Karlsson, R., Fenner, K., van de, W.H., 2005. Biosensor analysis of the interaction between drug compounds and liposomes of different properties; a two-dimensional characterization tool for estimation of membrane absorption. J. Pharm. Sci. 94, 25–37.

Gaidukov, L., Fish, A., Mor, A., 2003. Analysis of membrane-binding properties of dermaseptin analogues: relationships between binding and cytotoxicity. Biochemistry 42, 12866–12874.

Graneli, A., Edvardsson, M., Hook, F., 2005. DNA-based formation of a supported, three-dimensional lipid vesicle matrix probed by QCM-D and SPR. Chemphyschem. 5, 729–733.

Heyse, S., Ernst, O.P., Dienes, Z., Hofmann, K.P., Vogel, H., 1998a. Incorporation of rhodopsin in laterally structured supported membranes: observation of transducin activation with spatially and time-resolved surface plasmon resonance. Biochemistry 37, 507–522.

Heyse, S., Stora, T., Schmid, E., Lakey, J.H., Vogel, H., 1998b. Emerging techniques for investigating molecular interactions at lipid membranes. Biochim. Biophys. Acta 1376, 319–338.

Hong, Q., Gutierrez-Aguirre, I., Barlič, A., Malovrh, P., Kristan, K., Podlesek, Z., Maček, P., Turk, D., Gonzalez-Manas, J.M., Lakey, J.H., Anderluh, G., 2002. Two-step membrane binding by Equinatoxin II, a pore-forming toxin from the sea anemone, involves an exposed aromatic cluster and a flexible helix. J. Biol. Chem. 277, 41916–41924.

Honing, S., Ricotta, D., Krauss, M., Spate, K., Spolaore, B., Motley, A., Robinson, M., Robinson, C., Haucke, V., Owen, D.J., 2005. Phosphatidylinositol-(4,5)-bisphosphate regulates sorting signal recognition by the clathrin-associated adaptor complex AP2. Mol. Cell 18, 519–531.

Jung, L.S., Shumaker-Parry, J.S., Campbell, C.T., Yee, S.S., Gelb, M.H., 2000. Quantification of tight binding to surface-immobilized phospholipid vesicles using surface plasmon resonance: binding constant of phospholipase A2. J. Am. Chem. Soc. 122, 4177–4184.

Karlsson, R., Falt, A., 1997. Experimental design for kinetic analysis of protein–protein interactions with surface plasmon resonance biosensors. J. Immunol. Methods 200, 121–133.

Karlsson, O.P., Lofas, S., 2002. Flow-mediated on-surface reconstitution of G-protein coupled receptors for applications in surface plasmon resonance biosensors. Anal. Biochem. 300, 132–138.

Karlsson, R., 2004. SPR for molecular interaction analysis: a review of emerging application areas. J. Mol. Recognit. 17, 151–161.

Kim, K., Cho, S., Park, J.H., Byun, Y., Chung, H., Kwon, I.C., Jeong, S.Y., 2004. Surface plasmon resonance studies of the direct interaction between a drug/intestinal brush border membrane. Pharm. Res. 21, 1233–1239.

Knoll, W., Frank, C.W., Heibel, C., Naumann, R., Offenhausser, A., Ruhe, J., Schmidt, E.K., Shen, W.W., Sinner, A., 2000. Functional tethered lipid bilayers. J. Biotechnol. 74, 137–158.

Kremer, J.J., Murphy, R.M., 2003. Kinetics of adsorption of beta-amyloid peptide Abeta(1–40) to lipid bilayers. J. Biochem. Biophys. Methods 57, 159–169.

Lakey, J.H., Raggett, E.M., 1998. Measuring protein–protein interactions. Curr. Opin. Struct. Biol. 8, 119–123.

Lang, H., Duschl, C., Vogel, H., 1994. A new class of thiolipids for the attachment of lipid bilayers on gold surfaces. Langmuir 10, 197–210.

MacKenzie, C.R., Hirama, T., Lee, K.K., Altman, E., Young, N.M., 1997. Quantitative analysis of bacterial toxin affinity and specificity for glycolipid receptors by surface plasmon resonance. J. Biol. Chem. 272, 5533–5538.

MacKenzie, C.R., Hirama, T., 2000. Quantitative analyses of binding affinity and specificity for glycolipid receptors by surface plasmon resonance. Methods Enzymol. 312, 205–216.

Masson, L., Mazza, A., Brousseau, R., 1994. Stable immobilization of lipid vesicles for kinetic studies using surface plasmon resonance. Anal. Biochem. 218, 405–412.

McDonnell, J.M., 2001. Surface plasmon resonance: towards an understanding of the mechanisms of biological molecular recognition. Curr. Opin. Chem. Biol. 5, 572–577.

Mozsolits, H., Wirth, H.J., Werkmeister, J., Aguilar, M.I., 2001. Analysis of antimicrobial peptide interactions with hybrid bilayer membrane systems using surface plasmon resonance. Biochim. Biophys. Acta 1512, 64–76.

Mozsolits, H., Aguilar, M.I., 2002. Surface plasmon resonance spectroscopy: an emerging tool for the study of peptide–membrane interactions. Biopolymers 66, 3–18.

Mozsolits, H., Thomas, W.G., Aguilar, M.I., 2003. Surface plasmon resonance spectroscopy in the study of membrane-mediated cell signalling. J. Pept. Sci. 9, 77–89.

Myszka, D.G., 1997. Kinetic analysis of macromolecular interactions using surface plasmon resonance biosensors. Curr. Opin. Biotechnol. 8, 50–57.

Myszka, D.G., Morton, T.A., 1998. CLAMP: a biosensor kinetic data analysis program. Trends Biochem. Sci. 23, 149–150.

Myszka, D.G., 1999. Survey of the 1998 optical biosensor literature. J. Mol. Recognit. 12, 390–408.

Nedelkov, D., Nelson, R.W., 2003. Surface plasmon resonance mass spectrometry: recent progress and outlooks. Trends Biotechnol. 21, 301–305.

Papo, N., Shai, Y., 2003. Exploring peptide membrane interaction using surface plasmon resonance: differentiation between pore formation versus membrane disruption by lytic peptides. Biochemistry 42, 458–466.

Plant, A.L., 1993. Self-assembled phospholipid/alkanethiol biomimetic bilayers on gold. Langmuir 9, 2764–2767.

Plant, A.L., Brigham-Burke, M., Petrella, E.C., Oshannessy, D.J., 1995. Phospholipid/alkanethiol bilayers for cell-surface receptor studies by surface plasmon resonance. Anal. Biochem. 226, 342–348.

Puu, G., 2001. An approach for analysis of protein toxins based on thin films of lipid mixtures in an optical biosensor. Anal. Chem. 73, 72–79.

Rich, R.L., Myszka, D.G., 2000a. Advances in surface plasmon resonance biosensor analysis. Curr. Opin. Biotechnol. 11, 54–61.

Rich, R.L., Myszka, D.G., 2000b. Survey of the 1999 surface plasmon resonance biosensor literature. J. Mol. Recognit. 13, 388–407.

Rich, R.L., Myszka, D.G., 2001. Survey of the year 2000 commercial optical biosensor literature. J. Mol. Recognit. 14, 273–294.

Rich, R.L., Myszka, D.G., 2002. Survey of the year 2001 commercial optical biosensor literature. J. Mol. Recognit. 15, 352–376.

Rich, R.L., Myszka, D.G., 2003. A survey of the year 2002 commercial optical biosensor literature. J. Mol. Recognit. 16, 351–382.

Rich, R.L., Myszka, D.G., 2005. Survey of the year 2003 commercial optical biosensor literature. J. Mol. Recognit. 18, 1–39.

Rossi, C., Homand, J., Bauche, C., Hamdi, H., Ladant, D., Chopineau, J., 2003. Differential mechanisms for calcium-dependent protein/membrane association as evidenced from SPR-binding studies on supported biomimetic membranes. Biochemistry 42, 15273–15283.

Saenko, E., Sarafanov, A., Ananyeva, N., Behre, E., Shima, M., Schwinn, H., Josic, D., 2001. Comparison of the properties of phospholipid surfaces formed on HPA and L1 biosensor chips for the binding of the coagulation factor VIII. J. Chromatogr. A 921, 49–56.

Salamon, Z., Wang, Y., Tollin, G., Macleod, H.A., 1994. Assembly and molecular organization of self-assembled lipid bilayers on solid substrates monitored by surface plasmon resonance spectroscopy. Biochim. Biophys. Acta 1195, 267–275.

Salamon, Z., Wang, Y., Soulages, J.L., Brown, M.F., Tollin, G., 1996. Surface plasmon resonance spectroscopy studies of membrane proteins: transducin binding and activation by rhodopsin monitored in thin membrane films. Biophys. J. 71, 283–294.

Salamon, Z., Brown, M.F., Tollin, G., 1999. Plasmon resonance spectroscopy: probing molecular interactions within membranes. Trends Biochem. Sci. 24, 213–219.

Schlattner, U., Wallimann, T., 2000a. A quantitative approach to membrane binding of human ubiquitous mitochondrial creatine kinase using surface plasmon resonance. J. Bioenerg. Biomembr. 32, 123–131.

Schlattner, U., Wallimann, T., 2000b. Octamers of mitochondrial creatine kinase isoenzymes differ in stability and membrane binding. J. Biol. Chem. 275, 17314–17320.

Schmidt, E.K., Liebermann, T., Kreiter, M., Jonczyk, A., Naumann, R., Offenhausser, A., Neumann, E., Kukol, A., Maelicke, A., Knoll, W., 1998. Incorporation of the acetylcholine receptor dimer from *Torpedo californica* in a peptide supported lipid membrane investigated by surface plasmon and fluorescence spectroscopy. Biosens. Bioelectron. 13, 585–591.

Schuck, P., 1997. Reliable determination of binding affinity and kinetics using surface plasmon resonance biosensors. Curr. Opin. Biotechnol. 8, 498–502.

Sepčić, K., Berne, S., Rebolj, K., Batista, U., Plemenitaš, A., Šentjurc, M., Maček, P., 2004. Ostreolysin, a pore-forming protein from the oyster mushroom, interacts specifically with membrane cholesterol-rich lipid domains. FEBS Lett. 575, 81–85.

Silin, V., Plant, A.L., 1997. Biotechnological applications of surface plasmon resonance. Trends Biotechnol. 15, 353–359.

Stachowiak, O., Dolder, M., Wallimann, T., 1996. Membrane-binding and lipid vesicle cross-linking kinetics of the mitochondrial creatine kinase octamer. Biochemistry 35, 15522–15528.

Stahelin, R.V., Cho, W., 2001a. Differential roles of ionic, aliphatic, and aromatic residues in membrane–protein interactions: a surface plasmon resonance study on phospholipases A2. Biochemistry 40, 4672–4678.

Stahelin, R.V., Cho, W., 2001b. Roles of calcium ions in the membrane binding of C2 domains. Biochem. J. 359, 679–685.

Stahelin, R.V., Long, F., Diraviyam, K., Bruzik, K.S., Murray, D., Cho, W., 2002. Phosphatidylinositol 3-phosphate induces the membrane penetration of the FYVE domains of Vps27p and Hrs. J. Biol. Chem. 277, 26379–26388.

Stahelin, R.V., Burian, A., Bruzik, K.S., Murray, D., Cho, W., 2003. Membrane binding mechanisms of the PX domains of NADPH oxidase p40phox and p47phox. J. Biol. Chem. 278, 14469–14479.

Stenberg, E., Persson, B., Roos, H., Urbaniczky, C., 1990. Quantitative determination of surface concentration of protein with surface plasmon resonance using radiolabeled proteins. J. Colloid Interf. Sci. 143, 513–526.

Stenlund, P., Babcock, G.J., Sodroski, J., Myszka, D.G., 2003. Capture and reconstitution of G protein-coupled receptors on a biosensor surface. Anal. Biochem. 316, 243–250.

Stora, T., Lakey, J.H., Vogel, H., 1999. Ion-channel gating in transmembrane receptor proteins: functional activity in tethered lipid membranes. Angew. Chem. Int. Ed. 38, 389–391.

Svedhem, S., Pfeiffer, I., Larsson, C., Wingren, C., Borrebaeck, C., Hook, F., 2003. Patterns of DNA-labeled and scFv-antibody-carrying lipid vesicles directed by material-specific immobilization of DNA and supported lipid bilayer formation on an Au/SiO_2 template. Chemphyschem. 4, 339–343.

Terrettaz, S., Stora, T., Duschl, C., Vogel, H., 1993. Protein binding to supported lipid membranes: investigation of the cholera ganglioside interaction by simultaneous impedance spectroscopy and surface plasmon resonance. Langmuir 9, 1361–1369.

Terrettaz, S., Ulrich, W.P., Vogel, H., Hong, Q., Dover, L.G., Lakey, J.H., 2002. Stable self-assembly of a protein engineering scaffold on gold surfaces. Protein Sci. 11, 1917–1925.

Thomas, C.J., Surolia, N., Surolia, A., 1999. Surface plasmon resonance studies resolve the enigmatic endotoxin neutralizing activity of polymyxin B. J. Biol. Chem. 274, 29624–29627.

van der Merwe, P.A., Barclay, A.N., 1996. Analysis of cell-adhesion molecule interactions using surface plasmon resonance. Curr. Opin. Immunol. 8, 257–261.

Xenarios, I., Eisenberg, D., 2001. Protein interaction databases. Curr. Opin. Biotechnol. 12, 334–339.

Yamaji, A., Sekizawa, Y., Emoto, K., Sakuraba, H., Inoue, K., Kobayashi, H., Umeda, M., 1998. Lysenin, a novel sphingomyelin-specific binding protein. J. Biol. Chem. 273, 5300–5306.