Recent Trends in the Application of Evanescent Wave Biosensors

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Evanescent Waves and Biosensors

During the last few years biosensors have become important for the investigation of biomolecular interactions. In particular surface plasmon resonance (SPR) and resonant mirror (RM) techniques[1] are frequently used in biosensors. More than one thousand publications concerning the use of SPR or RM biosensors underline the importance of these techniques in the analysis of interactions between biomolecules.

SPR and RM techniques are optical methods which use evanescent waves[1] to primarily determine the refractive index of an (aqueous) solution. As a consequence, these methods are unspecific, however they have the advantage of being very sensitive. Specificity can be introduced by modification of the surface facing the solution, for example, by immobilization of a receptor protein. Extremely thin layers of gold, other precious metals or glasslike materials may be used as supporting surfaces. Due to the interaction with the immobilized protein a bioactive molecule, present in solution above the sensor, will be attracted to the surface. The obtained change of the refractive index of the solution close to the sensor surface is directly proportional to the increase or decrease in mass of the sensor during the ligand–receptor binding process (Figure 1).

The experiments can be performed within minutes and need only very small amounts of sample, making them interesting as pharmaceutical screening processes. However, for biological aspects the signal progress over time is much more interesting, since one can determine the equilibrium and time constants (the association rate constant or "on-rate", and the dissociation rate constant or "off-rate") of the ligand–receptor binding process from the curves. Due to the optical detection mode, one obtains insights into the time scale of the processes being monitored without labeling the molecules. The collected kinetic and thermodynamic information complement structural data of the systems under investigation, thus being of great importance for structure activity relationship (SAR) studies of biomolecular complexes.

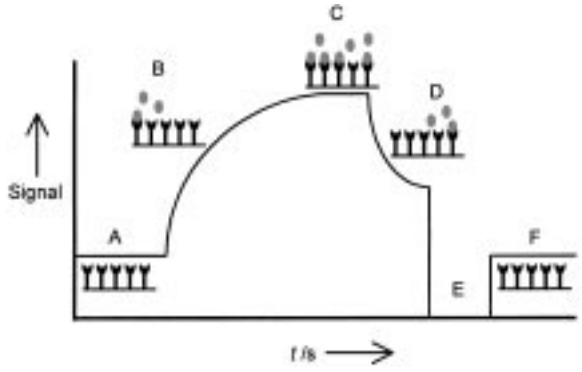

Figure 1. Schematic representation of the dependence of signal intensity on the surface properties during a resonant mirror or surface plasmon resonance experiment. A) Baseline after preparation of the sensor surface, for example, by immobilization of a protein. B) Addition of a binding partner to the solution results in complex formation. This process changes the refractive index at the sensor surface and the experimental signal increases. C) If the experiment is continued until equilibrium, a plateau is reached. D) Removal of the binding partner from solution results in complex dissociation. The experimental signal decreases. E), F) Termination of the experiment and regeneration of the surface without deactivation of the protein leads back to the baseline level. The association and dissociation rate constants of the ligand receptor binding process can be mathematically determined from the slopes of the curve.

Membrane Systems on Sensor Surfaces

Immobilization of a molecule onto the sensor surface results in reduced translational and rotational degrees of freedom. This fact distinguishes SPR and RM techniques from other biophysical methods where all interacting partners are dissolved. However, many important biomolecular recognition processes take place on surfaces, such as cell membranes. The logical consequence is, therefore, to build artificial membranes on sensor surfaces to study receptor–ligand interactions in an environment most closely resembling the natural one. To achieve this goal, different concepts have been developed to immobilize membranes and embedded proteins on sensor surfaces. The most apparent and simple one is to couple alkanes through a thiol group directly to the gold surface. These functionalized alkanes create a hydrophobic, self-assembling monolayer on the sensor surface, on which a lipid bilayer can be formed (Figure 2a). The use of these membrane systems allowed the study of interactions such as the binding of factor VIII[2] and antibacterial peptides[3] to membranes, the interaction of a protein kinase with phospholipids,[4] and the binding of lectins[5] and cholera-

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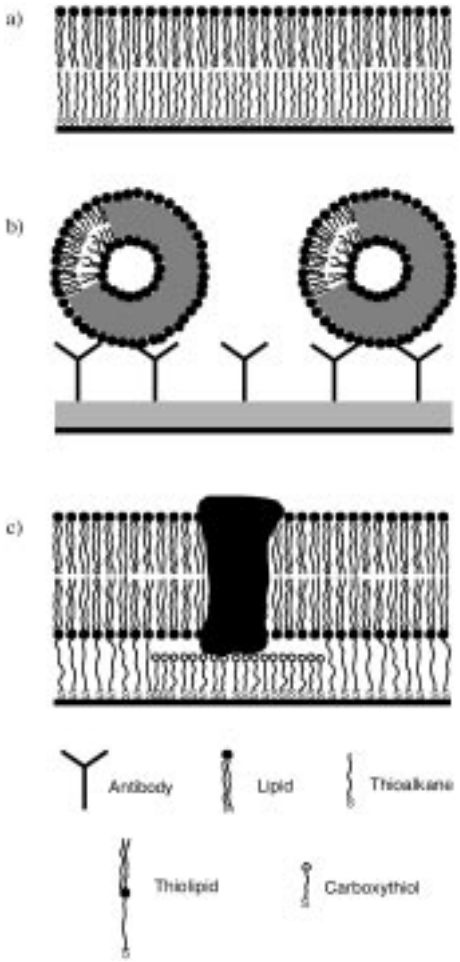

Figure 2. Different strategies for creation of artificial membrane systems on sensor surfaces. a) Thioalkanes directly immobilized on the sensor provide a hydrophobic surface, on which a lipid bilayer can be formed. Transmembrane proteins can not be introduced into this artificial membrane. b) Anti-lipopolysaccharide antibodies are immobilized on a modified dextrane matrix, to which liposomes containing lipopolysaccharides can bind.[10] This allows the study of molecules binding to the liposomes or membrane associated proteins. c) Special thiolipids and carboxythiols are used to build bilayers with fixed distances to the surface of the biosensor.[14] This is also a method to introduce transmembrane proteins into an artificial membrane and study it as a receptor.

toxin[6] to glycolipid systems. Furthermore, it was possible to place a functionally intact nicotinic acetylcholine receptor[7] as well as cytochrome C oxidase[8] in such artificial membranes. Recently, the formation of a multicatalytic protease complex (20-S-proteasom), which consists of 28 subunits, was achieved on such a lipid bilayer.[9]

A lipid bilayer which is attached directly to the sensor surface has the drawback that membrane-spanning proteins may not be introduced. If such proteins are to be studied in a bilayer system, one must fix the membrane at a certain distance above the sensor surface by special spacer molecules. One such spacer is the monoclonal *anti*-lipopolysaccharide antibody SE155-4, since its ligand, the lipopolysaccharide, possesses optimal properties to anchor micelles or lipid bilayers onto the biosensor (Figure 2b).[10] Alternatively, it is also possible to attach biotinylated lipids to the surface by immobilized avidin or streptavidin. Vesicles immobilized in this manner were used for binding mitochondrial creatin kinase[11] and recoverin.[12]

A new development is the direct modification of a gold-attached dextrane matrix with hydrophobic anchors through which liposomes may be immobilized.[13] Furthermore, special thiolipids were employed to fix the lipid bilayer to the sensor surface at a certain distance (Figure 2c). This way it was possible to position rhodopsin in predefined regions of a membrane and investigate the light-induced transformation of transducin by rhodopsin.[14]

Sample Volume and Sensitivity

In the meantime, recent developments in SPR technology by Biacore AB have lead to decisive improvements concerning sample consumption and sensitivity of the instruments. With current instruments the detection of mass changes of the sensor surface in the picogram range is possible. The surface of such a sensor equals approximately 1 mm^2. In addition, the volume within the flow cell could be decreased to approximately 21 nL, greatly reducing the consumption of the solubilized binding partner. Working with these SPR instruments, one can now study the binding of compounds with molecular weights considerably below 1000 Da and dissociation constants in the millimolar range to immobilized proteins. This was demonstrated with a monoclonal antibody raised against oligoglucose and small (di- to tetrasaccharide) ligands.[15] From these improvements new applications for the use of SPR instruments in screening processes or in binding studies of small, low-affinity ligands to receptor proteins will emerge.

Coupling of SPR Techniques and Mass Spectrometry

The use of SPR technology in the quest for bioactive compounds from libraries allows the determination of binding activity of one or more substances from such a mixture. However, it is not possible to unambiguously identify the bioactive compound directly from the complex mixture. Since mass spectrometry is one of the most sensitive analytical methods for providing information on structure and composition of a molecule, the coupling of SPR and MALDI-TOF (Matrix-assisted laser desorption/ionization time-of-flight) techniques enables the characterization of bioactive substances immediately following an SPR experiment. For this purpose, the sensor surface is treated with the matrix needed for the MALDI-TOF experiment and subsequently analyzed. This way, bioactive compounds may be characterized directly from the surface of the biosensor even in the subfemtomolar range.[16] However, the biosensor must be removed from the instrument after each positive SPR result and prepared for the MALDI-TOF experiment. This is time consuming and can become quite expensive, since the biosensor can not be used repeatedly after it has been modified for the MALDI-TOF experiment. Therefore, a new development concerning the SPR–MALDI-TOF coupling allows the recovery of the bioactive compound in a small volume directly from the biosensor.[17] The recovered substances can subsequently be analyzed by MALDI-TOF spectrometry, also in concentra-

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tions reaching the femtomolar range. This way, the biosensor is not destroyed and can be used for further experiments.

Mobile SPR Sensors

A wide-spread distribution of SPR and RM instruments to determine refractive indexes and applications derived thereof is often opposed by the size and, in particular, the price of the instruments. Here, an alternative are the SPREETA sensors developed by Texas Instruments.[18] These devices combine the sensor surface with all the optic and electronic components required for SPR experiments in a compact (approx. $3 \times 4.2 \times 1.5$ cm) and lightweight (approx. 7 g) assembly which can be produced cost efficiently (Figure 3). A likewise

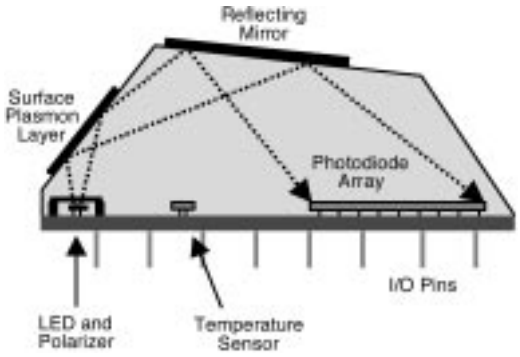

Figure 3. Structure and path of rays of a SPREETA sensor (kindly provided by Texas Instruments, Dallas, TX).

transportable interface handles data acquisition and data transfer to a personal computer, where the evaluation is achieved by use of an MS Windows program. The SPREETA sensors also allow the determination of the refractive index of a solution, and are hence useful for different purposes, such as monitoring the saccharide content of soft drinks. Analogous to the much larger stationary SPR instruments, these sensors may also be used to study biomolecular interactions. This was demonstrated for the binding of creatine kinase to an immobilized *anti*-creatine kinase antibody on the sensor surface.[19] Although, the sensitivity of state of the art SPR instruments may not be reached by the SPREETA sensors, these cost-effective and small SPR sensors will provide the basis for the development of a wide range of applications with SPR biosensor technology.

Conclusions

Since their presentation, SPR and RM biosensors have been continuously improved, and can thus be employed in different analytical areas. In many of these areas, such as environmental monitoring or product control in the food or beverage industry, custom-designed biosensor solutions already exist. For biologically oriented chemists or biochemists, the described advances in biosensor technology open new perspectives for the investigation of protein–ligand or other biomolecular interactions. Therefore, these techniques will become part of the standard equipment of biologically oriented laboratories in the near future.

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