Analysis of molecular recognition using optical biosensors

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Optical biosensors have made the analysis of molecular interactions rapid and convenient. The field is still young, with commercial instrumentation having been available for less than ten years. For this reason instrument development is still an important factor and the past year has seen continuing advances in instrumentation and particularly in novel sensor surfaces.

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Abbreviations

ASI	Artificial Sensing Instruments
DODA	dioctadecylamino
ITC	isothermal titration calorimetry
NTA	nitriloacetic acid
SPR	surface plasmon resonance

Introduction

Optical biosensors rely upon a phenomenon called the evanescent field to monitor changes in refractive index occurring within a few hundred nanometers of the sensor surface. Such changes are generated as a result of the binding of a molecule to a surface-immobilised receptor (or the subsequent dissociation of this complex), and real-time monitoring of these effects allows binding constants to be derived. A limited number of commercial instruments are available; currently, BIAcore (Uppsala, Sweden; World Wide Web URL: http://www.biacore.com), Affinity Sensors (Cambridge, UK; World Wide Web URL: http://www.affinity-sensors.com), and Artificial Sensing Instruments (ASI) (Zurich, Switzerland) account for most of the market. The instruments differ in the method used to generate this evanescent field. The BIAcore instrument [1•] utilises surface plasmon resonance (SPR), in which laser light is shone onto a glass prism in contact with a gold surface. Light is reflected at all angles except for the critical angle, at which the light excites the metal surface electrons (plasmons) generating the evanescent field and causing a dip in intensity of the reflected light. The critical angle is sensitive to refractive index changes occurring close to the sensor surface and thus, by monitoring the change in critical angle with time, details of the events at the surface can be probed. The IAsys from Affinity Sensors [2•] and the ASI BIOS-1 [3] also use the evanescent field to probe surface changes but generate it in a different manner using waveguiding techniques. In the IAsys, laser light is shone onto a glass prism, which has a layer of low refractive index material deposited on it and this in turn has a further high refractive index layer. This constitutes a waveguiding structure along which laser light at the critical angle will couple. The light emerges with a difference in phase, which can be detected using appropriate optics. The angle of laser incidence is monitored with respect to this phase change. The ASI also uses the principle of waveguiding but has a different coupling method, which employs a diffraction grating.

Despite the differences in generation of the evanescent field, the general design of experiments is essentially the same for each instrument. One of the interacting partners, the ligand, is covalently attached (or attached by a high affinity noncovalent interaction) to the sensor surface while the ligate binds to the ligand from free solution. As the sensors monitor refractive index changes occurring in real-time, the amount of ligand immobilised and the rate of ligate binding can be determined. Indeed, the determination of the interaction kinetics is one of the key advantages of this technique.

This review covers developments in the field of optical biosensors since mid-1998. Attention will be paid to the advances made in the kinetic analysis of interactions, the increase in the number of different sensor surfaces, and the variety of uses that the surfaces and instruments have produced.

Method development

Kinetic analysis

Over the past several years, much attention has been focused on kinetic analysis of biosensor data. Over the past year, however, the number of publications in this area has fallen, perhaps because the kinetics are now becoming better understood. In general, valid kinetic measurements are obtained provided that low levels of ligand immobilisation are used [4,5]. This is probably due to a mixture of factors. The low levels of immobilised ligand prevent any significant alteration in the concentration of ligate in solution, a situation which is essential when applying standard pseudo first order equations [6]. Furthermore, the rate of ligate solution flow (or stirring speed) should be sufficiently high to ensure that association rates are not limited by the rate at which ligate can reach the sensor surface (mass transport limitations). Ligate concentrations should be sufficient to obtain a measurable response. When these experimental conditions are met, reliable data can be obtained. Analysis of these data relies, in part, upon the knowledge of the user in determining the model of binding. A simple 1:1 interaction can be investigated using the integrated rate equation. More complicated analysis requires the use of numerical integration [7], for example when binding is bivalent [8] or involves the measurement of kinetics from a capture system (one where the ligand is anchored by a high affinity noncovalent interaction) showing capture/ligand dissociation [9•]. Further

analysis can be afforded by the use of global fitting, which involves fitting all data sets (response versus time at a number of different ligate concentrations) simultaneously [10].

In a similar manner to that described earlier using the IAsys system [11], equilibrium titration analysis has now also been performed on the BIAcore [12,13]. This technique allows measurement of ligate interactions with ligand that has poor resistance to the solutions used to remove the ligate and so regenerate the sensor surface (it is possible for the regeneration soloution to inactivate the ligand).

Comparison of kinetic constants from optical biosensors and solution based techniques

Because of the anchored nature of the ligand, there has often been concern as to whether the binding constants measured using optical biosensors reflect the situation in free solution. Although no systematic comparisons of biosensor data with solution techniques have yet been published, several researchers have made specific comparisons. For example, the interaction of lipopolysaccharide with various immobilised peptides was investigated by both SPR and isothermal titration calorimetry (ITC) [14•]. Close agreement between affinity constants from the two methods was observed, with typical affinities of around the micromolar range. Another comparison highlighted larger differences between affinity measurements from ITC and SPR [15•]. Here the interaction of a glutathione-*S*-transferase labelled protein involved in prevention of cell death, BAG-1, was attached to a carboxymethyl dextran matrix with immobilised anti-glutathione-*S*-transferase monoclonal antibody. The affinity of heat shock protein (HSP70) binding was determined to be 500 nM by SPR and 100 nM by ITC. The difference is likely to be due to the diffusional influence of the dextran. It should be noted that not all comparisons result in agreement. The kinetics of DNA triplex formation were compared by SPR and ITC and resulted in a 20-fold difference in affinity (0.09 μM compared to 2.2 μM with ITC) [16•]. This difference in affinity resulted from differences in the dissociation rate constant, as the association rate constant agreed with previously determined values.

Expanding the range of applications

Most of the published applications of biosensors have been attributable to interactions between soluble protein components and their ligands, as best illustrated by antibody–antigen interactions. The past year has seen increasing interest in extending this repertoire to include different types of biomolecular interactions.

Membrane systems

Binding to membrane-bound components has enormous biological importance, and several workers have described methods to measure binding to ligands within liposomes (spherical lipid bilayers over 25 nm in diameter). Liposomes have been anchored to a sensor surface using some form of biospecific interaction. Following this, the immobilised liposome has been used for further binding studies. For example, liposomes containing the cell-surface tumour antigen (disialoganglioside GD3) plus salmonella serogroup B lipopolysaccharide have been anchored to immobilised IgG that is specific for this salmonella antigen [17]. This system was used successfully to monitor binding of antibodies specific to the tumour antigen. In another experiment, biotinylated high-density lipoprotein within liposomes was anchored to a streptavidin-derivatised surface [18]. This was then used to study binding of natural and mutant lecithin cholesterol acyltransferase. An interesting development that will assist such studies is the recent availability of hydrophobic surfaces from Affinity Sensors and BIAcore. Such surfaces effectively provide one half of a bilayer covalently attached to the sensor surface, so allowing direct coupling of the second half of the bilayer to the sensor. These surfaces have been used to measure the binding affinities of the B subunit of cholera toxin B to a bilayer containing ganglioside G_{M1} within dioleoyl phosphatidylcholine and distearoyl phosphatidylcholine monolayers [19•] and membrane-bound nicotinic acetylcholine receptor to receptor-specific monoclonal antibodies, Fab′ or bispecific antibodies [20•]

A further interesting consideration with hydrophobic surfaces is the orientation of ligand. By preparing lipids with nitriloacetic acid (NTA), monolayers containing NTA can be formed allowing the binding of molecules containing a hexa-histidine tag. Such an approach has been employed with dioctadecylamino (DODA) and 1-stearoyl-2-oleylphosphatidylcholine (SOPC) to produce a monolayer of NTA–DODA/SOPC by vesicle fusion [21•]. The binding kinetics of histidine tagged green fluorescent protein (GFP) were found to be dependent upon the NTA–DODA composition of the surface. At 10 mol% concentration, the bindings were described by high order kinetics, whereas at 2 mol% concentration the bindings were fitted by the pseudo first order model. The authors ascribe this to two different situations. In the first, at 10 mol% and above, GFP is trapped at the surface; below 10 mol% the surface can be used for His-tagged proteins.

Interactions involving DNA

Although much of the early work with biosensors measured the binding of proteins, the technique is applicable to any binding interaction. Several workers have published papers in the past twelve months describing DNA–DNA and DNA–protein interactions. The kinetics of forming an intermolecular DNA triplex by immobilising a 5′-biotinylated 17-mer oligomer have been investigated [16•]. Formation of a 17 basepair duplex was achieved by the addition of a complementary 17-mer. The kinetics of triplex formation were monitored from the interaction of a third 11-mer strand. Duplexes have also been immobilised to streptavidin surfaces via biotinylation of the duplex to study the interaction with zinc finger peptides [22].

Other factors

There is constant interest in extending the scope of biosensor methods to an increased range of affinities or to smaller molecular weight systems. Interactions with dissociation equilibrium constants in the millimolar range have been successfully measured using analytes with molecular weights of less than 1000 Da [23•]. At the other extreme, Echovirus 11 (M_r 8000 kDa), an *Enterovirus*, has been immobilised to the dextran matrix in order to study the interaction with the cell receptor CD55 (decay-accelerating factor) [24].

The fact that ligate can be selectively removed from solution by specific binding to the ligand and then eluted allows the biosensors to perform as a microaffinity column. This has been successfully employed and the eluted products analysed using matrix-assisted laser desorption/ionisation (MALDI) mass spectrometry [25•].

Biosensors can also be used for fermentation monitoring, where their use increases the speed at which a process can be followed. Samples are taken from the reactor and the concentration of specific product determined [26•]. The technique can also be used to determine the breakthrough point for chromatographic purification [27].

The biosensor as a routine laboratory tool

In many early publications, the optical biosensor itself was the prime focus of the article. It is, however, apparent that in most recent publications that involve such instrumentation, the biosensor is used more as a routine tool to provide binding measurements. This reflects the growing acceptance of the methodology and the increased availability of instrumentation. For example, the interaction of mutant and wild type yeast calmodulin has been measured using various immobilised peptides [28]. The binding of a peptide-containing phage to immobilised antibody has been monitored [29], and the specificity of a dimeric, bispecific miniantibody has also been investigated [30]. For each of these communications the focus is the interaction in question, with the biosensor being just a convenient way to characterise this. Despite the growing acceptance of biosensor data, however, it is worth noting that the values derived from the data analyses must still be interpreted with caution. As with any complex technique, correct experimental design is essential if the results are to be meaningful. For example, the amount of ligand loaded onto the dextran surface should be as low as possible in order to generate meaningful results [31]. The issues highlighted by Shuck and Minton [32] are also still relevant.

Advances in sensor surfaces

Over the past year there has been progress in the development of sensor surfaces, which will increase the range of experiments that can be performed. In addition to the widely used carboxymethyl dextran, the following surfaces are also now available: planar surfaces (carboxylate, biotin, and amino); nickel chelating surfaces (NTA); and streptavidin coated dextran surfaces. The hydrophobic surface has been mentioned earlier, and promises to extend the biosensor methodology to applications involving membrane-bound components. More subtle surface variations, including lower molecular weight dextran and lower charge dextran, have also been commercialised and could be more suitable for certain applications than are the existing surfaces. The lower molecular weight dextran allows large ligates to approach the surface more closely while retaining their activity; the lower charge dextran allows the immobilisation of highly charged ligands such as DNA.

The use of planar surfaces can eliminate certain problems relating to diffusion of ligates through a dextran surface [33], and is very important if measuring whole cell binding. However, other uses have been reported. Rabaptin-5, a 100 kDa protein that acts as an effector for the small GTPase Rab5, was immobilised on an aminosilane cuvette [34]. The biosensor showed a marked difference in binding response (fivefold higher) for Rab5–GTPγS and Rab5–GDP. This is consistent with the importance of the switch from the GDP-bound to the GTP-bound state in vesicle transport mechanisms.

Conclusions

With the advent of the new surfaces, an increased range of interactions can be analysed. The key issue to be addressed in the future is the ever increasing demand for higher sensitivity to allow the interactions of small molecules to be monitored reliably in real-time, thus allowing kinetic parameters to be determined. Developments in instrumentation will facilitate such measurements; over the past year we have seen the release of the new BIAcore 3000 instrument which, it is claimed, is able to measure the binding of molecules with molecular weights as low as 180 Da. Further increased sensitivity, increased throughput and improvements in the user interface of the biosensor devices can be expected in coming years. As the field matures and optical biosensors become more widely available there is an increasing shift to applications rather than method development. It seems likely that the trend will soon be for biosensor use being seen as the routine way to measure biomolecular interactions.

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