Abstract of the seminar "Commercial Biosensors" (MB-JASS 2006)

1 Introduction

A biosensor is a device, which uses a biological component to determine the concentration of a certain substance. Apart from this biological component –the bioreceptor- a detector is needed in order to convert the physical or chemical information in an output signal. This detector is called transducer.

The question may rise, why one would make the trouble of obtaining biological material – which is only stable under certain conditions and has a limited lifetime- and integrating that in a transducer device. The answer as why to use biological reagents is simple: because they are designed to react to and distinguish between (in)organic substances in an extremely sensitive and selective way. The advantage of biosensors over traditional analysis, which is based on manual or semi-automatical addition of a reagent to the probe, is the immobilization of the reagents within the system. Thus biosensors allow automated and therefore easy and fast analysis, which in turn makes continuous monitoring possible.

Unfortunately, it is exactly the specificity and sensitivity that often fails yet. For example, a lot of biosystems react to pesticides in the same way they react to heavy metals. This is one of the reasons why the commercialization of biosensors is still limited.

2 The biological component

The substance to be measured is also referred to as the analyte or substrate. Any substance that is consumed or produced in a biochemical process can in principle be analyzed by a biosensor, if one can be constructed. Some examples of analytes are gases, ions and all kinds of organic compounds, like aminoacids, proteins, urea, paracetamol and DNA. This list can be expanded by bigger biological systems –like microbial cells- that stay whole, while part of them reacts with the receptor.

The biological sensing element must above all be selective to the analyte. Other import features are a reasonably long lifetime – a couple of months or more – and sufficient stability under pH and temperature variation. Four main groups of biomaterials qualify for this: enzymes, antibodies, nucleic acids and chemoreceptors. These may be used in purified form or as part of their natural environment.

- **Enzymes** are the most regularly used bioreceptors. They work as catalysts for a reaction, which changes the concentration of the analyte. Most commonly, this reaction is a redox reaction or can be combined with a redox reaction. Thus the concentration can be converted into an electrical signal with the aid of an electrochemical cell.

- **Antibodies** bind very strongly with the corresponding antigen. Providing a signal for the transducer is not as obvious as in the case of enzymes. A possible solution is using labelled antibodies or antigens in a competitive assay. Labelling may be done with radioisotopes, enzymes, fluorescent probes or chemiluminescent probes.

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- **Nucleic acids** operate selectively because of their base-pairing properties: they are the building blocks of DNA and RNA. They could be utilized for identifying genetic disorders, cancers and viral infections. As with antibodies, a DNA assay often involves the addition of labelled DNA.

- **Chemoreceptors** are proteins inside a cell membrane that can recognize either neurotransmitters or hormones. The binding of the analyte to the chemoreceptor activates a physiological response, such as ion channel opening, a second messenger system or the activation of enzymes. Thus they can be seen as natural biosensors, regulating the electrical signals in our nerves. It is difficult to isolate them, but whole nerve cells can be used as well, for instance to determine the presence of drugs.

The lifetime of biosensors can vary between several hours and over a year. That does not only depend on the type of receptor chosen and the conditions under which it is kept, but also on the method of immobilization used.

3 Immobilization

The process of attaching the biological component to the transducer is known as immobilization. The simplest method of immobilization is adsorption. However, this bonding is very weak: the surface density is very low and it typically lasts for only one day.

Another method is entrapment. Here a solution consisting of monomers is mixed with the bioreceptors. Then the mixture is polymerized to a gel, so that the biological material is trapped in a network of chains. A disadvantage is that hereby part of the biomaterial is blocked from participating in the reactions.

Microencapsulation has already been used in early biosensors. With this method the biomaterial is placed behind a membrane, which is only permeable to small molecules. An incidental advantage is the protection of the bioreceptors from contamination.

One can also chemically bond the biomaterial to polymer chains, so that a close-packed chain structure is formed. This is called cross-linking and is useful to stabilise adsorbed biomaterials.

Another method involves a carefully designed bond between the biomolecules and the transducer surface or membrane. The surface density of the biomolecules can be made quite large with this method. Moreover, their active sites are all neatly facing away from the surface, thus enhancing the bioactivity. The typical lifetime of a biosensor with covalent bonded receptors is 4 to 14 months.

4 Transducers

4.1 Electrochemical transducers

4.1.1 Potentiometric transducers

Potentiometric transducers are based on an electrochemical cell. Each electrochemical cell has two separated electrodes where oxidation resp. reduction takes place. The electrical circuit is closed by a salt bridge or membrane.

At the indicator electrode substances like ammonia, carbon dioxide, hydrogen ions and iodine can be detected. These substances may –amongst other reactions- be part of a reaction in which the analyte is involved. The bioreceptor may be an enzyme, which allows the reaction with the analyte to take place at the indicator electrode. If the analyte is present in the sample, the ratio of concentrations will be altered massively. Hence, on the basis of the measured concentration of the inorganic substance, one can deduce the concentration of the analyte. The required ratio of concentrations, which depends on the chemical equilibrium under the outer circumstances, can be derived by calibration.

The potential difference between the indicator and reference electrode is measured at equilibrium, when the netto current is zero. The potentials E ("electromotive force") of the half-cells can be calculated with the Nernst equation, which can be derived from the equations for the Gibbs free energy. According to thermodynamics, the Gibbs free energy must be a logarithmic function of the ratio of activities of the oxidator and reductor. For dilute solutions, the activities can be taken to be the same as the concentrations.

In a real measurement set-up, the electrodes are usually made of silver-silverchloride, because silverchloride has the advantage of being hardly soluble into water. The measured voltage is the sum over the potential jumps at each junction (i.e. each solid-liquid or liquid-liquid interface at the electrodes and ion-selective membranes).

4.1.2 Amperometric transducers

Now if an increasing voltage is applied to the electrochemical cell, at a certain point the reaction will be forced to evolve in the opposite direction. This causes a sharp rise in the cell current. If the voltage is further increased, the current will cease to rise. Obviously, the limiting factor is the diffusion rate of the substances from the bulk of the solution to the electrode. The peak current is directly proportional to the concentration of the substance to be measured. This is made use of in amperometric transducers. If the potential at which the reaction is forced to "turn around" is known, one may step V directly to a value just above that potential and observe the current. This method is also known as chronoamperometry and is the most widespread transducer method.

The current will decay over time because of Fick's second law of diffusion (see slide nr. 21), where C is the concentration and D is a constant. This is illustrated in the picture on the slide, where the grey area is the electrode surface, the horizontal axis is the distance to it and the vertical axis is the concentration C(x,t). The slope of the concentration is decreasing in time, meaning the spreading-out of the diffusion layer. The simplest solution to the diffusion equation, which can be obtained analytically, shows that the decay is proportional to one over the square root of time. From this current-time profile C can be derived.

4.1.3 Other electrochemical transducers

In a conductimetric transducer, the change in resistance of the solution after a reaction with the analyte is used to measure the concentration.

Field Effect Transistors are devices in which the current from source to drain strongly depends on the gate voltage. Thus, the transistor can be switched on and off with the gate voltage. This device can be transformed into a sensor by building in a biological element between source and gate, by which the effect of V_G is lessened or increased.

4.2 Optical transducers

Optical methods have become increasingly popular due to the development of optical fibres. A change in optical properties can be achieved by using the absorption of light in a medium, luminescent labels, Internal Reflection Spectroscopy or laser light scattering.

An innovative example of a luminescence-based method is the combination of chemiluminescence and fluorescence in a competitive immunoassay. Chemiluminescence occurs by the oxidation of certain substances. Thus it produces visible light in the cold and in the absence of any exciting illumination – that is, in the dark. A fluorescent substance could absorb this light and change it into light with another wavelength. This occurs for instance if a chemiluminescent-labelled antigen binds to a fluorescent-labelled antibody. Hence the ratio of the original chemiluminescent vs. altered wavelength equals the ratio of unlabelled vs. labelled antibodies. One knows the number of added labelled antibodies, so the concentration of unlabelled antibodies can be calculated.

Total Internal Reflection (TIR) methods are based on the complete reflection of a ray of light that hits a less dence medium. Remembering Snell's law, we see that the transmitted light beam vanishes for angles larger than the critical angle θ_c. According to quantummechanics, there is a finite probability for a photon, which should be reflected, to reach into the classically forbidden medium. Thus there is an exponentially decaying (=evanescent) wave on the other side.

One can use this evanescent wave for example in an immunoassay, where the antigens are located at the surface in the less dence medium. Part of the corresponding antibodies can be labelled with fluorescent particles. When the antibodies bind themselves to the antigens, the fluorescent particles can absorb the energy of the evanescent wave and re-emit it into the dark medium, which can be detected.

Another way to use the evanescent wave in a biosensor is by using Surface Plasmon Resonance (SPR). A plasmon is a collective excitation of conductance electrons in a crystal. A plasmon is localized and propagates slowly through the crystal. Surface plasmons are – as their name says - bound to the surface of a bulk crystal. A surface plasmon can be excited by an evanescent wave, if the quantum energy of the photons is equal to the energy of plasmons in that material. This so-called resonance leaves a gap in the reflected light intensity at one particular frequency.

Apart from the energy, the momentum must be conserved. For a plasmon that is confined to a plane, this means that the angle of the incoming light must be $sin\ \theta = k_{Plasmon}(E)/ k_{Photon}(E)$. The wave vectors k are functions of the energy.

Now plasmons are excited in a thin gold film on the surface of a prism in which the light propagates. The layer is usually made of gold, because gold is chemically inert and gives a signal at convenient combinations of angle and wavelength. The bioreceptors are placed on the other side of the gold film. The binding of the analyte to the receptors changes the refractive index of that side. Since the plasmons can fall back into photons and vice versa, they interact strongly with the evanescent wave on the side of the biomaterial. Therefore a change in the refractive index there causes a large shift in the wave vector of the plasmons and the angle of the light absorption. The latter can be measured accurately.

5 Application areas

Biosensors can be used in the health and food industry and for monitoring the natural environment. The field of biosensors started in 1962 with the invention of the blood glucose biosensor by Leland Clark. Although it took quite long before Yellow Springs Institute commercialized this biosensor in 1974, its success has not been beaten by other biosensors: the share of blood glucose sensors in the world market for biosensors is now about 85% (see [4]). This is due to their use for diabetes patients, the number of whom is ever growing: in 2006 there are approximately 171 million people suffering from diabetes worldwide, in 2030 there will be twice as much. Patients with diabetes type 1 should measure their blood glucose level regularly in order to adjust the necessary insulin injections to the result. Patients with diabetes type 2 are strongly recommended to measure their blood glucose level regularly in order to adjust their diet appropiately. Single use biosensors have a lot to offer: they are easy to use, disposable and thus hygienic and small enough to be integrated in a wristwatch.

6 References

An introduction to biosensors and general overview of their applications can be found in [1], [2] and [11].

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