literature survey

Status of monitoring in biotechnology

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Traditional methods of monitoring bioprocesses rely either on removing samples with subsequent analytical chemistries or indirect monitoring by sampling the gas phase.[1] In recent years powerful new techniques have been developed for fabricating probes that have a high degree of biochemical specificity and provide the capability to monitor directly key substances that influence and control cell behavior.[2]

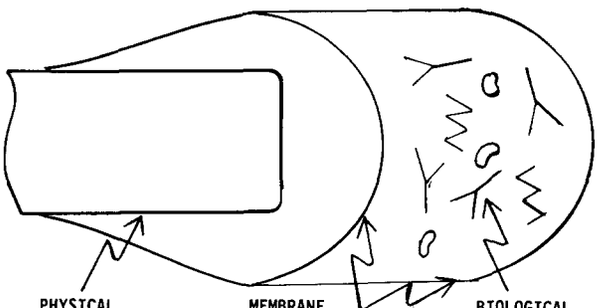
Nearly all of today's biosensors (Figure 1) are comprised of a biologically active material (e.g., enzyme, antibodies, binding protein) in intimate contact with a suitable optical or electronic system to convert the biochemical event into a quantifiable electrical signal that can be processed and transmitted.[3] The transducer element usually contains the chemistry that provides the selectivity of the device. The detector element is usually physical in nature, optical, thermal or frequency sensitive, primarily determines the sensitivity of the device, and selective membranes control the immediate environment of these subsystems.[4]

Biochemical transducer elements

The biochemical reaction system (containing enzymes, organelles or cells) is chosen to generate a readily detectable species from the specific analyte of interest. If the generated species is an ion, then a potentiometric detector such as a pH electrode or ion selective electrode might be used; on the other hand, if the reaction product is either oxidizable or reducible, then an amperometric detector could be employed. The literature of this area is quite extensive with the groups of G. Rechnitz,[5] S Suzuki[6] and G. Guilbault[7] being very prolific.

The use of bioreceptors (antibodies or binding proteins[8] in conjunction with electrochemical and optical detectors for the purpose of bioanalytical sensing has been pursued over the past 10 years. Numerous workers have attempted to devise a variety of indirect "immunosensor" devices for detecting drugs (haptens), hormones or antibodies. For example, several groups have employed electrodes to monitor enzyme-linked immunoassays in either homogeneous[9-11] or heterogeneous configurations.[12-15]

Solsky and Rechnitz[16] immensely improved on the overall concept of directly measuring antibodies by modifing an ion-selective carrier (e.g., crown ether) with a low molecular weight analyte (hapten) and impregnating this carrier–hapten conjugate into a poly(vinyl chloride) membrane electrode. In the presence of anti– hapten antibodies, the membrane potential was altered because the carrier's ability to complex and transport sodium and potassium was changed.[17-19] Additional experiments



	PHYSICAL COMPONENT	MEMBRANE COMPONENTS	BIOLOGICAL COMPONENT
FUNCTION	DETECTOR	BARRIER	TRANSDUCER
EFFECT ON CHARACTERISTICS	SENSITIVITY	RESPONSE TIME	SELECTIVITY
TYPICAL COMPONENTS	ELECTRODE SEMICONDUCTOR FIBER-OPTIC QUARTZ CRYSTAL PIEZOELECTRIC CRYSTAL	DIALYSIS MEMBRANE HYDROPHILIC POLYMER HYDROPHOBIC POLYMER ION-SELECTRIC MEMBRANE CONDUCTIVE POLYMER	ENZYME ANTIBODY MITOCHONDRIA BINDING PROTEIN CELLS

Figure 1

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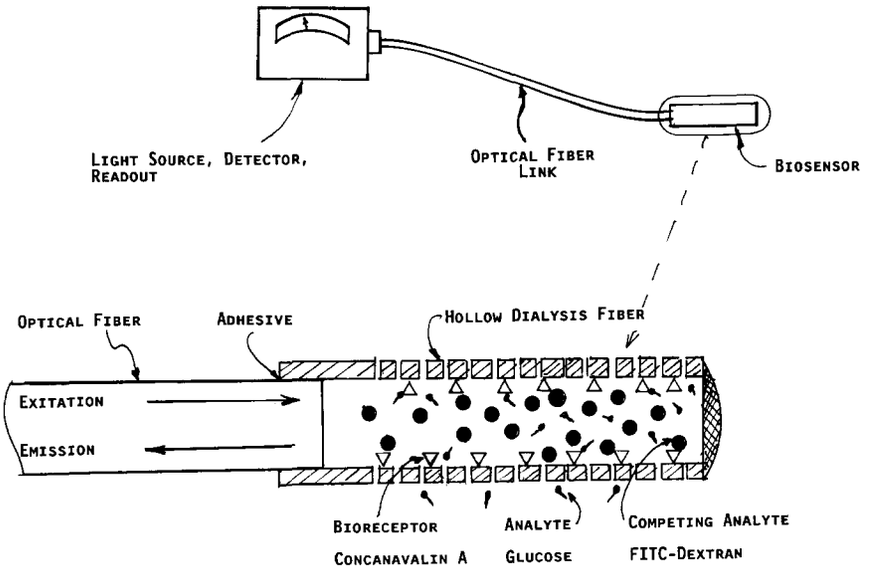

Figure 2

have shown that such an antibody sensing electrode can also be used to measure the concentration of hapten present in a test solution by a competitive binding principle, i.e., the more hapten present, the less antibodies bind to the carrier–hapten conjugate in the membrane, and the smaller the potential change observed.

Detector technology

Recently increasing attention is being given to miniaturization of these electrochemical sensors by the use of thin-film integrated circuit technology.[20-22] Research papers have demonstrated the feasibility of measuring on the order of 100 substances by electrochemical biosensors, yet very few have reached commercialization as continuous on-line sensors. Some of the problems relate to stability of the components, e.g., enzymes, organelles, materials (membranes, adhesives), to sterilization and exposure to biological fluids, as well as the drift of standard potentials. The main advantage of these devices, in addition to size, is that many modes of handling electrical signals are readily available, e.g., amplifiers, high impedance potential measuring devices or electrical signal processing circuits.

Ion-selective electrodes have primary utility for inorganic cations and anions; however, organic compounds can also be measured if they exist in ionic form. Senker *et al.*, Buck and Freiser (Cunningham and Freiser, 1982) have demonstrated that many basic biochemicals can be monitored by ion-selective electrodes that are "doped" with the compound of interest, that is, the carrier in the liquid membrane contains this compound.[23-25]

By using optical fiber wave guides, spectrophotometric, fluorometric and light scattering methods can be miniaturized to monitor very small samples, since the active portion of the sensor at the tip of the fiber is on the order of 1 lambda in volume. This capability has taken on special significance in recent years due to the ready availability of a variety of optical fibers and optoelectronic devices from the telecommunications industry.[26] Optical fibers have been used to fabricate microcolorimeters and microfluorimeters.[27] However, it is only recently that optical fibers have been coupled to biochemical reactions for the purpose of developing miniaturized biosensor detector elements.

There are two principal mechanisms by which light from an optical fiber interacts with the external environment. In one mode, light emanates from the end of a cleaved fiber core, and behaves like a miniature flashlight, with a useful sampling volume confined to a distance of about 5 mm from the end of the fiber.

Fiber-optic based analytical devices for physical properties such as temperature and simple analytes such as pH, CO_2 and O_2 have been demonstrated.[28-30] An approach to measure biochemicals was introduced by Schultz and Sims.[31] These developments in optical biosensors have been reviewed by Seitz,[32] Peterson and Vurek[33] and Wolfbeis.[34]

Fluorescence immunoassays can be converted to continuous biosensors by placing the bioreagents within a miniature hollow fiber dialysis compartment. An example of an optical biosensor for glucose based on this concept is shown in Figure 2.[35] This system is based on the reversible competition between glucose and fluorescein labeled dextran for receptor sites on Concanavalin A (an antibody-like protein). In this sensor the receptor is immobilized on the inner wall of the hollow dialysis fiber, so as to separate spatially the bound FITC-dextran from the free FITC–dextran, allowing the latter to be measured by the optical system, which mainly "sees" the solution in the central region of the hollow fiber. This system is "reagentless," i.e., there is no net consumption of chemicals required for its operation, and artifacts due to diffusional resistances do not effect the equilibrium response.

Second mode of illumination is achieved by removing the cladding from the central core of an optical fiber, which produces an evanescent "halo" of light energy that penetrates the fluid near the core and is effective for measuring adsorbed species or solutes on or near the surface (about 100 Å). Place *et al.* have reviewed a variety of techniques that could be utilized for developing optical immunossays on surfaces.[36] Of particular interest in this regard is the utilization of total internal reflectance fluorescence (TRIF) as for biosensors as demonstrated by Andrade and coworkers.

Conclusion

A wide variety of biochemical systems, e.g., enzymes, antibodies, binding proteins and bioreceptors, can be utilized with a number of detector elements to provide many opportunities for selective, sensitive and miniature probes. With advances in microfabrication of electronic as well as optic devices, on the one hand, and the capability (by genetic engineering) to produce and modify proteins to serve as analytical reagents, on the other, one can expect a new generation of biosensors that will provide the capability of monitoring and controlling bioprocesses both at the laboratory research level and at the commerical production level.

Literature

1 Wang, H. Y. in *Comprehensive Biochemistry* (Moo-Young, M., ed.) Pergamon Press, Oxford, 1985, pp. 423–431
2 Turner, A. P. F., Karube, I. and Wilson, G. S. eds. *Biosensors: Fundamentals and Applications*, Oxford University Press, Oxford, 1986
3 Lowe, C. R. *Trends in Biotechnology* 1984, **2**, 59
4 Ross, P. *Bio techniques* 1983, 204–207
5 Rechnitz, G. A. *Science* 1981, **214**, 287–291
6 Suzuki, S., Satow, I. and Karube, I. *Appl. Biochem. Biotech.* 1982, **7**, 147–155 (1982)
7 Guilbault, G. G. *Handbook of Enzymatic Methods of Analysis*, Dekker, New York, 1976, 460–509
8 Bachas, L. G., Tsalta, C. D. and Meyerhoff, M. E. *Bio Techniques* 1986, **4**, 42–56
9 Heineman, W. R. and Halsall, H. B. *Anal. Chem.* 1985, **57**, 1321
10 Gebauer, C. R. and Rechnitz, G. A. *Anal. Chem. Acta* 1980, **115**, 61
11 Eggers, H. M., Halsall, H. B. and Heineman, W. R. *Clin. Chem.* 1982, **28**, 1848
12 Alzawa, M., Murioka, A. and Suzuki, S. *Anal. Chem. Acta* 1980, **115**, 61
13 Renneberg, R., Schobleu, W. and Scheller, F. *Anal. Lett.* 1983, **16**, 1279
14 Masani, M., Zolesi, F. and Palleschi, G. *Anal. Let.* 1982, **15**, 101
15 Meyerhoff, M. E. and Rechnitz, G. A. *Anal. Biochem.* 1979, **95**, 483
16 Solsky, R. L. and Rechnitz, G. A. *Science* 1979, **204**, 1308
17 Solsky, R. L. and Rechnitz, G. A. *Anal. Chim. Acta* 1981, **123**, 135
18 Keating, M. Y. and Rechnitz, G. A. *Anal. Chem.* 1984, **56**, 801
19 Connell, G. R., Sanders, K. M. and Williams, R. L. *Biophys. J.* 1983, **44**, 123
20 Janata, J., and Huber, R. J. in *Ion-Selective Electrodes in Analytical Chemistry*, vol. 2, Plenum, NY, pp. 107–174
21 Lauks, I., *SPIE Intl. Soc. Optical Eng. Proc.*, vol 387 of *Critical Reviews of Technology*, 1983
22 Cheung, P. W. *et al.*, eds. *Therory, Design and Biomedical Applications of Solid State Sensors*, CRC, (1978)
23 Senker, J. *et al. Anal. Chem.* 1979, **51**, 786–790
24 Buck, R. P. and Cosofret, V. Proc. Symp. Biosensors, Los Angeles, Sept. 15–17, 1984.
25 Cunningham, L. and Freiser, H. *Anal. Chem. Acta* 1982, **139**, 97–103
26 Chabay, L. *Anal. Chem.* 1982, **54**, 1071A–1080A
27 Vurek, G. and Bowman, R. *Anal. Biochem.* 1969, **29**, 238–247
28 Lubbers, D. and Opitz, N. Proc. Intl. Mtg. Chem. Sensors, Fukuoka, Sept. 19–22, 1983, Elsevier, Amsterdam
29 Peterson, J. *et al. Anal. Chem.* 1980, **52**, 864–869
30 Saari, L. and Seitz, W. R. *Anal. Chem.* 1982, **54**, 821–823
31 Schultz, J. and Sims, G. *Biotech. Bioeng. Symp.* 1979, **9**, 65–71
32 Seitz, W. R. *Anal. Chem.* 1984, **56**, 16A–34A
33 Peterson, J. and Vurek, G. *Science* 1984, **224**, 123–126
34 Wolfbeis, O. S. *Pure and Appl. Chem.* 1987, **59**, 663–672
35 Schultz, J. S., Mansouri, S. and Goldstein, I. *Diabetes Care* 1982, **5**, 245–253
36 Place, J., Sutherland, R. and Dahne, C. *Biosensors* 1985, **1**, 321–353
37 Andrade, J. *et al. IEEE Trans. Elect. Devices* 1985, **32**, 1175–1179