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Recent Developments in Enzyme and Microbial Biotechnology — Strategies in Bioprocess Design

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Microorganisms have been used traditionally by industry as sources of natural products, or as sources of enzymes capable of mediating specific chemical transformations. This situation has changed radically in recent years, a time during which we have seen a dramatic increase in the number and range of potential biotechnological applications of enzymes and their genetically-engineered variants. An increasing number of enzymes, receptors and other proteins have now been structurally characterized, and their genes isolated as a basis for producing recombinant proteins for genetic analysis of their structure and function. These innovations have necessitated development of associated technologies for large-scale production of proteins in bioreactors, appropriate strategies for quality control, and new analytical tools for structural characterization of recombinant gene products. Some recombinant proteins are already in an advanced stage of development for use either as new-generation therapeutics, as target molecules for "intelligent" drug screening, or as biological components of biosensors. As the predictive power of protein model building improves, the diversity of applications of such technology will increase further as it becomes feasible to generate totally synthetic proteins with specifically-tailored properties.

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1 Introduction

1.1 Historical Perspective

"Biotechnology", in the broadest sense of the word, is almost as old as civilization itself. For thousands of years man has made use of naturally-occurring microorganisms to produce foodstuffs and beverages. The plant kingdom has additionally provided a wealth of pharmacologically active substances of medicinal value, almost all of which were used to advantage at the time without knowledge of their mechanism of action. These early "biotechnological" activities are a tribute to man's ability to control his environment through ingenuity and the adventitious use of available natural resources.

1.2 Growth of Microorganisms in Bioreactors

In modern times we have seen a considerable expansion in the scale, scope and innovative uses of biotechnology, paralleled by a more rational approach to biotechnologically-important questions as it became possible to grow the relevant microorganisms under controlled conditions on an industrial scale. Microbiology provided the means to identify and cultivate a wide range of hitherto uncharacterized organisms capable of producing novel substances with potent biological activities, such as antibiotics. Organisms have been discovered with enzymes which can generate efficiently important substances such as vitamins and amino acids from simple substrates, and yet other organisms have been found which can bring about relatively complex, stereospecific chemical transformations with astounding efficiency. Biochemistry has been important in defining the nature and complexity of the biosynthetic pathways which lead to a particular natural product, and studies of the physiology of cell growth have been essential for optimizing production rates of these substances in bioreactors. Finally, classical genetics has proved to be an invaluable means of generating new mutant cell lines optimized for product yield and large-scale cultivation.

1.3 Bioprocess Control

These essential contributions of the Biological Sciences to the development of modern Biotechnology have been matched by an equally significant contribution from the Physical and Engineering Sciences. New engineering problems have been addressed in the design of efficient production plants for large-scale cultivation of microorganisms. It has become necessary to develop novel, sensitive sensor devices for monitoring rapid changes in biological parameters such as growth rate, pH, oxygen tension and nutrient supply in order to optimize microbial growth for maximal production of a substance. The use of bioreactors to generate chemical substances and more complex biomolecules has also presented entirely new problems associated with product purification, treatment of biomass and waste disposal.

1.4 Impact of Molecular Biology

The last decade has seen a further biotechnological revolution following the application of knowledge gained from Molecular Biology. When examined in a historical context, this can be seen as an additional extension of the contribution that advances in basic knowledge in the Biological Sciences have made to biotechnological innovation. Nonetheless, these recent advances have added a new dimension to the application of biotechnology within the pharmaceutical and chemical/agrochemical industries. For the first time we are presented with the possibility of creating new, specifically engineered macromolecules with desirable biological properties either for use as therapeutics, as tools for the development of novel bioprocesses and construction of biosensors, or as a basis for development of novel and efficient screening systems for drug discovery.

The following review summarises some advances in several of the above areas of biotechnology and bioprocess development, based partly on the proceedings of a recent Meeting [1].

2 Chemical Synthesis Using Enzymes from Microbial Sources

2.1 Specific Chemical Transformations

Many microorganisms contain specific enzymes capable of directing particular chemical transformations, the most well-known being those leading to the synthesis of amino acids and cofactors. As in the case of antibiotic-producers, natural habitats have traditionally provided a rich source of the relevant microorganisms. In some parts of the world this concept is no longer considered to be as fashionable as previously, but in Japan this painstaking but pragmatic, intuitive approach has continued to lead to the identification of a range of new and useful enzymes from a diverse selection of organisms of different genera (Table 1), and the list continues to grow yearly. In some instances different organisms can be found containing enzymes capable of efficiently synthesising alternative stereochemical forms of a compound (e.g. synthesis of D- or L-cysteine; Table 1). In other cases growth conditions for the same organism can be manipulated to produce different enzymes. *Rhodococcus rhodochrous*, for example, produces either nitrile hydratase or nitrilase, depending on the growth conditions employed; the addition of crotonamide and cobalt chloride selectively induces the production of nitrile hydratase, whereas isovaleronitrile or isobutyronitrile are potent inducers of nitrilase. These, and other organisms such as *P. chlororaphis* and *Brevibacterium* can be used to produce a range of amines with diverse applications, such as acrylamide or nicotinamide and biologically-active amines such as L-Dopa [2–5] (Table 1). Many of these compounds are important products in their own right, whereas others are important stereospecific intermediates required for further steps in chemical syntheses of additional compounds.

Table 1. Chemical production using enzyme synthesis. The Table shows a representative collection of chemical compounds generated by enzyme synthesis, together with yields. The functional enzyme and bacterial source are indicated. (Table courtesy of Dr. H. Yamada, Laboratory of Agricultural Chemistry, Kyoto University, Kyoto, Japan)

Product	Enzyme (source)	Yield	
		$g\ l^{-1}$	mol%
Amino acids			
D-*p*-Hydroxyphenylglycine	Dihydropyrimidinase (*Bacillus* sp.)	4.9	(74)
D-Phenylglycine	Dihydropyrimidinase (*Bacillus* sp.)	6.2	(91)
L-Tyrosine	β-Tyrosinase (*Erwinia herbicola*)	61	
L-Dopa	β-Tyrosinase (*Erwinia herbicola*)	53	
L-Tryptophan	Tryptophanase (*Proteus rettgeri*)	100	(95)
L-Cysteine	Cysteine desulfhydrase (*E. cloacae*)	50	(86)
L-Cysteine	Cysteine synthase (*B. sphaericus*)	70	(82)
D-Cysteine	β-Chloro-D-alanine lyase (*P. putida*)	22	(88)
L-Cystathionine	Cystathionine γ-synthase (*B. sphaericus*)	42	(92)
L-Serine	Serine transhydroxymethylase (*Hyphomicrobium* sp.)	35	(25)
R-4-Chloro-3-hydroxy-butyric acid	Aldehyde reductase (*Sporoboromyces salmonicolor*)	72	(95)
Amides			
Acrylamide	Nitrile hydratase (*P. chlororaphis*)	400	(98)
Methacrylamide	Nitrile hydratase (*P. chlororaphis*)	200	
Crotonamide	Nitrile hydratase (*P. chlororaphis*)	200	
Nicotinamide	Nitrile hydratase (*Rhodococcus rhodochrous*)	1465	(100)
Nicotinic acid	Nitrilase (*Rhodococcus rhodochrous*)	172	(100)
Pyrogallol	Gallic acid decarboxylase (*Citrobacter* sp.	23	(100)
Theobromine	Oxygenase (*P. cepacia*)	14	(72)
D-Pantoyl lactone	Carbonyl reductase (*Candida parapsilosis*)	100	(83)
Coenzymes			
Coenzyme A	Multi-step enzyme system (*Br. ammoniagenes*)	115	(100)
S-Adenosylmethionine	AdoMet synthetase (*Saccharomyces sake*)	12	(45)
S-Adenosylhomocysteine	AdoHcy hydrolase (*Alcaligenes faecalis*)	74.2	(97)
FAD	FAD pyrophosphorylase (*Arthrobacter globiformis*)	18	(28)
Pyridoxal 5′-phosphate	PMP oxidase (*P. fluorescens*)	0.15	(98)
NADH	Formate dehydrogenase (*Arthrobacter* sp.)	30	(90)
NADPH	G6P dehydrogenase (a methanol-utilizing bacterium)	7	(75)
Polyunsaturated fatty acids			
Dihomo-γ-linolenic acid	Multi-step conversion (*Mortierella alpina*)	2.2	
Arachidonic acid	Multi-step conversion (*Mortierella alpina*)	3.6	
Eicosapentaenoic acid	Multi-step conversion (*Mortierella alpina*)	1.4	

2.2 Current and Future Developments

Current emphasis is being placed on development of flexible, efficient multi-step processes involving both enzymes and conventional chemistry. This is a good example of the synergy between biology and traditional industrial chemistry which can lead

to novel solutions to problems associated with particular complex organic syntheses. Japan currently has one of the leading positions in this new biotechnological approach.

A number of additional future developments could be envisaged in this area of Biotechnology. Apart from strain improvement using classical genetics or recombinant genetics, where such industrially-important enzymes are in short supply the techniques of Molecular Biology could be used to clone the relevant gene and over-produce the enzyme in appropriate homologous or heterologous genetic expression systems. Knowledge of the structure of such proteins could also be used to generate genetically-engineered variants with improved properties specifically tailored for application in bioreactors. These might include molecules with improved heat-stability or altered selectivity within a given range of substrates. Structural studies have already been initiated on a number of biotechnologically-important enzymes identified as potential targets for this type of protein engineering [6-10].

3 Recent Advances in Protein Structure Determination and its Biotechnological Applications

3.1 X-ray Diffraction

Protein structure determination has for many years provided an important tool to aid drug discovery in the pharmaceutical industry. Where an enzyme has been identified as a potentially important biochemical target, knowledge of the stereochemical configuration of its active-site and the location of important amino acid residues involved in the enzymic mechanism can provide the critical information necessary for the design of highly-specific chemical inhibitors.

X-ray diffraction of single crystals of a purified protein has been the traditional method of obtaining this three-dimensional structural information, once the primary amino acid sequence was known. During the early phase of development of this technology in the late 1950s and 1960s, X-ray structures of proteins accumulated slowly, since available methodologies were rather slow and unsophisticated by present-day standards. Primary sequence information was obtained by painstaking analysis of overlapping polypeptides purified after proteolytic or chemical cleavage; reliable methods were lacking for the generation of protein crystals appropriate for X-ray diffraction analysis; data collection techniques and computing facilities were primitive. Dramatic improvements have been made in virtually all these aspects of X-ray diffraction technology, so that today X-ray analysis of much larger, complex protein structures and protein assemblies can be attempted with a high degree of success. The time-scale required to complete such structural analyses has also been reduced considerably as a consequence; in contrast to early studies taking several years to complete the most recent innovations permit the elucidation of the structure of an "average" protein in favourable cases in less than six months. Additionally, the advent of recombinant DNA technology allows the necessary primary polypeptide sequence information to be deduced from the cloned complementary DNA (cDNA) sequence encoding the protein of interest in a fraction of the time needed to obtain similar information directly from the protein itself. The most uncertain step in X-ray diffraction analysis

remains that of obtaining suitable single crystals of the purified protein; on occasion crystals can be obtained easily, in other cases it proves difficult or impossible. No hard-and-fast rules appear to govern the suitability of a protein for crystallization; the best crystals often result from a process of trial and error.

3.2 Nuclear Magnetic Resonance

An alternative method of protein structure determination, nuclear magnetic resonance (NMR), is now receiving increased attention. The method yields valuable complementary data to that obtained using X-ray diffraction analysis, providing a greater insight into protein structure/function relationships than previously available using X-ray analysis alone. One major advantage of the approach is that protein specimens are analysed in solution or in other non-crystalline states, avoiding the requirement for single crystals on which X-ray analysis is dependent. For some proteins extensive similarities between the X-ray crystal structure and solution NMR structure are observed [11–13], but in other cases NMR analysis predicts major conformational differences between crystal and solution structures [14–15]. In some instances detailed comparison between crystal and solution structures reveals only subtle conformational differences which, nevertheless, can be significant with regard to the functional properties of the protein [11].

For those proteins where no single crystals are available NMR will, at least in some instances, be the only alternative method to obtain 3-dimensional structural information. The method also has a marked advantage over X-ray crystallography in that NMR data analysis can be performed over a much shorter timescale, and it can provide unique information on the dynamics of protein structure. However, technical constraints currently restrict the application of the NMR method to protein structures with a molecular weight less than 20,000–30,000 Da which can form stable, relatively concentrated solutions (>1 mM [16]).

3.3 Structure of Rare Proteins

Taken together, these recent developments allow X-ray diffraction analysis and NMR to be used as ever more powerful analytical tools for protein structure determination. These methods have already been used to solve the structures of a significant number of proteins of basic biological interest and, increasingly, proteins which are prime targets of pharmaceutical and biotechnological interest as discussed above. However, until recently only naturally-occurring proteins that are relatively easy to isolate and available in sufficient quantity have been amenable to such detailed structural analysis. A small number of additional studies had already been made of rare proteins, obtained only after painstaking purification from large quantities of source material.

Molecular Biology has changed this picture in a revolutionary way. Where a protein/enzyme is available only in minute amounts it is now possible in many instances to clone its gene or cDNA and produce a recombinant form of the protein in non-limiting amounts using a number of alternative genetic expression systems. Thus, for the first time it has become possible to derive information about the structure

of a whole range of rare and often complex proteins hitherto unobtainable by conventional means. Examples include cell receptors for biologically and pharmaceutically-important ligands [17, 18], target enzymes for the design of specific therapeutics [19], hormones [20], recombinant growth factors [21] and many others.

Efforts worldwide have increased considerably the range of vectors that can be used for the manipulation of recombinant DNA molecules, and the range of organisms that can be used for DNA cloning and expression. These include Gram-negative microorganisms (especially *Escherichia coli*), *Bacillus*, *Staphylococcus*, *Streptococcus*, *Haemophilus*, *Neisseria*, *Cyanobacteria*, and a wide range of other organisms including fungi, yeasts, plants and various animal-cell systems [22]. Additional DNA cloning vectors have also been developed, termed "shuttle vectors", which permit their replication in two or more organisms of different genera [22]. Such vectors have proved useful for several applications, especially for genetic manipulation in *E. coli* of genes from microorganisms that are difficult to handle in the laboratory. This wide choice of alternative expression systems has proved necessary because genetic factors such as codon usage, for example, can effect the efficiency with which a given cloned gene can be expressed in a heterologous expression system. Another way around such problems is to generate a totally synthetic gene with appropriate codon bias to facilitate expression but which nevertheless encodes a polypeptide sequence of identical primary structure to the original protein [9].

3.4 Mutational Analysis of Protein Structure: Protein Engineering

3.4.1 Site-directed Mutagenesis

Recombinant DNA methods have likewise revolutionized mutational analysis of proteins. There is no longer a need to rely on classical genetics to generate informative functional mutants. A complete range of new, fast and often sophisticated experimental techniques permit the generation, at will, of precise site-directed genetic changes in polypeptide sequences such as point mutations and deletions. Together with appropriate recombinant gene expression systems for generating the mutant proteins, and rapid methods for their physical characterization, these new procedures offer incisive new tools for the study of protein structure and function using mutational analysis.

3.4.2 Predicting Changes in Protein Structure

In some cases recombinant genetics leads to mutants which may have a particularly useful property, such as an enzyme with enhanced stability or altered substrate specificity. Synthetic variants of the polypeptide protease inhibitor Eglin-C, for example, have an altered preference for particular proteases [23]. Knowledge of the three-dimensional shape of such proteins is an essential aid to making useful mutants, but even with this information unpredictable changes in structure are sometimes seen [24]. This unpredictability stems at least in part from deficiencies in the current methods for computer modelling, which fail to foresee conformational changes which can occur in the three-dimensional structure of a protein even as a result of the introduction of a single point mutation. However, rapid advances are now being made in the development of new and more powerful computer software systems which can

take advantage of the availability of a progressively increasing data base of protein structures for the formulation of reliable empirical rules for model building [25,26]. The significant financial investment and high level of technical expertise required to ensure the success of this approach has resulted in the development of a number of "Centres of Excellence" for protein engineering, notably in the United Kingdom and in Japan [27], where costs are shared between Government and Industry.

Computer-assisted molecular modelling, although still very much in its infancy, will probably play an increasingly important role in the future in allowing the protein chemist to make more predictable changes in the structure of a given protein/enzyme which can then be exploited for biotechnological purposes. Certain enzymes with improved heat-stability, for example, may be more useful than their natural counterparts for incorporation into biosensor devices (Sect. 4.2.2). It can likewise be envisaged that alterations in other properties such as cofactor requirements, pH optimum and substrate specificity could also have important consequences for bioprocess development.

3.4.3 Antibody Engineering

Antibody engineering is one area where the perceived usefulness of protein structure manipulation has already been realized. It has been possible to exploit the vast amount of basic information available on antibody structure and function to generate many different kinds of engineered molecules. These include chimaeric antibody molecules, in which the antigen-combining domains of the heavy- and light-chain immunoglobulin (Ig) polypeptides from one animal species are combined with Ig structural domains of another [28–30]. It has also proved possible to generate Ig:non-Ig chimaeric protein structures [31,32] and to graft the relevant oligopeptide segments (complementarity determining segments) responsible for antigen specificity from one antibody on to a second Ig molecule [33]. Additionally, antibodies which recognise transition-state intermediates of simple chemical reactions can be generated and shown to act as primitive enzymes [34,35]. Manipulation of antibody structures illustrates the potential versatility of protein engineering in general and paves the way for future studies in which it may be possible to create a new generation of entirely synthetic proteins with defined, exploitable properties. Studies on such artificial proteins have already begun [25].

As an illustration of this philosophy, Rees and de la Paz [36] have suggested how site-directed mutagenesis of a given pair of immunoglobulin Heavy- and Light-chain genes, together with functional analysis of the proteins in an appropriate expression system and computer graphic modelling, could be used to explore the structure/function relationships of the antigen-combining sites of antibodies (Fig. 1). From such approaches it is possible to conceive how empirical rules for structure prediction of variable-region domains of antibodies could be devised and refined. Such an approach could be extended to other proteins where similar structural information and functional assays become available.

3.4.4 Quality Control in Production of Recombinant Proteins

An increasing number of recombinant polypeptide structures with specific biological activities have also been generated for therapeutic application in man. These include

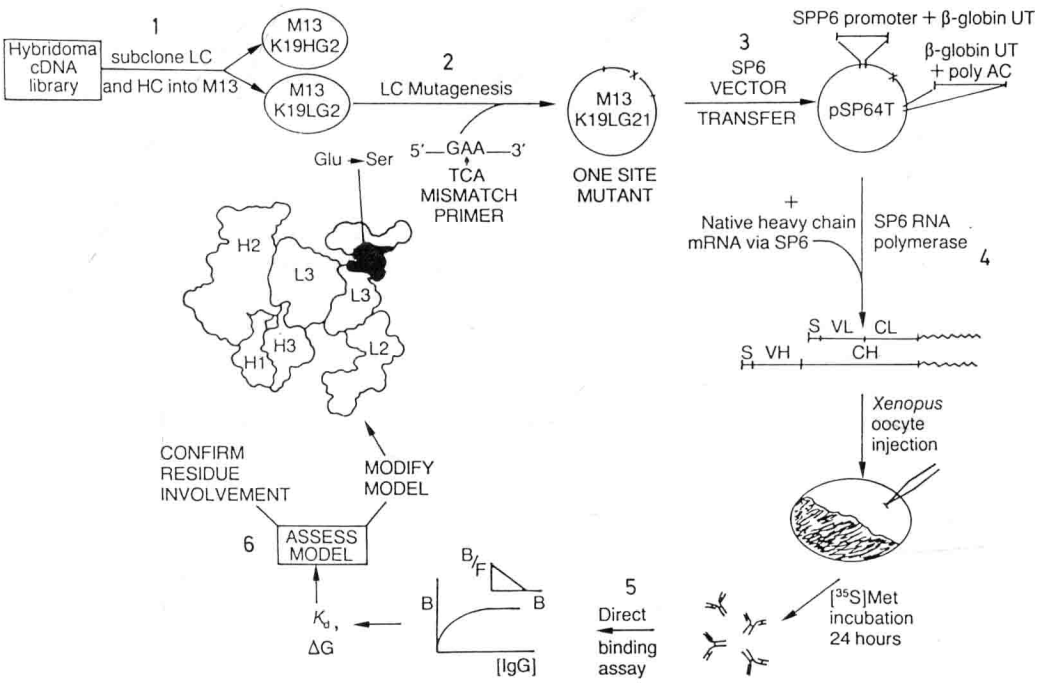

Fig. 1. Probing the structure of an antigen-combining site of a monoclonal antibody using site-directed mutagenesis. The figure shows a scheme, taken from Rees and de la Paz (1986) [36], illustrating a hypothetical experimental cycle devised to determine the relationship between the structure of an antibody light-chain and its antigen-combining properties. A cDNA library is constructed using template mRNA isolated from the hybridoma which generates the monoclonal antibody, from which heavy chain (HC) and light chain (LC) immunoglobulin cDNAs are identified (*1*). In oder to study the functional consequences of incorporating a specific change in the LC sequence (Glu to Ser), the LC cDNA is subjected to site-directed mutagenesis using a mutagenic oligonucleotide, which creates a mismatch in the coding sequence when incorporated into DNA and replicated. This step can be performed either with the aid of M13 bacteriophage vectors (as illustrated) or with alternative vectors used for this purpose (*2*). The mutant cDNA, after sequence analysis, is incorporated into an SP6 vector with appropriate transcriptional and translational control signals to permit the in vitro synthesis of functional mutant cDNA (*3*). The mutant LC mRNA is then injected into *Xenopus* oocytes, together with normal HC mRNA prepared in a similar manner in vitro (*4*). Radioactively-labelled antibody isolated from the oocytes is used in antigen-binding assays to determine the effect(s) of the LC mutation (*5*). This either confirms, or otherwise, the involvement of the specific residue in question and three-dimensional computer model building can be used to refine the empirical rules governing antibody-antigen recognition (*6*). Similar experiments could be used to probe the structure/function of the HC sequence. The scheme could be extended to include 3D-structural analysis of antibody Fab fragments using X-ray diffraction analysis or an alternative procedure. (Reproduced with permission of the original Publishers, Elsevier Press Ltd.)

recombinant vaccines, hormones and even larger protein structures: human insulin, somatostatin, β-urogastrone, human growth hormone, tissue plasminogen activator, human interferons and recombinant antibodies are but a few examples. In the majority of cases these molecules are produced in bioreactors using either bacteria, yeast or animal-cells to express the required recombinant protein.

The production of these molecules as therapeutics using these new production systems has necessitated the introduction of appropriate procedures for quality control, purification and toxicological testing of the recombinant protein products, since the possibility exists that they may be contaminated with cellular impurites such as other proteins, polysaccharides, DNA or possibly viruses, derived from the bioreactor. Regulatory authorities therefore require detailed analytical information on the degree of purity of recombinant proteins that are destined for use as in vivo diagnostics or as therapeutics in man. Regulations for quality control in the production of recombinant proteins are now reasonably well established and are being continuously updated [37–39]. For smaller protein molecules, amino acid analysis and peptide mapping may provide adequate analytical evidence of purity. Reverse phase HPLC can be used effectively for large proteins such as antibodies [40], and methods such as circular dichroism can provide useful additional analytical information which correlates not just with product purity, but also with biological activity, to take beta-urogastrone as an example [41]. Additional standard protein analytical methods are also used to assess product purity, including SDS-PAGE, isoelectric focusing, ELISA tests, and immunoblotting for minor protein contaminants (reviewed by Thomas [42]). Nucleic acid hybridization techniques are used to estimate the level of DNA contamination [43,44], and viruses are detected using a combination of infectivity tests and specific assays for virus-specific enzymes [39]. Finally, the product must be shown to contain tolerably low levels of pyrogens/endotoxins [45].

In the bioreactor itself it is necessary to demonstrate that altered forms of the recombinant product do not arise as a result of spontaneous mutation. Hence, it is necessary to confirm the sequence of the recombinant protein and/or gene for each batch culture, or in samples taken periodically from continuous cultures. The required methodologies for the rapid determination of the primary nucleotide sequence/polypeptide sequence of the gene and protein have already been developed and automated. However, fast and routine methods for obtaining three-dimensional structural information could also be useful as a means to demonstrate structural identity (or reveal structural dissimilarities) between recombinant and natural protein, and to detect impurities or contaminating mutant forms arising in the bioreactor. In this regard the NMR method, because of its speed and applicability to a wide-range of globular proteins, could have considerable potential as a future analytical tool for bioprocess control.

4 Sensors, Biosensors and Their Applications

4.1 Concept and Applications

Sensor and biosensor devices have a wide range of potential applications, particularly in the human and veterinary health care industries. They were conceived as a result of a medical need for rapid analysis of specific substances as diagnostic indicators of disease, or for continuous on-line in vivo monitoring of critical body functions. The first medical biosensors were produced in the United States and although novel, these early devices were often cumbersome and somewhat limited in their application.

Modern biosensors benefit from the considerable technological innovation that has taken place in this field during the past decade.

The medical care industry is still one of the major markets for the application of biosensors. Current estimates of the world market for medical diagnostics are in the region of $ 4,000 million [47]. Interestingly, in a recent review containing a list of the current 24 major producers of medical and commercial biosensors twelve were located in Japan, seven in the U.S.A., one in Canada, one in Eastern Europe, one in Scandinavia, but only two in Western Europe [47].

With regard to other applications, there is an increasing demand worldwide for specific and sensitive methods for monitoring levels of toxic substances, heavy metal ions and pesticides in the environment. Likewise modern and highly-mechanized food production processes require reliable and sensitive methods to detect microbial contamination and to monitor concentrations of nutrients and various food additives. Finally, there are several potential applications of biosensors in bioprocess control, for monitoring biomass, the rate of utilization of substrates and the production of the microbial product.

4.2 Structure of Biosensors

4.2.1 General Aspects

In most instances biosensors have been adapted from simpler devices designed originally for the measurement of relatively simple analytes: Oxygen, CO_2, pH, sodium, potassium or calcium, NH_3. Although limited in their analytical potential, such sensors provide extremely valuable information for the clinician wishing to monitor patient condition continuously in a critical situation, for example following heart attack or in a patient receiving kidney dialysis.

The basic concept behind the construction of a biosensor is shown schematically in Fig. 2 [46]. Biosensors differ from simpler gas, vapour and ion sensors in that they have a mechanism to detect selectively any given substance in a complex matrix containing a mixture of other components. The selectivity of the device for the complex analyte is made possible by molecular recognition using a biological component such as an enzyme, antibody, cell membrane or whole cell located in the biosensor matrix. A signal is thus generated as a result of the interaction which is then transduced to an appropriate detector. The recognition event is designed either to produce/consume a simple secondary molecule whose level is monitored using a gas- or ion-sensitive sensor [47], or to induce some physical change which can be detected by an optical or electronic device.

All biosensors share this basic design concept, and ingenious solutions to problems of biosensor construction have arisen from the interface between electronic engineering, biochemistry/enzymology and molecular biology. For new medical applications current biosensor technology is being focused on a few central issues: Ideally medical biosensors should be low-cost, sterilizable and disposable, give reproducible results, and in many cases be suitably miniaturized for on-line monitoring of patient condition. Such miniaturized devices avoid the trauma associated with the introduction of larger, more impractical monitoring instruments into the blood vessels of a patient.