Industrial and Medical Applications of Bioelectrochemistry

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Applications of electron transfer between biological systems and electrodes

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The demonstration of rapid and reversible electron transfer between cytochrome c and a gold electrode in the presence of 4,4'-bipyridyl (Biddowes & Hill, 1977; Albery et al., 1981) offered the opportunity for development of a new generation of bioelectrochemical devices. Extension of this work on 'promoted' electron transfer to other redox proteins such as bacterial ferredoxin (Armstrong et al., 1982), together with successes in coupling oxidoreductases such as nitrate reductase to these modified electrodes (Hill et al., 1981), provided a means of tightly linking enzyme-catalysed reactions to solid electrodes. A complementary method of achieving direct electron transfer is the use of chemical 'mediators', which, unlike the 'promoters' they replace, undergo redox reactions during translocation of electrons between proteins and electrodes (Plotkin et al., 1981; Turner et al., 1982a,b). Mediators may also be immobilized on or within the electrode surface, which when coated with an appropriate oxidoreductase provide commercially important catalytic electrodes.

Bioelectrocatalysis

Ex vivo biotransformation is a well-proven tool in the chemical industry, e.g. in the hydroxylation of progesterone in the production of cortisone and the oxidation of glucose to gluconic acid. Of particular interest for future applications are a variety of alkane, aromatic and steroid hydroxylations catalysed by external mono-oxygenases (Higgins et al., 1980a). A major problem in the exploitation of external mono-oxygenases, however, is their requirement for reduced cofactor, e.g. cytochrome P-450 enzyme systems:

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\begin{align*}
\text{NAD(P)H} & \rightarrow \text{Fe}^{2+} \cdot \text{protein} \\
\text{NAD(P)}^+ & \rightarrow \text{Fe}^{2+} \cdot \text{protein} \\
\text{O_2} & \rightarrow \text{H}_2\text{O}
\end{align*}
\]

Where fp is flavoprotein, and P-450 is cytochrome P-450.

The majority of mono-oxygenases require reducing equivalents in the form of NADH or NADPH, which are both inherently unstable and expensive. Naturally, attention has focused on methods of regenerating these cofactors. Coupled reactions whereby NAD(P)H is replenished by a dehydrogenase-catalysed reaction, e.g. formate dehydrogenase, provide a relatively inexpensive solution (Davies & Mosbach, 1974). Oxidation of formate produces only CO₂ and water, and stable immobilized enzyme preparations are readily available. Chemical and electrochemical methods of regeneration both face the problem of achieving efficient two-electron reduction of the biologically relevant 4-position of the cofactor, without chemical contamination of the product. NAD(P)H has been recycled by using dithionite (Jones et al., 1972), but this method is relatively non-specific and can interfere with the coupled enzyme. Useful regeneration of reduced nicotinamide ('pyridine') nucleotides has been demonstrated by electrochemical reduction (Alzawa et al., 1976). This latter technique promises to provide the simplest and cheapest means of regenerating cofactors; it is possible, however, to avoid these unstable reducing agents altogether, by direct electrochemical reduction of the enzyme prosthetic group (Higgins & Hill, 1978, 1979). Early work demonstrating the feasibility of this approach (Higgins et al., 1980b) has been verified by using cytochrome camphor P-450 hydroxylase as a model enzyme. It is clear that electrons may be introduced directly into the hydroxylase component via an oxidized graphite electrode (A. E. G. Cass & H. A. O. Hill, personal communication). This technology is being developed and extended to other enzyme catalysed reactions.

Biological fuel cells

As a result of recent technical advances, interest has been revived in fuel cells based on biological systems (Bennetto et al., 1982; Karube et al., 1981; Turner et al., 1982a,b; Wingard et al., 1982). These devices fall into two main categories, those utilizing microbial products such as hydrogen in conventional fuel cells and those incorporating biocatalysts either at or near the electrodes. Twenty years of research pursuing the ideal of energy transduction without the limitations imposed by the Carnot cycle, has resulted in commercially viable hydrogen/oxygen fuel cells. Suitable catalytic anodes for the oxidation of other fuels, however, have proved elusive. Biological systems offer the advantage of being able to oxidise a wide range of potential fuels under chemically mild conditions at ambient temperatures. They may find application generating small quantities of power from wastes or other dilute solutions of organics. True biological fuel cells, where the enzyme-catalysed redox reactions are coupled to the electrode, face many of the same problems encountered in biocatalysis; principally the density and frequency of protein–electrode interactions must be maximized with the minimum input of power. Transfer of electrons from enzymes and organisms to an electrode, however, has proved a more tractable problem than their passage in the

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determined in the range $3 \times 10^8$–$2 \times 10^9$ bacteria $\cdot$ ml$^{-1}$ with a 2σ precision of 16%. The use of phenzamine ethosulfate as a mediator in the work reported here was probably the main cause of greater sensitivity ($6 \times 10^6$–$6 \times 10^8$ bacteria $\cdot$ ml$^{-1}$).

This work has demonstrated that bioelectrochemical detectors, working in either the poised-potential or fuel-cell mode, can rapidly and reproducibly determine bacterial concentrations in the range of $10^6$–$10^8$ bacteria $\cdot$ ml$^{-1}$.

Similar principles may be applied to the construction of broad-specificity whole-organism-based sensors, whereby the inhibition or stimulation of microbial activity is monitored electrochemically. Methylophobic bacteria provide an ideal basis for such an instrument, being extremely stable and capable of oxidizing a wide range of organic substances (Higgins et al., 1980b). Their stimulation or inhibition should provide a valuable pollution monitor.

**Enzyme-based sensors**

The most immediately commercially interesting application of electron transfer between proteins and electrodes is in the field of enzyme-based sensors. This area has attracted considerable interest and has been the subject of several recent reviews (Carr et al., 1982; Suzuki et al., 1982; Turner et al., 1982a; Wingard et al., 1981). Systems have been devised to monitor the inhibition or activation of enzyme activity using a variety of secondary detectors such as thermistors, photocells, field-effect transistors, ion-selective electrodes and potentiographic techniques. Considerable effort has been devoted to immobilizing enzymes on suitable secondary detectors, which provide either an amperometric or potentiometric response. Although many of the proposed sensors incorporate oxidoreductases, the more direct approach to an amperometric sensor, i.e. exploiting direct electron transfer between the redox centre of the enzyme and a suitably modified electrode, has been neglected. One of the most commercially successful enzyme-based sensors operates by the amperometric detection of hydrogen peroxide produced by glucose oxidase; the reaction is dependent on the presence of oxygen and the sample must be diluted to bring the glucose concentration within the range of the electrode and to minimize chemical interference. A superior sensor may be constructed utilizing the same enzyme, but replacing the natural electron acceptor, oxygen, with a chemically modified electrode. Initial work utilizing chloramin in a carbon-paste electrode (Higgins et al., 1981) has been refined to produce a commercially competitive electrode. Mediators which are stable in the reduced form have been co-immobilized at a solid-carbon electrode with a monolayer of kinetically modified glucose oxidase; resulting electrodes have generally similar characteristics to their predecessors, but are oxygen-insensitive and respond over a much greater range (0.5–70 μM-glucose in blood). It is clear that this technology may be applied generally to oxidase and dehydrogenase enzymes, furnishing a wide range of highly specific and sensitive sensors. The use of electrochemically coupled enzyme antibody complexes promises to extend further these possibilities and to rival the projected use of capacitance measurements in immunology.

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**Novel electrochemical sensors for clinical analysis**

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In recent years there has been a gradual shift in clinical practice away from the diagnosis and treatment of established disease towards pre-symptomatic diagnosis and preventative measures.

Biochemical profiling for pre- and post-symptomatic diagnosis now embodies the execution of a range of routine biochemical tests on serum or urine samples. Tests such as blood glucose, urea, uric acid, albumin, cholesterol, triglycerides, creatinine and electrolytes are now fully automated and interface with computerized data logging and output systems. Many of these routine tests now exploit the unique specificity of enzyme-catalysed reactions, since with appropriate immobilization