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Structure and electrochemistry of oxidoreductases

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The principles on which Nature has developed multifunctional redox centres, covering a large range of potentials, protected from water and oxygen and surrounded by highly specific proteins, are demonstrated. Structures and accessibilities of the active sites of iron–sulphur proteins, sulphur proteins, flavoproteins, cytochromes and copper proteins are correlated with their possibilities and modes of electron exchange with natural partners, artificial mediators and (modified) electrodes. The participation of charge-transfer and tunnelling processes in electron transport is demonstrated, and a suitable relative orientation of the partners is recognized as one of the most important requirements for electrochemical communication between large molecules and electrodes. The use of specifically modified electrode surfaces, for example those based on electroconductive polymers, is proposed as one of the aspects of future developments for direct electron transfer to proteins.

1. Introduction

Among the intrinsic features of living systems are the separation, transport and storage of electrical charge. Their importance is illustrated by the fact that the redox state of a living cell of today is probably the same as in the early days of life. Before oxygen was a main constituent of the Earth's atmosphere, the ratio of reduced to oxidized glutathione was 10^5, as it still is in human cells, whereas it would be 10^{-16} under atmospheric conditions. The early energy support of cells had to be supplied in an anaerobic environment, and the capacity to bring about simple dehydrogenating reactions and to transfer electrons to acceptors such as sulphur, sulphate or fumarate was probably among the characteristics of ancestral life.

The early catalysts and mediators must have been iron–sulphur proteins, some kinds of ribonucleotide, deazaflavins (still in Archaebacteria) and, later on, flavins (Visser 1980). They had multiple functions, as is still so for ubiquitous and very conservative proteins, such as thioredoxin (Holmgren 1985). The evolution of photosynthesis supplied a new high-potential acceptor, oxygen, and the respiratory chain, with new mediators and enzymes, evolved. Before the appearance of oxygen, a potential difference from *ca.* -500 mV to $+200$ mV was available as a source of energy for living systems. This difference was extended to 1300 mV, equal to ΔG values of 250 kJ mol^{-1} O_2, when oxygen appeared.

In parallel with the increasing possibilities of using redox energy, Nature had to solve the problem of the transfer of one or more electrons, while avoiding the formation of highly reactive and cytotoxic radicals. This was obviously achieved in two separate steps: in the anaerobic area at low potentials by the use and combination of iron–sulphur proteins, thioredoxins and flavoproteins, and later on by the elements of photosynthesis in the membrane system of chloroplasts and of the respiratory chain in mitochondria. Membranes and hydrophobic microenvironments are the tools developed to separate charge from the surrounding dielectricum (water), to transport energy without dissipation, and to create the differentiation of redox potentials of mediators and catalysts.

The need for specific recognition sites implied the construction of large molecules around charge carriers, and appropriate ways of charge transport had to be developed. Charge can be transported by electrons, protons, cations, anions and neutral compounds. In large molecules a direct contact of the charge carriers is not possible and the transport is achieved by mechanisms involving the organic moiety of the macromolecules. Charge-transfer mechanisms and tunnelling processes have been reviewed by Gutman (1986) and by Marcus (1979), respectively.

Oxidoreductases are enzymes which catalyse the transfer of reduction equivalents from a donor to an acceptor. Attempts to create electron transport between redox couples and oxidoreductases have to take into account the intrinsic natural properties of these enzymes. An excellent review of the structure and function relations of electron-transport proteins has been given by Salemme (1979). Data on their electrochemical properties have been collected and reviewed (Eddowes & Hill 1981; Berezin 1983; Hill 1983; Turner *et al.* 1983; Marcus & Sutin 1985; Higgins & Hill 1985).

The purpose of this summary is to combine these structural and biochemical features with electrochemical data, to develop general aspects for further research.

2. PROTEIN REDOX CENTRES AND PRINCIPLES OF THEIR BEHAVIOUR IN NATURAL AND ARTIFICIAL ELECTRON TRANSFER

(*a*) *Iron–sulphur proteins*

Ferredoxins, whose electroactive group is an iron–sulphur cluster, are widely distributed redox mediators in anaerobic as well as in aerobic organisms. They are involved in multiple, low-potential electron transport functions such as NADP-photoreduction in algae and plants, hydroxylation reactions, nitrogen reduction and many other metabolic processes. The ferredoxins of plants normally transfer one electron: those of anaerobes (e.g. clostridia), two. Another group of iron–sulphur proteins are the conjugated iron–sulphur proteins, which contain other prosthetic groups, such as flavins, haem and/or metal ions other than iron, as well as the iron–sulphur centres.

The type and number of iron–sulphur clusters (1Fe–1S, 2Fe–2S, 3Fe–3S and 4Fe–4S) (Cammack 1980), and their combination with FMN and/or FAD and proteins, form a multifunctional body of mediators and catalysts, which spans a potential difference of 750 mV. The interaction of clusters and protein determines the potential of the mediator. Different potentials of iron–sulphur clusters may occur even in the same protein molecule. A ferredoxin of *Azobacter vinelandii* contains two different clusters with redox potentials of +320 mV and −424 mV respectively. Two practically identical 4Fe–4S clusters are the electroactive components in the high-potential iron protein of *Chromatium vinosum* (HIPIP, +350 mV) (Carter *et al.* 1974*a*, *b*) and the ferredoxin of *Peptococcus aerogenes* (−420 mV) (Adman *et al.* 1976;

Carter 1977). The HIPIP contains one, and *P. aerogenes* ferredoxin, two identical clusters. In both proteins the clusters are positioned by 4 cysteine residues and isolated by clouds of hydrophobic amino acids (Carter 1977), which prevent access of solvent. In HIPIPs of different origins the cavity-forming amino acids seem to be invariant and the spatial arrangement of the four cysteine residues is practically identical (Tedro *et al.* 1976). Two tyrosine residues in *P. aerogenes* ferredoxin, as well as a tyrosine residue in HIPIP, are in intimate contact with an Fe_2S_2 face of their clusters (Carter 1977 and references therein). The electrical access to the cluster is apparently achieved by this tyrosine and two NH–S hydrogen bonds. In ferredoxin the cavity, except for the tyrosine, is formed by aliphatic, and in HIPIP by aromatic and aliphatic, non-polar amino acids. The arrangement of the amino acids in β-sheets and helical areas forms a different diastereomeric surrounding to the cluster and thus alters the free energy of electron transfer to the protein-bound clusters (enthalpy and entropy changes). Depending on these microenvironmental differences, the cluster can exist in three redox states: oxidized, reduced by acceptance of one electron, and reduced by two electrons, whereby the reduced form of the cluster of HIPIP and the oxidized of ferredoxin are both clusters in the same redox state, reduced by one electron. The intracluster electron exchange occurs with a frequency of more than 10^{16} s^{-1} (Holm *et al.* 1974) but the self-exchange rates for HIPIPs are low, reflecting the buried nature of the Fe_4S_4 cluster (which is 0.45 nm in distance from the surface, less in ferredoxins) (Adzamli *et al.* 1981). In electron transfer between HIPIPs and cytochromes, orientated tunnelling is suggested as a transfer mechanism (Cusanovich & Mizrahi 1979) and non-polar specific interactions seem to dominate the contact between the two proteins.

The direct and reversible electron transfer between mercury (Miller & Werber 1979; Chien 1976; Kakutani *et al.* 1980), carbon (Armstrong *et al.* 1982; van Dijk *et al.* 1984), gold (Landrum *et al.* 1977; Crawley & Hawkridge 1983) and platinum (Gladyshev *et al.* 1981) as electrodes, and a variety of ferredoxins, has been shown. The electrodes may also be non-modified or modified by viologens (Holm *et al.* 1974; van Dijk *et al.* 1984 and references therein) or bivalent cations (Armstrong *et al.* 1982). Direct unmediated electrochemical reduction of *Clostridium pasteurianum* ferredoxin at a glassy carbon electrode does not occur until Mg^{2+} forms a bridge between the negatively charged ferredoxin and the carboxylate groups of the carbon surface, thus producing adsorption and proper orientation, which in turn enables electron transfer to occur.

An example of a one-electron step is the rate-limiting reaction, at the electrode, in spinach ferredoxin with a heterogeneous electron-transfer rate constant in the range from 1×10^{-4} to 6×10^{-4} cm s^{-1} depending on the experimental conditions (Crawley & Hawkridge 1983). Only adsorbed molecules are involved in this transfer, as shown with two ferredoxins of halobacteria. The adsorption of these ferredoxins is diffusion-controlled. The maximum adsorption is 6.3 ± 0.4 picomol cm^{-2}; one ferredoxin molecule covers approximately 26.5 nm^2 (Miller & Werber 1979). The interaction is influenced by the ionic strength, the pH, chelating agents and the surface of the electrode. AC-polarograms of *Clostridium pasteurianum* ferredoxin show that in basic media (pH 8) the mechanism is a two-step one-electron transfer (EE mechanism) of the iron–sulphur clusters, whereas in media of pH less than 7 the reaction has to be assigned to a 2 RSH/RSSR mechanism of cysteine residues of the apoferredoxin (Kakutani *et al.* 1980).

(*b*) *Sulphur proteins*

Protein disulphides (thioredoxin, glutaredoxin and other proteins) as well as mediators of low molecular mass (glutathione, lipoate), transport reduction equivalents by dithiol–disulphide transhydrogenation reactions:

$$\text{thioredoxin–S}_2 + \text{NADPH} + \text{H}^+ \xrightarrow[\text{reductase}]{\text{thioredoxin}} \text{thioredoxin–(SH)}_2 + \text{NADP}^+.$$

Although there is a great variety of thioredoxins even in one organism (Ziegler 1985), these proteins have in common the amino acid sequence Cys–X–X–Cys, which forms a protruding active site. Bacteria, yeasts, and plant and animal cells contain thioredoxins with homologous primary structures (M_r 12000), and thus they probably possess similar higher structures. Additional evidence comes from the three-dimensional structures (determined by X-ray crystallography) of two thioredoxins of no detectable homology, namely *E. coli* thioredoxin and bacteriophage-induced thioredoxin, which also show very similar spatial structures. This clearly shows the importance of interaction of function and structure. Examples of structure–function relations are discussed in the review by Holmgren (1985). The exchange or modification of single amino acids in thioredoxin alters its interaction with other proteins.

Some of the protein disulphides not only serve as mediators or have regulatory effects but also have enzymatic properties. Dithiothreitol reduces thioredoxin but not a number of other disulphides. Thioredoxin reduces these disulphides (e.g. cystine, glutathione, insulin) thus acting as an enzyme catalysing the reaction via a 'ping-pong' mechanism (Holmgren 1979).

$$\text{dithiothreitol}_{\text{red}} + \text{protein–S}_2 \xrightarrow{\text{thioredoxin}} \text{dithiothreitol}_{\text{ox}} + \text{protein–(SH)}_2.$$

Disulphide mediators of low molecular mass have been used in combination with enzymes for electrochemical reductions. Lipoamide and dithiothreitol at a tungsten electrode mediated the electron transfer to immobilized dihydrolipoamide: NAD$^+$-oxidoreductase [EC 1.8.1.4] for the electrochemical reduction of NAD (Shaked *et al.* 1981).

(*c*) *Flavoproteins*

In flavoproteins, the interaction of the π-electrons of the isoalloxazine ring with those of the pyridine nucleotides plays an important role in the electrochemical properties of these enzymes. The isoalloxazine ring of FMN and FAD can exist in three redox states: oxidized, and reduced by one or by two electrons. The flavin-catalyzed splitting of a C–H bond in substrates is probably achieved by the formation of a proton and a carbanion, whereas the pyridine-nucleotide-dependent reaction forms a carbocation and a hydride ion (Ghisla & Massey 1980). The electron transfer takes place via a charge-transfer mechanism after the close approach of pyridine nucleotide and enzyme-bound flavin. The flat isoalloxazine ring of the flavin moiety (Schulz 1984; Vervoort 1986) is in a parallel position to the nicotinamide ring of the pyridine nucleotide at a distance of 0.34 nm. In the well-understood glutathione (GSSG) reductase [EC 1.6.4.2] reaction, the reduction equivalents from a pyridine nucleotide are used for the formation of a dithiol. The protein itself has, in addition to an FAD, a disulphide group able to distribute electrons in a charge-transfer intermediate.

$$\text{NADPH} + \text{GSSG} + \text{H}^+ \xrightarrow[\text{reductase}]{\text{glutathione}} \text{NADP}^+ + 2\ \text{GSH}.$$

The reaction takes place in two separated steps at opposite sides of the enzyme (Schirmer & Schulz 1983), a mechanism which transports only reduction equivalents (probably only 2 e). At the same distance as the NAD at the opposite side of the flavin is the sulphur of a cysteine residue, which is the next electron acceptor. Flavoproteins with a protruding prosthetic group, such as flavodoxins, can be reduced directly at a cathode, because the FMN is in immediate contact with the medium. In larger flavoproteins the prosthetic group is often anchored in a 'barrel' formed by the protein, as was shown for glycollate oxidase [EC 1.1.3.1] (Lindqvist & Brändén 1984). In enzyme–substrate complexes, the substrate (preferably a small molecule) is stereospecifically oriented relative to the prosthetic group.

Efforts to connect oxidoreductases of this type to electrodes have to take into account these stereochemical conditions. Correct orientation of the active site relative to the electron-donating or -accepting surface determines the success of such experiments. In this respect, the immobilization of flavin at electrodes is probably useful only in cases where, in Nature, flavin nucleotides act as soluble mediators for enzyme reactions (Myawaki & Wingard 1985).

Artificial soluble mediators which are regenerable at an electrode are useful for experiments even on a preparative scale. Enoate reductase [EC 1.3.1.31] (Simon *et al.* 1985), an NAD^+-dependent iron–sulphur flavoprotein, accepts electrons from reduced viologens, which in turn are reduced at the cathode of an electrochemical cell, and reduces unsaturated acids. Results of kinetic measurements with enoate reductase are in good agreement with a 'ping-pong' bi-bi mechanism (Brühler & Simon 1985). When a natural electron acceptor, such as tiglate, is not available, enoate reductase also reduces NAD, forming NADH in a methylviologen-dependent electrochemical reduction. Similarly, a recently isolated NADH oxidase from *Streptococcus faecalis* (Schmidt *et al.* 1986) is able to accept electrons from methylviologen and to reduce NAD, thus being a potential tool for electrochemical NADH-regeneration.

In glucose oxidase [EC 1.1.3.4] the prosthetic group is at least partly inaccessible to the solvent (Schopfer *et al.* 1982). Nevertheless, mediated analytical and micropreparative redox processes have been developed on the basis of this enzyme (Laane *et al.* 1984). Glucose oxidase is irreversibly adsorbed to the surface of gold, glassy carbon and mercury electrodes, and in this state is capable of either transferring electrons from glucose to the electrode or reducing O_2 electrochemically (Scheller *et al.* 1984). The mechanism of this reaction is not quite clear not least because the tertiary structure of glucose oxidase has not yet been elucidated.

(*d*) *Cytochromes*

Cytochromes are proteins with haem as a prosthetic group; four ligands of the Fe^{2+}/Fe^{3+}-ion are provided by the porphyrin ring, and the last two are contributed by the surrounding peptide chain. The functions of cytochromes range from electron mediation to oxygenase activity; the most well-known representatives are parts of electron transport chains and multienzyme complexes.

(i) *Electron-transporting porphyrin proteins*

In the respiratory chain and other electron transport chains, *c*-type cytochromes are the natural mediators between oligomeric electron-transferring proteins (Hatefi 1985). Subunits of these multienzyme complexes are commonly cytochromes, for communication with the mediators. For example, in ubiquinol-cytochrome *c* oxidoreductase (complex III of the chain), a cytochrome c_1-subunit protrudes into the intermembrane space. This protein has polypeptide chains with a high glutamic acid content. A similar accumulation of negatively charged groups is present in a corresponding subunit of cytochrome *c*: O_2 oxidoreductase.

The mediator between these two multiprotein complexes of the respiratory chain is cytochrome *c*. The cytochrome *c* protein from horse heart is a prolate spheroid protein with a haem group inserted vertically into a small crevice with an aperture of about 0.5 × 1.8 nm (Marcus & Sutin 1985). A small part of the haem is exposed, surrounded by the surface of the polypeptide. At pH 7.5 the oxidized molecule has a net charge of +9. The exposed haem edge is surrounded by asymmetrically distributed charges provoking a dipole moment which causes cytochrome *c* to orientate relative to its redox partners (cytochrome *c* reductase, cytochrome *c* oxidase, cytochrome *c* peroxidase) (Koppenol & Margoliash 1982). The interaction domain of cytochrome *c* for docking with its partners comprises several lysine residues. Therefore polylysine is a competitive inhibitor for the interaction, and the ionic strength of the medium influences the reaction rate (Smith *et al.* 1981).

Electron transfer between cytochromes takes place close to the prosthetic group. Computer modelling and corresponding calculations show that two molecules of cytochrome *c* could approach to a haem-to-haem edge (C–C distance) of 0.94 nm, this would correspond to an iron–iron distance of 1.77 nm and an electron-transfer distance of 0.64 nm (Marcus & Sutin 1985). Distances between the haem centres of other interacting cytochromes (Vanderkooi *et al.* 1980) are between 0.8 and 2.5 nm; tunnelling must therefore be the main process for electron transfer (Chance *et al.* 1979).

The electron transfer between non-biological metal complexes and cytochromes seems to proceed through the exposed haem edge (Marcus & Sutin 1985); its velocity is inversely proportional to the ionic radius of the complexes. In flavins, the reduction rate of cytochrome *c* is correlated to the solvent exposure of their redox centres (Meyer *et al.* 1983), and flavosemiquinones probably interact *near* the exposed haem edge (Meyer *et al.* 1984). This is in line with the observation that cytochrome *c*, even when bound to large proteins by the exposed haem site, can be reduced by hydrophobic mediators (Meyer *et al.* 1985; Osheroff 1983).

With unmodified metal electrodes, multihaem cytochromes of low molecular mass, such as cytochrome c_3, with exposed haem moieties, are rapidly reduced in a diffusion-controlled process (Gutmann 1986; Bianco & Haladjian 1981). Other cytochromes need mediators or orientating aids. Hill and co-workers (Eddowes & Hill 1979; Eddowes *et al.* 1979; Albery *et al.* 1981) reduced cytochrome *c* reversibly at a gold electrode in the presence of 4,4′-bipyridyl or derivatives. They observed an inhibitory effect of polylysine as described for the reaction between cytochrome *c* and cytochrome oxidase (Eddowes *et al.* 1980) and interpreted the effect of bipyridyl as an orientation of the cytochrome *c* on the electrode surface. The bipyridyl derivatives bind to the electrode material (I. Taniguchi *et al.* 1982; I. Taniguchi *et al.* 1984) and, as investigations of Kuznetsov *et al.* (1985) with mercury electrodes showed, are oriented in the pores in the adsorbed protein monolayer. Other systematic investigations with pretreated but uncoated electrodes (Bowden *et al.* 1984) revealed the importance of asymmetrically distributed charges on the surface. The maximum possible distance for an electron transfer between such a surface and the exposed haem edge of cytochromes was 0.6–0.9 nm; this distance corresponds to half the value for the electron exchange between two cytochrome molecules mentioned before.

(ii) *Porphyrin proteins with enzyme activity*

The cytochromes P-450 [EC 1.14.15.n] are monooxygenases with a *b*-type haem thiolate prosthetic group (Wagner 1983). Two electrons required for the activation of O_2, normally originating from NAD(P)H, are transferred to the cytochrome by means of specific reductases. In eukaryotic microsomes a single protein, NADPH-cytochrome P-450-reductase [EC 1.6.2.4], containing FMN and FAD as prosthetic groups, is found; in mitochondrial and in many microbial systems its function is performed by an FAD-containing reductase and an $Fe_2S_2Cys_4$-type redoxin. In the microsomal reductase the enzyme-bound FAD is the acceptor for electrons from NADPH; FMN is responsible for their transfer to the cytochrome (Blumberg *et al.* 1982; Mason 1983). The same mechanism probably applies to the introduction of electrons from non-natural mediators. In the bacterial system the redoxin could probably also communicate with mediators, because it belongs to the type with buried iron but with a ligand accessible to the surface (Tsukihara *et al.* 1981).

Very recently, the tertiary structure of the substrate complex of the camphor-hydroxylating monooxygenase from *Pseudomonas putida* (P-450_{cam}) has been resolved (Poulos *et al.* 1985). The protein possesses an unexposed iron protoporphyrin IX prosthetic group 'sandwiched between two parallel helices'. From its natural macromolecular donor the haem can obviously accept electrons only by participation of protein groups. In agreement with this structure, Huang *et al.* (1986) could not realize a direct electrode reduction of isolated cytochrome P-450; however, they succeeded with mediated one-electron transfer (benzylviologen, indigo carmine). This confirms the experiences with electrode-promoted hydroxylations by cytochromes P-450: whereas with an isolated cytochrome only the mediated (methyl viologen, mercury cathode) oxygen activation has been possible (Mohr *et al.* 1982), the unmediated electrochemical reduction of cytochromes P-450 (gold or oxidized graphite electrode) has so far only been performed in the presence of the reductase systems (Hill & Higgins 1981; Turner *et al.* 1983).

Lactate dehydrogenase from yeast [EC 1.1.2.3] is a tetramer flavocytochrome, in which the hydrogen from the substrate is accepted by the flavin moiety, from where the electrons are transferred to the cytochrome part. Cytochrome *c* is the natural electron acceptor; the enzyme has a high-affinity binding site for this protein (Vanderkooi *et al.* 1980). In the enzyme–cytochrome *c* complex the distance between the haem centres is 1.8 nm. The haem centre of the enzyme can transfer electrons to $[Fe(CN)_6]^{3-}$, a reaction which has been used in a commercial lactate analyser (Racine *et al.* 1975). Recently direct connections between both centres of the enzyme with electrodes have been found. Durliat & Comtat (1982) demonstrated that an electron transfer to the flavin centre can be done with electrochemically pretreated Pt-electrodes. Also, from electroconductive organic complexes (which adsorb the enzyme) the haem moiety is produced (Kulys & Samalius 1983). In the latter reaction, a rapid first-order electrochemical oxidation of lactate is possible, and a pretreatment of the electrodes with polyethylenimine increases the reaction velocity, probably because of an orientation of the enzyme towards the surface.

(*e*) *Cu-proteins*

Like cytochromes, Cu-containing proteins cover a wide range of redox potentials and functions. Cu-proteins with relatively low molecular masses (azurins, stellacyanins and plastocyanins), containing only one ESR-detectable 'blue' (type I) Cu^{2+}, are electron carriers, for example in the chloroplast electron-transport chain. More complex proteins function in O_2

transport (haemocyanins), monooxygenation and O_2 reduction (laccase, tyrosinase, ascorbate oxidase, ceruloplasmin) (Mason 1983). These proteins have in addition at least a binuclear diamagnetic 2-Cu centre for the binding of O_2 (Lu Bien *et al.* 1981).

In plastocyanins the Cu^{2+} is inaccessible to water; however, the edge of one of the coordinating residues is exposed (Cookson *et al.* 1980) and amino-acid residues may participate in the electron transport by hydrophobic channels or tunnelling. Plastocyanine can readily accept electrons from metal complexes, including cytochromes; the reduction rate depends on the pH-value of the medium (Marcus & Sutin 1985). For azurin, self-exchange rates of 2×10^6 M^{-1} s^{-1} have been measured (Canters *et al.* 1984).

Correspondingly, in oxygen-reducing Cu-proteins, such as laccase ([EC 1.10.33.2]) (Reinhammer 1984) or tyrosinase ([EC 1.10.3.1] and [EC 1.14.18.1]) (Robb 1984) the 'blue' Cu^{2+} site is accessible to various mediators (V. T. Taniguchi *et al.* 1982; Wynn *et al.* 1984). However the O_2-binding centre also accepts electrons from one-electron donors, e.g. diphenols, and transfers them to O_2 (Cline *et al.* 1983; Himmelwright *et al.* 1980).

Correspondingly these Cu-enzymes can accept electrons from solid surfaces. Laccase from fungi, adsorbed to graphite electrodes, catalysed the electrolytic reduction of O_2 (Tarasevich *et al.* 1979). Systematic studies with modified graphite electrodes (Čénas *et al.* 1984) indicate a direct electron transfer to the enzyme, and cyclic voltammography in the absence of O_2 demonstrates reversible enzyme reduction (Lee *et al.* 1984). As with cytochromes, the electrochemical reduction is enhanced by bipyridyl derivatives; this result implies an orienting effect. Laccase and similar Cu-proteins have also been used with analytical electrodes for substrate determinations (Wasa *et al.* 1984).

3. CONCLUSIONS

Oxidoreductases, representing the most common types of redox centres, have been studied by electrochemical methods. Whenever a mediated or direct electron transfer between electrodes and the proteins has been observed, the results are consistent with their structural properties and the facts known about their communication with their natural partners. This is especially true for distances and mechanisms of electron transfer, for steric data and for the surface polarization as a prerequisite for contact and orientation.

Therefore, to advance further practical application of oxidoreductase bioelectrochemistry, one must systematically combine information on protein structure and active-site conformation with observed electrochemical behaviour. In this context it has to be mentioned that in some oxidoreductases the redox state has a modulating or regulatory effect on their activity. This is known for the ratio of thiol to disulphide groups in some proteins (Ziegler 1985) but has also been observed in flavoproteins. Similarly, substrate binding can cause conformational changes and shifts of redox potentials, and influence the electron-transport mechanism and kinetics (Lambeth 1982). In addition these facts have to be considered in the context of contact of proteins with solid surfaces and their immobilization (Martinek & Mozhaev 1985). Computer modelling will be a valuable aid to understanding these effects.

On the other hand, systematic studies on electrode surfaces (Faulkner 1984) and on their modification, on the electrochemical behaviour of adsorbed proteins and on the developments of new binding methods, are needed (Razumas *et al.* 1984). This should include the use of conducting polymers (Wrighton 1985, 1986) and of biomimetic polymers with catalytic properties, based on the principles of oxidoreductases (Imanishi 1985). Finally, as has been shown by Hill *et al.* (1987) and by Albery (this symposium), the orientated immobilization of small biomolecules (cytochrome *c*-551, azurins), with accessible redox centres, on electrode surfaces could be the way to transfer electrons to larger oxidoreductases with buried centres, thus imitating the natural mechanisms of electron transport.

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