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Understanding interfacial electron transfer to monolayer protein assemblies

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Abstract

Recent advances in the study of protein interfacial electron transfer are described. Special emphasis is given to cytochrome *c*/self-assembled monolayer/electrode assemblies. Results on the ionic strength dependence of the formal potential, the change of electron transfer mechanism with increase of electron tunneling distance, and the most probable electron tunneling pathway are discussed.

Keywords: Interfacial protein electron transfer; Cytochrome *c*; Formal potential; Friction control of electron transfer; Proton-coupled electron transfer; Conformational gating

1. Major recent advances

The development of strategies for controlling the electronic communication between biomolecules and electrodes is discussed by reviewing recent developments for cytochrome *c* immobilized on electrodes, namely changes in the protein's apparent surface charge and chemical manipulation of the electron tunneling pathway between the protein and the electrode.

2. Introduction

Electron transfer is a fundamental reaction type in biological systems, especially in bioenergetic processes like respiration and photosynthesis. The energy conversion in these processes is carried out by a series of proteins and enzymes that form electron transfer chains. During the past few decades our knowledge about biological electron transfer processes has improved profoundly. In general, a protein regulates electron transfer by modulating the formal potentials of its redox moieties, defining the local environment to control the reorganization energy, and providing proper electron tunneling pathways between redox centers. Biological electron transfer occurs at protein–protein interfaces and many proteins or enzymes can only maintain their activity in biological membranes. For these reasons, electron transfer of proteins that are immobilized at chemically modified electrode surfaces provides a useful model system for addressing some fundamental aspects of protein electron transfer.

Other motivations for studying protein electron transfer at an electrode are the demands for clinical assays, drug screening, and biofuel cells [1–7]. Many supramolecular assemblies of proteins/enzymes, which maintain necessary bioactivity and display Faradaic current, have been created. For example, nanoparticle/nanotube arrays, hydrogels, molecular sieves, redox active polyions, oligonucleotide assemblies and others have been constructed. Table 1 provides some examples of strategies for immobilizing protein into redox active assemblies. The electron transfer in these systems occurs either directly between the target redox species and the electrode or is mediated by other redox species. Such systems require theoretical developments that include the coupling of one or more diffusion and charge transfer steps to account for the multi-charge transfer dynamics [5].

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Table 1
Examples of immobilized protein assemblies on electrodes

Type	Promoters	Substrates	Proteins/enzymes	Examples
Direct		Au	Yeast iso-1 cytochrome *c*	[14–16]
			Cytochrome c^{m}_{555}	[17]
			Azurin mutants K27C, S118C	[18,19]
			Disulphide/cysteine tagged plastocyanin	[19]
		PG	Cytochrome *c*	[20]
		ITO	Cytochrome *c*	[21]
SAM	Ni-NTA terminated thiol	Au	Poly-histidine tagged photosynthetic center	[22]
	OPV	Au	Azurin	[23]
	Alkanethiol/hydroxyl terminated thiol	Au	Subunit II of cytochrome *c* oxidase azurin	[24]
	Epoxysilane	ITO	*E. coli* O157:H7	[25]
	MPA or cysteine	Au(111)	*Pyrococcus furiousus* terredoxin	[13]
Lipid film		PG	Redox cofactor of spinach photosystem 1	[26]
		Ni-NTA coated Ag	L-Cys tagged cytochrome *c*	[27]
Nano material	TiO_2, SnO_2 nanocrystalline	Conducting glass	Cytochrome *b* maquette flavodoxin	[28–30]
	Ag nanoparticle	PG	Myoglobin	[31]
	Carbon nanotube	GC	Glucose oxidase	[32]
	Carbon nanotubel arry	SAM/Au	Microperoxidase-11	[33]
DNA	Thiol modified DNA	Au	Photolyase	[34]
			Glycosyslases	[35]
Gel	Silica sol–gel	CP	Myoglobin	[36]
	Agarose hydrogel	EPG	Myoglobin, hemoglobin, horseradish peroxidase	[37]
Polymer	Polyaniline	PG	Hemoglobin	[38]
		CP	Horseradish peroxidase	[39]

NTA: nitrilotriacetic acid; OPV: oligomeric phenylenevinylene; PG: pyrolytic graphite; GC: glassy carbon; CP: carbon paste.

Effective electronic communication ('wiring') between proteins and electrodes is a key issue for immobilization of proteins into electroactive assemblies. Protein immobilized into a monolayer or submonolayer assembly provides a better platform for elucidating the redox properties and catalytic mechanism of enzymes, and it has been exploited extensively in fundamental studies to elucidate the mechanism and kinetics of protein electron transfer [8,9]. This protein immobilization provides better control of the protein's orientation and position, yielding a high level of homogeneity. Self-assembled monolayer (SAM) films and protein–metal junctions allow diffusion to be eliminated from the kinetic modeling and provide more transparent display of the fundamental charge transfer [1,10,11]. Recent work uses in situ STM to study individual protein molecules [12,13]. Such studies can provide important information on the heterogeneity of electron transfer rates and generate population distributions of protein activity [12].

This review discusses recent advances in understanding heterogeneous electron transfer with proteins immobilized on electrodes, with a special emphasis on cytochrome *c*/SAM assemblies.

3. Sub-monolayer protein assemblies

A straightforward way of 'wiring' a protein to an electrode is to attach it directly to the electrode surface. Whether a protein can be immobilized in this way and retain its function depends on its structure. For example, immobilization of horse heart cytochrome *c* directly to a gold electrode surface causes it to denature and lose its electroactivity, however yeast iso-1 cytochrome *c* can be immobilized on a gold surface by forming a Au–S bond with the Cys 102 amino acid on its surface. STM and cyclic voltammetry show that the yeast cytochrome *c* retains much of its structure and electrochemical activity in the assembly; in particular, the protein is well localized (covalently linked) and the redox potential is close to that for the native protein [16,40]. Heering et al. reported that the electron transfer rate constant of this immobilized cytochrome *c* is $1.8 \times 10^3\ s^{-1}$, and it efficiently relays electrons to its natural partner cytochrome *c* peroxidase, as well as two other enzymes, cd_1 nitrite reductase and NO-reductase from *Paraccocus denitrificans* [15]. Baymann reported on the immobilization of cytochrome c^{m}_{555} to Au by a thiol bond to cysteine 18, with an electron exchange rate constant of $1.4 \times 10^4\ s^{-1}$ [17]. Not all proteins have surface accessible linking groups and can be immobilized directly like yeast iso-1 cytochrome *c*, hence other strategies are needed.

A number of workers have used protein engineering to create mutants which can be directly linked to electrodes; e.g., a cysteine group can be introduced on the protein surface by site directed mutagenesis. Davis et al. reported introducing cysteine residues onto the surface of Azurin (two mutants, K27C and S118C, were studied) and compared their electron transfer behavior to that of the native protein, which can also be immobilized. The assemblies