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Review

Direct electron transfer between copper-containing proteins and electrodes

Sergey Shleev[a,b], Jan Tkac[a,c], Andreas Christenson[a], Tautgirdas Ruzgas[a], Alexander I. Yaropolov[b], James W. Whittaker[d], Lo Gorton[a,*]

[a] *Department of Analytical Chemistry, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden*
[b] *A.N. Bach Institute of Biochemistry, RAS, Leninsky prospekt 33, 119071 Moscow, Russia*
[c] *Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta 9, 812 37 Bratislava, Slovak Republic*
[d] *Department of Environmental and Biomolecular Systems, Oregon Health and Science University, Beaverton, OR 97006, USA*

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Abstract

The electrochemistry of some copper-containing proteins and enzymes, viz. azurin, galactose oxidase, tyrosinase (catechol oxidase), and the "blue" multicopper oxidases (ascorbate oxidase, bilirubin oxidase, ceruloplasmin, laccase) is reviewed and discussed in conjunction with their basic biochemical and structural characteristics. It is shown that long-range electron transfer between these enzymes and electrodes can be established, and the mechanistic schemes of the DET processes are proposed.

Keywords: Ascorbate oxidase; Azurin; Bilirubin oxidase; Ceruloplasmin; Direct electron transfer; Galactose oxidase; Gold electrode; Graphite electrode; Hemocyanin; Laccase; Redox potential; T1, T2 and T3 sites; Tyrosinase

Contents

Abbreviations: ET, electron transfer; DET, direct electron transfer; DPV, differential pulse voltammetry; IET, intraprotein electron transfer; CV, cyclic voltammetry; SWV, square wave voltammetry; AO, ascorbate oxidase; BOD, bilirubin oxidase; Cp, ceruloplasmin; GalOD, galactose oxidase; Tyr, tyrosinase; Hc, hemocyanin

* Corresponding author. Tel.: +46 46 222 7582; fax: +46 46 222 4544.
E-mail address: lo.gorton@analykem.lu.se (L. Gorton).

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1. Introduction

This review focuses on one of the most intriguing phenomenon that has been intensively studied over the last 25 years in the boundary between electrochemistry and biology, viz. the electronic coupling between the redox cofactor of the protein and an electrode through a direct electron transfer (DET) reaction. Understanding "bioelectrocatalysis" at atomic resolution is not only an intellectual challenge but has already contributed greatly to improving the quality of life since it forms the basis for a range of biosensors, of which the personal blood sugar monitor is perhaps the most successful (Wang, 2001). Extending this methodology will depend on determining the parameters that modulate a direct electron transfer mechanism between an electrode and redox active proteins or protein clusters (Schmidt and Günther, 1987). The very first reports on DET with a redox active protein were published in 1977 when Eddowes and Hill (1977) and Yeh and Kuwana (1977) independently showed that cytochrome *c* on bipyridyl modified gold and tin doped indium oxide electrodes, respectively, showed virtually reversible electrochemistry as revealed by cyclic voltammetry. Cytochrome *c* is a small redox protein, which is active in biological electron transfer (ET) chains but has no intrinsic catalytic activity (Scott and Mauk, 1996). These first publications were soon followed in 1978/1979 by reports from Russian scientists that provided indirect evidence that DET was also possible for larger redox proteins with enzyme activity (oxidoreductases or 'redox enzymes'). They showed that, laccase-modified (Berezin et al., 1978; Tarasevich et al., 1979) and peroxidase-modified (Yaropolov et al., 1979) carbon electrodes exhibited DET in the presence of the substrates dioxygen and hydrogen peroxide, respectively. These findings were reported some 10 years after the first papers were published on combining redox enzymes and electrodes (enzyme-based amperometric biosensors or "enzyme electrodes" (Updike and Hicks, 1967)). The electronic coupling between redox enzymes and electrodes for the construction of devices for practical applications (enzyme electrodes, biofuel cells, bioelectroorganic synthesis) has, however, in most cases, not been based on DET but rather on the electroactivity of the enzyme primary or secondary substrate or product (*first generation biosensors*) or through the use of non-physiological redox mediators (*second generation biosensors*), most typically illustrated by numerous biosensors based on glucose oxidase (Wang, 2001).

A high percentage of all enzymes are redox active revealing their great importance for all living organisms. Efficient DET reactions with electrodes have been demonstrated for many redox proteins, which have no intrinsic catalytic activity but act as electron transfer components in biochemical pathways (e.g., ferredoxins, flavodoxins, cytochrome *c*, and azurin) (Guo and Hill, 1991; Hill and Hunt, 1993; Bond, 1994; Hill et al., 1996). In contrast, efficient DET reactions with electrodes have only been reported for a restricted number of redox enzymes even though the number has increased substantially the last few years (Guo and Hill, 1991; Sucheta et al., 1992, 1993; Hill and Hunt, 1993; Hirst et al., 1996; Ghindilis et al., 1997; Hirst and Armstrong, 1998; Gorton et al., 1999; Armstrong et al., 2000; Armstrong, 2002; Aguey-Zinsou et al., 2003; Ferapontova et al., 2003). There are in principle two experimental approaches to establishing whether DET is occurring between redox enzymes and electrodes:

1. Indirect evidence based on observing a catalytic response current in the presence of the enzyme substrate.
2. Direct evidence from observation of independent electrochemical activity of the redox cofactor comprising the active site in the absence of substrate.

The vast majority of redox enzymes with known DET properties contain redox active metallocenters in their active site, e.g., heme, iron–sulphur cluster, and copper (Gorton et al., 1999). The number of redox enzymes with DET properties lacking metallocenters and having only an organic cofactor (e.g., a flavin or a quinone) is small (Gorton et al., 1999). Many of the redox enzymes that can communicate via DET with electrodes are intracellular enzymes located in membranes, where they participate in biological electron transfer pathways. Interprotein electron transfer generally involves complementary docking sites on each of the redox partners that minimises the electron transfer distance between the two redox active metal or organic cofactor centres and therefore enhances the ET rate. Many of the copper-containing redox proteins and enzymes show efficient DET, making the bioelectrochemistry of these enzymes especially interesting for further study in order to correlate their ability for DET with common electrode materials (naked and surface modified carbon, gold, platinum, etc.) with their 3D-structure. This will also lead to a better understanding of their function and regulation in natural processes.

Copper is an essential trace element in living systems, present in the parts per million-concentration range. It is a key cofactor in a diverse array of biological oxidation–reduction reactions and oxygen transport (Lewis and Tolman, 2004). A very notable feature of the copper proteins is that they function almost exclusively in the metabolism of O_2 or NO_x compounds and are frequently associated with oxidising organic/inorganic radicals including amino acid side chain radicals. Cu^{3+} is not a biologically relevant oxidation state, because the formal redox potential ($E^{\circ\prime}$) for the Cu^{3+}/Cu^{2+} redox couple is generally very high. Cu^{2+} centres tend to adopt a six-coordinate tetragonal (distorted octahedral) geometry or five-coordinate (square pyramidal or trigonal bipyrimidal) geometry (Rorabacher, 2004), whereas for Cu^{+} centres trigonal coordination is typical (Mirica et al., 2004). The relatively open trigonal coordination of Cu^{+} no doubt contributes to the exceptional O_2 reactivity of the reduced copper ion involving fast inner-sphere reduction of oxygen. The $E^{\circ\prime}$ of the Cu^{2+}/Cu^{+} redox couple can be modulated by ligand type and coordination geometry (up to 500 mV) and by the extended amino acid environment (up to 500 mV) compared to