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Alternating electron transfer mechanism in the case of high-performance tetrathiafulvalene–tetracyanoquinodimethane enzymatic electrodes

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ABSTRACT

The electron transfer mechanism in enzymatic electrodes employing tetrathiafulvalene–tetracyanoquinodimethane (TTF–TCNQ) complex is despite of numerous publications, still a matter of controversy. To clarify this issue, enzymatic electrodes based on TTF, TCNQ and TTF–TCNQ have been prepared in an identical manner and their electrochemical behavior and activity have been tested. The enzymatic electrodes containing the respective mediator, glucose oxidase and Vulcan nanoparticles dispersed in a gelatin matrix show high activity for glucose oxidation in terms of both currents and oxidation onset potential. The observed electrochemical features, supported by infrared spectroscopy measurements, indicate that the activity of the TTF–TCNQ electrodes can be ascribed to TTF and TCNQ species released from the organic salt. The two mediators are active in different potential regions, implying an alternating electron transfer mechanism. TTF is active at more negative potentials and generates higher current densities, while TCNQ exhibits activity only at potentials more positive than 0 V vs. SCE. This work analyzes and summarizes different aspects of the most recent electron transfer hypotheses by providing the respective experimental evidences in an effort for better understanding of this puzzling bioelectrochemical system.

1. Introduction

Charge transfer complexes (CTCs) are characterized by partial transfer of electrons from a donor to an acceptor molecule. CTCs belong to the group of organic conductors together with organic conductive polymers [1]. The history of conductive organic molecules could be dated back to 1954, when the first molecular crystal (perylene–bromine complex) with high conductivity was reported [2]. However, the field of organic conductors was practically initiated by the discoveries of the tetracyanoquinodimethane (TCNQ) and the tetrathiafulvalene (TTF) molecules [3] and benchmarked by the first report of near-metal conductivity of the TTF–TCNQ complex [4]. TTF–TCNQ is a CTC (also known as organic salt or organic metal) with room temperature conductivity in the range of $400 \pm 100\ S\ cm^{-1}$ due to efficient overlapping of the π-orbitals of the respective molecules [5]. The conductive salt is composed of segregated parallel stacks of TTF and TCNQ and the π-orbitals interact mainly along the stacking direction, which results in a quasi-one-dimensional conductor [3].

The unique electrical properties of TTF–TCNQ have naturally prompted studies on its utilization as electrode material. Jaeger and Bard first studied the behavior of TTF–TCNQ electrodes in different aqueous solutions and found a stable potential range of about 0.7 V [6]. Since this groundwork study, the TTF–TCNQ complex evolved as a highly efficient electrode material for more specific, namely bioelectrocatalytic applications. Pioneering works in this direction were done by Kulys and co-workers with other organic salts – complexes between N-methylphenazinium (NMP^+) or N-methylacridinium (NMA^+) and TCNQ [7,8]. Later on, Albery and co-workers investigated different CTCs as electrode materials for the oxidation of glucose oxidase (GOx). They found that the TTF–TCNQ salt had the best performance [9] and exploited it further as an electrode material for the regeneration of other flavoenzymes [10].

Since then, the enzymatic electrocatalysis on TTF–TCNQ electrodes has been the subject of extensive research, almost exclusively for biosensor applications. TTF–TCNQ has been mostly used in combination with GOx for the determination of glucose concentration, but studies involving other substrates and enzymes have been also reported. The efforts in the development of amperometric biosensors based on TTF–TCNQ have been summarized in a recent review [11].

Despite of numerous publications, the electron transfer (ET) mechanism in enzymatic electrodes employing TTF–TCNQ remains

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still controversial. Enzymes may exhibit *direct electron transfer* (DET) if they are able to exchange electrons directly with the electrode surface. However, in most of the cases additional redox active species called *mediators* have to be introduced in order to ensure efficient ET. In that case the process is termed *mediated electron transfer* (MET) [12]. GOx immobilized on TTF–TCNQ is a challenging case due to the unique properties of the CTC. GOx in general is believed to lack DET due to the thick carbohydrate shell, which isolates its redox center (flavin adenine dinucleotide, FAD), although there are some studies reporting the occurrence of DET in the case of GOx as discussed recently [13]. Deglycosylation of the enzyme partially exposes FAD, which facilitates DET, as demonstrated by Mano and co-workers [14]. TTF–TCNQ exhibits high electrical conductivity and no visible redox processes (e.g. due to salt decomposition) in the catalytically relevant potential region, so it can be regarded as an inert electrode material like, e.g. glassy carbon. On the other side both TTF [15] and TCNQ [16] are known to act as mediators for GOx.

Early studies on CTCs suggested homogeneous MET [7,8]. Further works, specifically addressing TTF–TCNQ, claimed direct regeneration of the reduced enzyme by means of DET [9]. Later on, the same group assumed heterogeneous redox catalysis (opposed to the homogeneous case) with TCNQ as a mediator adsorbed on the CTC surface [17]. This hypothesis was further supported by electrochemical and quartz crystal microbalance (QCM) measurements and soluble TTF species were completely ruled out of the possible ET pathway [18]. More recent experimental observations indicated that TTF should be also involved in the mechanism and two independent modes of MET were suggested: homogeneous with TTF^{+} and heterogeneous with $TCNQ^{0}$ [19]. Another study excluded the possibility of homogeneous MET or heterogeneous redox catalysis and propounded the hypothesis of an electroactive enzyme, modified by incorporation of a hydrophobic mediator (TTF and possibly TCNQ), released from the CTC [20]. This suggestion was based on the successful modification of GOx with TTF by hydrophobic interactions [21]. Later on, Albery and coworkers developed a theory for a homogeneous MET with mediators being supplied from the bulk or generated *in situ* and considered the theoretical possibility to apply this mechanism in the case of organic salts [22].

After all, despite of the long debate, more recent works reported third generation (mediatorless) glucose biosensors based on TTF–TCNQ, whereby the assumption of DET corresponded well to the obtained experimental data [23,24]. Contrary to the latter publications, a very recent study suggested the concept of an alternating MET mechanism with different mediator species, depending on the applied potential, but without experimental evidences [11].

As can be seen, the mechanism of ET in the case of enzymatic electrocatalysis on CTC and especially the GOx/TTF–TCNQ system is still a matter of controversy in the scientific community. In order to provide a better understanding of this challenging issue, a simple, yet unexplored, approach has been used in the present work. The possible mediators TTF, TCNQ and TTF–TCNQ have been incorporated into a three-dimensional electrode architecture including a gelatin matrix and carbon black, which allows for a detailed characterization and direct comparison of their electrochemical behavior and glucose oxidation activity and results in high performance. In addition the conclusions based on the electrochemical tests have been supported by infrared spectroscopy.

2. Materials and methods

Glucose oxidase (EC 1.1.3.4, GOx) from *Aspergillus niger* was supplied by Fluka. Vulcan XC72R (Cabot) was supplied by QuinTech (Germany). TTF, TCNQ and TTF–TCNQ were of analytical reagent grade and purchased from Sigma–Aldrich. Ultrapure water from Millipore was used in all experiments.

Stainless steel discs, degreased with acetone before modification, were used as a mechanical and electrical support for the preparation of enzymatic electrodes. For the electrochemical tests the discs were mounted in a sample holder for rotating disc electrode (RDE) with an opening of 6 mm (0.28 cm^2 working area).

The ink used for modification of the enzymatic electrodes had the following composition: 20 mg Vulcan XC72R, 10 mg of the respective mediator (TTF, TCNQ or TTF–TCNQ) and 10 mg GOx (1920 U) in 1 ml of 2% w/v gelatin aqueous solution (heated to 35 °C before use). The mediators were ground in an agate mortar before use. Suspension of the ink components in the gelatin solution was assisted by mechanical stirring and ultrasonication for about 5 min. For preparation of the enzymatic electrodes 50 μl of the respective ink was applied on the stainless steel electrode and left to dry under ambient conditions. After that the electrode assembly was cross-linked by dipping into a glutaraldehyde solution (5% in water) for 60 s, washed carefully with water and dried again. The enzymatic electrodes were kept in plastic bags at −20 °C before use.

All electrochemical experiments were carried out in a conventional double-jacketed electrochemical cell (Radiometer Analytical). The RDE was used as a working electrode, platinum wire as a counter electrode, and saturated calomel electrode (SCE) as a reference electrode. The potential values in the text are referred to the SCE scale. All tests were done at a rotation rate of 400 rotations per minute (rpm) in order to ensure defined mass transport conditions in the investigated system. Electrochemical experiments were performed by a computer controlled potentiostat PGSTAT302 (Eco Chemie/Autolab).

Fourier transform infrared spectroscopy (FTIR) measurements were performed with a Nicolet 6700 that was equipped with ATR and DTGS detector (Thermo Electron GmbH, Germany).

3. Results and discussion

The preparation of enzymatic electrodes based on TTF–TCNQ and GOx usually involves the dispersion of the conductive salt in an inert polymer and a suitable solvent to form slurry or thick paste [9,25–27]. In some cases the CTC has been also grown directly over a conductive polymer [23,24]. The procedure described in the present study is conceptually similar to previous procedures but it involves a biopolymer and a carbon material, in addition. The enzymatic electrodes are based on Vulcan XC72R, GOx and the respective mediators, dispersed in a gelatin matrix, which altogether form a three-dimensional stable electrode architecture. Conductive high-surface area carbon materials such as Vulcan are well established as catalyst supports in fuel cell research. They have been recently adopted also in the preparation of enzymatic electrodes for biofuel cell applications as a mean to facilitate ET and to increase current densities. For instance, Vulcan XC72R has been recently used as a support for the immobilization of bilirubin oxidase (BOD) in Nafion [28]. Gelatin, on the other side, has a hydrophilic nature and swells in presence of water. The biocompatible hydrogel polymer network provides a suitable environment for the entrapped enzymes, in addition to good film-forming properties, non-toxicity and mechanical stability [29]. The utilization of Vulcan enhances the actual surface area and the ET rate and the resulting electrode demonstrates improved characteristics compared to previously reported TTF–TCNQ bioelectrodes, which will be discussed in detail in another publication.

In order to clarify the ET mechanism, enzymatic electrodes based on TTF, TCNQ and TTF–TCNQ as mediators have been prepared according to an identical procedure. This approach allows