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Direct Electrochemistry of Cytochrome *c* at Nanocrystalline Boron-Doped Diamond

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The cyclic voltammetric response for cytochrome *c* has been studied at a variety of electrode surfaces. It has been found to undergo rapid electron transfer with metal (e.g., Au, Pt, Ag), carbon, and metal oxide electrodes, as well as with chemically modified metal electrodes.[1−5] As a well-characterized, electron-transfer protein, cytochrome *c* has been used extensively as a test system for redox reactions of proteins. The electrode kinetics of this system are known to be strongly dependent upon a combination of interfacial electrostatic and chemical interactions, which are derived from the enzyme structure and the nature of the electrode surface. It has been shown that two factors are generally necessary to observe rapid electrode reaction kinetics for cytochrome *c* at bare electrodes: (i) a pure cytochrome *c* solution (i.e., free from oligomeric contaminants) and (ii) electrode pretreatment to produce a clean, hydrophilic surface.[4] Kinetic studies of cytochrome *c* at chemically modified SAM electrodes require negatively charged COO^- terminal groups, showing a decrease in the rate at mixed surfaces containing hydroxyl groups.[6,7] Armstrong and co-workers observed a marked difference in the electron-transfer rate of cytochrome *c* at the edge versus basal plane of pyrolytic graphite. The authors report a 2-fold increase in the kinetics at the polished "edge" plane, which has higher surface O/C ratio, than at the freshly cleaved, hydrophobic "basal" plane.[8] Taken together, the above-mentioned studies indicate that cytochrome *c* electron transfer at an uncharged, hydrophobic surface should be sluggish, if a response is observed at all.

Herein, we report a somewhat different finding, in that we observe quasi-reversible, stable, diffusion-controlled kinetics at an uncharged, hydrogen-terminated electrode surface. We observed this behavior as part of our research work to develop boron-doped diamond optically transparent electrodes (OTEs) for use in infrared spectroelectrochemical studies to probe protein structure−function relationships. We began our study with cytochrome *c*, as it is a structurally and electrochemically well-characterized heme protein that serves as a simple model for the more complicated heme systems we intend to study. One of the first steps in our multitasked project is to characterize the electrochemical kinetics of cytochrome *c* at an untreated boron-doped diamond electrode. The spectroelectrochemistry of cytochrome *c* in the UV/vis and IR at boron-doped diamond OTEs will be described in another publication.

Boron-doped diamond is a relatively new electrode material.[9−11] Good quality, hydrogen-terminated diamond thin-film electrodes exhibit properties well-suited for protein electrochemical studies such as: (i) a low and stable background current over a wide potential range and (ii) a resistance to fouling, due to weak adsorption of polar molecules on the nonpolar surface.[12]

There are two types of diamond thin-film electrodes, micro- and nanocrystalline. The microcrystalline films are grown by microwave-assisted chemical vapor deposition using a CH_4/H_2 (∼0.5%) source gas mixture. Under these growth conditions, the rate of crystal growth generally exceeds the rate of nucleation, producing large (1−20 μm), well-faceted diamond grains. The nanocrystalline films are grown using a CH_4/Ar (∼1%) source gas mixture with little or no H_2 added.[13] Under these growth conditions, the rate of nucleation generally exceeds the rate of crystal growth, resulting in the formation of small grains (∼15 nm). Such films are very smooth, with a 30−50 nm surface roughness and have a higher fraction of grain boundaries than do the microcrystalline films, due to the smaller grain size.[14,15] The contact angle measured for water (74.0°) indicates a hydrophobic surface. XPS data, for other films deposited using similar conditions, indicated surface oxygen levels of less than 2 atom %. Electrical conductivity is imparted to both types of films by boron doping (∼10^{19}−10^{21} B cm^{-3}). Both types of films exhibit excellent electrochemical properties. We have exploited these properties and demonstrate that nanocrystalline boron-doped diamond is a viable material for studying bioelectrochemical redox reactions—in particular, the reduction and oxidation of cytochrome *c*.

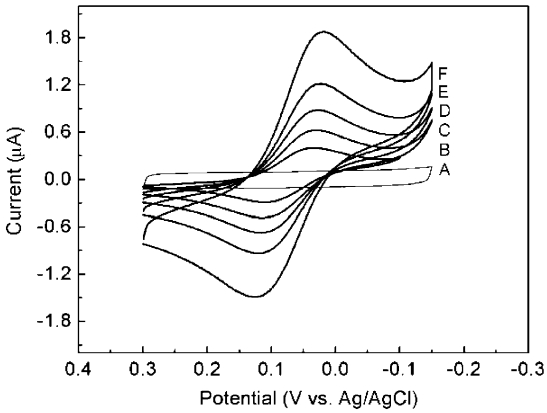

Figure 1. (A) Background CV at 20 mV/s for a 20 mM NaCl aqueous solution and (B−F) CVs for 200 μM horse heart cytochrome *c* in 20 mM NaCl, 1 mM Tris HCl pH 7 buffer at different scan rates: (B) 2 mV/s, (C) 5 mV/s, (D) 10 mV/s, (E) 20 mV/s, and (F) 50 mV/s. Electrode area = 0.2 cm^2.

Figure 1 shows a series of cyclic voltammetric $i-E$ curves for cytochrome *c* at a nanocrystalline boron-doped diamond electrode at different scan rates from 2 to 50 mV/s. The solution concentration was 200 μM in 1 mM Tris HCl buffer (pH 7) containing 20 mM NaCl. A well-defined voltammetric response, characteristic of a diffusion-controlled reaction, is observed in the potential range of 0.3 to −0.2 V. This response was observed immediately upon introducing cytochrome *c* solution into the electrochemical cell, and no time-dependent changes in the response were observed. The nanocrystalline boron-doped diamond electrode was used as deposited, with no extensive cycling required to activate or

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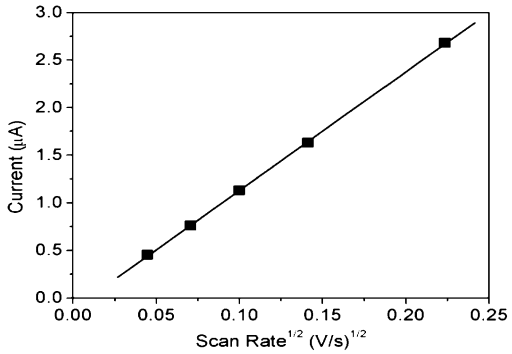

Figure 2. Plot of cathodic peak current versus the square root of the scan rate using the same conditions as in Figure 1. Linear regression coefficient = 0.9998. Slope = 1.22×10^{-5} μA $(V/s)^{-1/2}$.

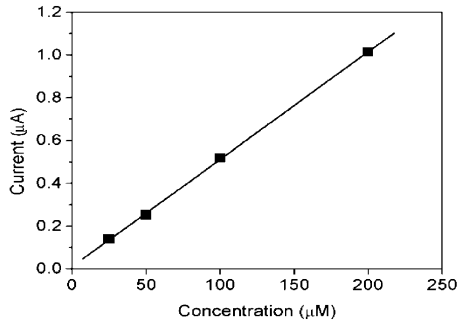

Figure 3. Plot of cathodic peak current versus cytochrome *c* concentration from 25 to 200 μM at 10 mV/s. Linear regression coefficient = 0.9995. Slope = 5.03×10^{-3} μA μM^{-1}.

precondition the electrode surface. It has been shown that reversible kinetics can be achieved for purified cytochrome *c* solutions at bare metal electrodes only after pretreatment.[3] Without special cleaning procedures, quasi-reversible cytochrome *c* voltammetry has been measured at gold and glassy carbon electrodes only after long equilibration times.[16]

A background scan for a 20 mM NaCl solution at 20 mV/s is also shown in Figure 1. This scan was obtained after the cyclic voltammetric measurements of cytochrome *c* were conducted. It is important to note that, even after exposure to the protein solution, the background is featureless. The apparent heterogeneous rate constant, k°_{app}, was calculated from the scan rate dependence (2−50 mV/s) of the peak splitting via the Nicholson method, using the data presented in Figure 1.[17] The value obtained from this method, 1.0×10^{-3} ($\pm$0.2) cm/s, is in accordance with the rate constant reported for other bare electrodes.[3] Computer simulations (DIGISIM) confirmed this value for the rate constant. The standard reduction potential, measured as the average of the anodic and cathodic peak potentials, remained independent of scan rate over this range. The value of 73 mV vs Ag/AgCl (270 mV vs NHE) is in very good agreement ($\pm$10 mV) with that reported in the literature for cytochrome *c* in solution.[18]

The cathodic peak currents are shown to vary linearly with the square root of the scan rate (Figure 2) and the concentration (Figure 3), as expected for a diffusion-controlled process. The diffusion coefficient, D_{ox}, for cytochrome *c* was calculated from the slope of the plot in Figure 3 and was found to be 7.5×10^{-7} cm^2/s (the value used in the computer simulations). This value is lower than the published value of 11×10^{-7} cm^2/s, owing to the quasi-reversible electrode kinetics.[19,20]

The reported results demonstrate that quasi-reversible, diffusion-controlled electron-transfer kinetics for cytochrome *c* are observed at a boron-doped nanocrystalline diamond thin-film electrode without the requirement of long equilibration times or potential cycling. Also, no electrode pretreatment, such as mechanical polishing, is needed. The electrode response is stable over time with no indication of fouling. This is an interesting result, considering that previous measurements of cytochrome *c* electrochemical kinetics at bare and modified electrodes have indicated the necessity of a hydrophilic, negatively charged, and oxygen-rich electrode surface. Preliminary measurements at microcrystalline boron-doped diamond films show slightly lowered rates of electron transfer for cytochrome *c* under the same conditions. Further studies are required to fully understand the factors that are involved in the heterogeneous electron transfer of cytochrome *c* at boron-doped diamond. These studies include the effect of (i) ionic strength, (ii) surface oxygen introduced by anodic polarization, (iii) solution pH, (iv) surface morphology, and (v) boron-doping level.

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Supporting Information Available: Information regarding nanocrystalline diamond film growth and protein purification (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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