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Magnetic Field Effects on Cytochrome *c*-Mediated Bioelectrocatalytic Transformations

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Abstract: Constant magnetic fields affect many biological transformations, but we lack mechanistic understanding of the processes. The magnetohydrodynamic effect may account for the enhancement of bioelectrocatalytic transformations at interfaces. This is exemplified by the bioelectrocatalyzed cytochrome *c*-mediated reduction of oxygen and oxidation of lactate in the presence of cytochrome oxidase and lactate dehydrogenase, respectively. We observe significant magnetic field effects on the rates of bioelectrochemical transformations (ca. 3-fold increase) at the functionalized interfaces at field strengths, *B*, up to 1 T. We show that the limiting current is proportional to the $B^{1/3}C^{*4/3}$, where C^* is the concentration of electroactive species. The results may have important implications on the understanding of the magnetic field effects on natural biocatalytic processes at membranes and on the enhancement of biotransformations in biotechnology.

Introduction

Biological systems of different complexity (such as microorganisms, plants, fish and animals) are sensitive to constant magnetic fields.[1] For example, the orientation of birds could originate from the sensing of the Earth's magnetic field.[2] The influence of magnetic fields on plant growth has also been demonstrated.[3] Exposition of a human body to strong magnetic fields could result in abnormal physiological processes and could even trigger some diseases (e.g., cancer).[4] Although extensive empirical data on the effects of constant magnetic fields on biological systems are available, there is no mechanism that explains these phenomena. It was recently suggested that a possible mechanism could involve changes of the rates of biochemical reactions upon the exposure of the biological systems to magnetic fields,[5] but real experimental examples of such biochemical reactions have not been yet demonstrated. Cytochrome *c*, Cyt *c*, is a central cofactor that mediates electron exchange with many redox enzymes, and thus the observation of magnetic field effects on Cyt *c* may reveal the possible influence of the magnetic field on electron-transfer cascades in nature. Biological electron transport processes (including those with the participation of Cyt *c*) occur at membrane surfaces, and these interfacial phenomena could be modeled by electrochemical reactions. It is known that a magnetic field could affect the rate of electrochemical reactions through the magnetohydrodynamic effect. This effect was well documented for simple one-step electrochemical reactions involving inorganic (e.g., ferrocyanide) or organic (e.g., acetophenone) reactants.[6] However, to the best of our knowledge, the magnetic field effect on bioelectrochemical systems and particularly on multistep biocatalytic processes was never observed before. Here we wish to report the novel observations on magnetic field effects on bioelectrocatalytic transformations (the electrocatalyzed cytochrome *c*-mediated reduction of O_2 or oxidation of lactate in the presence of cytochrome oxidase or lactate dehydrogenase, respectively). The observed magnetic field effects are explained in the terms of the magnetohydrodynamic effect.

Experimental Section

Cytochrome *c*, Cyt *c*, (from bovine heart) and lactate dehydrogenase, LDH, (from Baker's yeast, *S. cerevisiae*, EC 1.1.1.27) were purchased from Sigma and used without further purification. Cytochrome oxidase (COx) was isolated from a Keilin−Hartree heart muscle and purified according to a published technique.[7] All other chemicals including 4,4′-dipyridyl disulfide were purchased from Sigma and Aldrich and used as supplied. Ultrapure water from Barnstead NANOpure Diamond system was used in all of the experiments.

A glass support (10×10 mm^2) coated with a chromium sublayer (∼5 nm) and a gold layer (∼50 nm) supplied by Analytical-μSystem (Germany) was used as an electrode. The electrode was modified with a monolayer of 4-mercaptopyridine by soaking in a 0.01 M solution of 4,4′-dipyridyl disulfide in ethanol for 1 h. The modified electrode was

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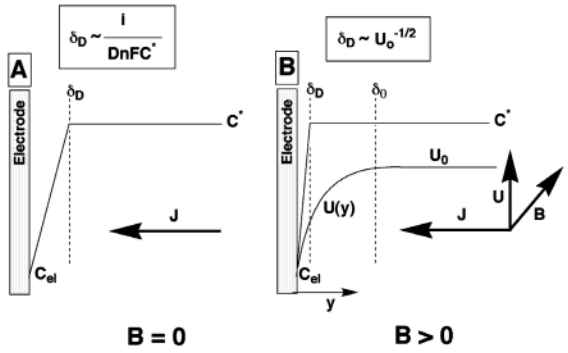
Scheme 1. Schematic Illustration of the Mechanism of Enhanced Mass-Transport of Electroactive Species[a]



[a] Under an applied magnetic field **B**, where **J** is the diffusional flux of the substrate; C^* and C_{el} are the substrate concentrations in the bulk solution and at the electrode surface, respectively; δ_D and δ_0 are the Nernst diffusion layer thickness and the hydrodynamic boundary layer thickness, respectively; and $\mathbf{U}(y)$ and $\mathbf{U_0}$ are the fluid velocity on the distance y from the electrode surface and on the outer edge of the hydrodynamic boundary layer, respectively.

rinsed with ethanol and water. The 4-mercaptopyridine-functionalized-working electrode (active area ca. 0.2 cm^2) was introduced in an electrochemical cell that included a Pt-wire counter electrode and Ag-wire quasi-reference electrode. The quasi-reference electrode was calibrated[8] according to the potential of dimethyl viologen, $E^\circ = -0.687$ V versus SCE, measured by cyclic voltammetry in a separate experiment, and the potentials are reported versus SCE. The cell was inserted between the poles (diameter of 6 cm) of an electromagnet (Model DPS-175, Scientific Equipment Roorkee, India) providing a constant magnetic field (±1% homogeneity) of variable strength that was measured with a digital gaussmeter (model DGM-102, manufactured by Sestechno, India). The working electrode was positioned parallel to the direction of the magnetic field. Electrochemical measurements were performed using an electrochemical analyzer (EG&G, VersaStat) linked to a computer (EG&G Software #270/250). All the data were obtained in a 0.1 M phosphate buffer, pH 7.0, at room temperature, ca. 24 ± 2 °C.

Theoretical Background

We describe concisely the mechanism of the magnetic field effect on mass-transport-limited electrochemical reactions. Provided that the bulk concentration of the electroactive substrate is C*, Scheme 1A, a concentration gradient is formed at the electrode surface along a distance δ_D (Nernst approximation).[9] The flux of redox-active species directed toward the electrode is expressed by eq 1,

$$j = D(C^* - C_{el})/\delta_D \tag{1}$$

where D is the diffusion coefficient, and C^* and C_{el} correspond to the concentrations of the electroactive substrate in the bulk solution and at the electrode surface, respectively. The limiting current density generated in the system, $\mathbf{i}_L$, is given by eq 2 (assuming that $C_{el} = 0$):[9]

$$\mathbf{i}_L = nFDC^*/\delta_D \tag{2}$$

where n is the number of electrons involved in the redox process and F is the Faraday constant.

The application of a magnetic field on a solution that includes moving ions (net current in our system) produces a magnetic body force, $\mathbf{F}_L$, acting on the system.[10] This force, $\mathbf{F}_L$, is given by the Lorentz equation, eq 3, that corresponds to the vector product of the net current density, $\mathbf{i}_L$, and the magnetic field strength, **B**.[10]

$$\mathbf{F}_L = \mathbf{i}_L \times \mathbf{B} \tag{3}$$

Under conditions where the flux of ions occurs orthogonal to the electrode surface and the applied magnetic field is directed parallel to the surface, a Lorentz force is exerted perpendicularly to these two vectors. This results in a momentum transfer to the solvent and leads to the formation of the solution flow mainly tangential to the electrode surface, thus yielding a flow velocity gradient along a layer of thickness δ_0, Scheme 1B. As this hydrodynamic boundary layer is formed only in the presence of an applied magnetic field, and since Levich[11] has shown that the diffusion layer thickness is inversely proportional to the square root of the flow velocity, the decrease of the Nernst diffusion layer thickness is anticipated. This leads to the accelerated mass-transport of the electroactive species to the electrode surface and to the enhanced electrochemistry. In fact, this is known as the magnetohydrodynamic effect on electrochemical reactions.[6]

In a recent study we formulated a theoretical model that accounts for the effects of a static homogeneous magnetic field, directed parallel to the planar semi-infinite electrode surface, on electrochemical reactions at interfaces.[12] Using the hydrodynamic boundary layer theory[11] and the Nernst diffusion layer approximation,[9] we formulated the relation between the limiting current density of the redox-active substrate, the magnetic field strength and the substrate concentration as given by eq 4,

$$\mathbf{i}_L \propto (\rho R)^{-1/3} D^{8/9} \nu^{-2/9} (nFC^*)^{4/3} B^{1/3} \tag{4}$$

where n is the number of the electrons involved in the Faradaic process, F is the Faraday's number, R is an electrode characteristic size, ρ and ν are the fluid specific density and kinematic viscosity, respectively, D and C^* are the diffusion coefficient and the bulk concentration of the redox species, respectively, and B is the magnitude of the imposed magnetic field.

This theoretical model was successfully applied to analyze the magnetic field effects on simple electrochemical processes (e.g., the reduction of ferricyanide, the reduction of acetophenone or the electrochemical deposition of Cu). It was found that the applied magnetic field leads to the shrinkage of the Nernst diffusional layer, and this accelerates the electrochemical reaction at the interface as the magnetic field intensity increases (according to eq 4, the reaction rate $\propto B^{1/3}$). Furthermore, at a constant magnetic field, the rate of the reaction relates to the concentration of the substrate according to the functional relation: the reaction rate $\propto C^{*4/3}$, where C^* is the substrate bulk concentration. These theoretical conclusions were experimentally verified for bioelectrochemical systems that include Cyt *c* as a typical electron relay in biological electron transport chains.

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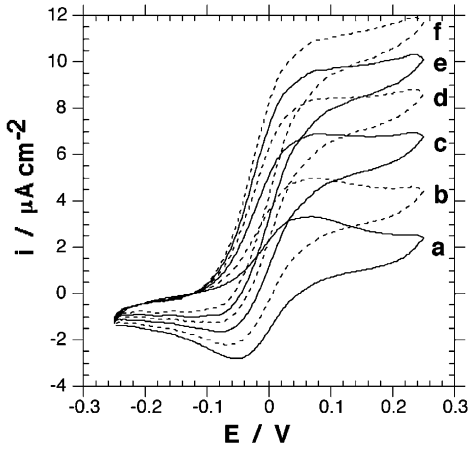
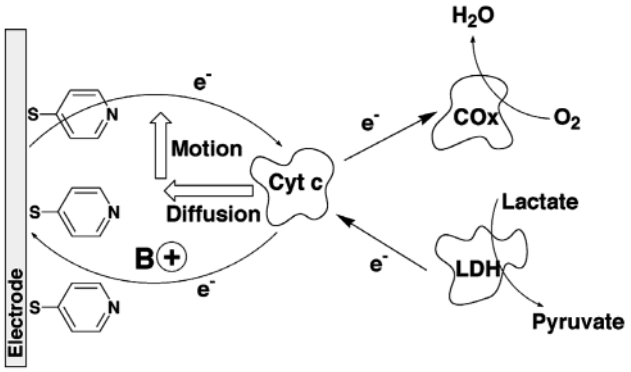


Figure 1. Cyclic voltammograms corresponding to Cyt *c*, 1×10^{-4} M, at a 4-mercaptopyridine-modified Au electrode upon application of the magnetic field perpendicular to the diffusion path of Cyt *c*: (a) 0 T, (b) 0.068 T, (c) 0.13 T, (d) 0.26 T, (e) 0.52 T, (f) 0.92 T. The data were recorded in 0.1 M phosphate buffer, pH 7.0, under Ar. Potential scan rate 50 mV s^{-1}.

Scheme 2. Electrochemical Reaction of Cyt *c* at the 4-Mercaptopyridine-Modified Electrode and the Secondary Bioelectrocatalyzed Cyt *c*-Mediated Reduction of O_2 or Oxidation of Lactate by Cytochrome Oxidase (COx) or Lactate Dehydrogenase (LDH), Respectively



Results and Discussions

The hemoprotein Cyt *c* lacks direct electrical contact with the electrode surface due to the nonappropriate orientation of the heme site in respect to the electrode surface. Modification of a Au-electrode with 4-mercaptopyridine yields a promoter layer on the electrode that binds reversibly the hemoprotein to the electrode.[13] It should be noted that the Cyt *c* electrochemical process at the promoter-modified electrode surface is a diffusionally limited process (peak currents in the cyclic voltammograms increase proportionally with the square root of the potential scan rate[13b]), and the redox reaction at the electrode would be affected by the external magnetic field, Scheme 2. Figure 1 shows the cyclic voltammograms of the Cyt *c* at different applied magnetic field strengths. Upon scanning the potential from −0.25 V to +0.25 V, the anodic current increases as the field strength increases. While the anodic current increases, the cathodic wave of Cyt *c* decreases upon enhancing the magnetic field. The mirror image of these cyclic voltammograms is observed upon scanning the potential from +0.25 V to −0.25 V, and the cathodic wave corresponding to the reduction of Cyt *c* increases with the increase of the external field strength. The cyclic voltammograms at the high magnetic field strengths are reminiscent of those typically obtained with a rotating disk electrode and show the shape characteristic of a steady-state mass-transport-limited current.[6f,14] These results were analyzed according to eq 4. The log *i* vs log *B* plot reveals a slope that corresponds to ca. 0.33 ± 0.02, as predicted by the model ($i \propto B^{1/3}$), Figure 2A. The log *i* vs log *C** plot at a constant magnetic field of 0.92 T reveals a slope of ca. 1.26 ± 0.01, nearly as expected from the theoretical model ($i \propto C^{*4/3}$), Figure 2B (see also the Supporting Information).

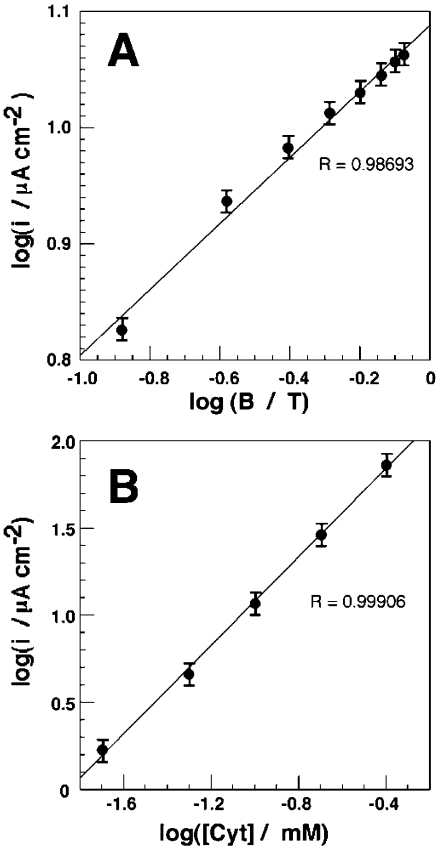


Figure 2. (A) Dependence of the limiting current densities of the Cyt *c* cyclic voltammograms on the magnetic field strength at [Cyt *c*] = 1×10^{-4} M. (B) The dependence of the limiting current densities on the Cyt *c* concentration at $B = 0.92$ T.

The redox transformations of Cyt *c* at the 4-mercaptopyridine-modified electrode are enhanced by the magnetic-field-stimulated increase of the mass-transport at the electrode interface. Since Cyt *c* mediates secondary electron-transfer processes that activate enzymes, one would expect that the coupled bioelectrocatalytic processes would be promoted by the external magnetic field too. Cytochrome oxidase, COx, is activated by Cyt *c* toward the biocatalyzed reduction of O_2 to H_2O, Scheme 2.[15] The bioelectrocatalytic current generated by Cyt *c*/COx in the presence of O_2, Figure 3A, curve b, is

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(14) The sigmoidal shape of cyclic voltammograms could also, in principle, originate from radial diffusion of redox species to pinholes or from an electrocatalytic process. Nonetheless, the fact that all of the present waves originate from the same electrode and that Cyt *c* is inactive as an electrocatalyst rules out these possibilities and implies that the observed wave shapes are controlled only by the magnetic field strength.

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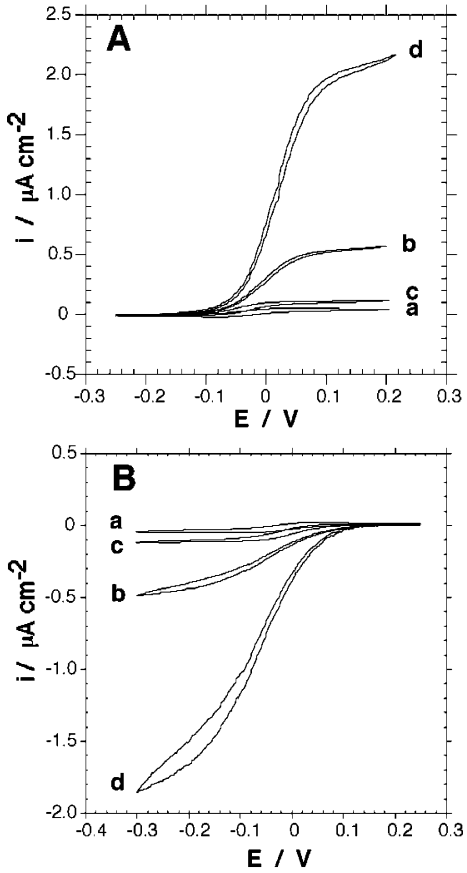

Figure 3. Cyclic voltammograms corresponding to (A) the Cyt c/COx system at the pyridine-functionalized electrode: (a) without O_2 in the background solution and with no applied magnetic field, (b) with the background solution equilibrated with air and with no applied magnetic field, (c) without O_2 and with an applied magnetic field of 0.92 T, and (d) with the background solution equilibrated with air and with an applied magnetic field of 0.92 T. (B) The Cyt c/LDH system at the pyridine-functionalized electrode: (a) without lactate and with no applied magnetic field, (b) with lactate, 1×10^{-2} M, and with no applied magnetic field, (c) without lactate and with an applied magnetic field of 0.92 T, (d) with lactate, 1×10^{-2} M, and an applied magnetic field of 0.92 T. Data recorded in 0.1 M phosphate buffer, pH 7.0, scan rate 5 mV s^{-1}. The concentrations of the biomaterials in the respective systems: Cyt *c*, 1×10^{-4} M; COx, 1 mg mL^{-1}, LDH, 2 mg mL^{-1}.

significantly higher than the cathodic current originating from the Cyt *c* reduction in the absence of O_2, Figure 3A, curve a. Application of the magnetic field (0.92 T) enhances the mass-transport of Cyt c and yields a substantially higher electrocatalytic current in the presence of the Cyt c/COx/O_2 system, Figure 3A, curve d. The current of the Cyt c/COx system in the absence of O_2 and upon application of the magnetic field (0.92 T) is shown for comparison, Figure 3A, curve c.

Cyt *c* also mediates oxidative bioelectrocatalytic processes, e.g., oxidation of lactate to pyruvate in the presence of lactate dehydrogenase, LDH, Scheme 2.[15a,16] Figure 3B, curve b shows the electrocatalytic anodic currents upon the oxidation of lactate by the Cyt *c*/LDH system at the 4-mercaptopyridine monolayer-functionalized electrode in the absence of an applied magnetic field. The application of the magnetic field (0.92 T) increases the bioelectrocatalytic anodic current of lactate oxidation, Figure 3B, curve d. The currents of the Cyt *c*/LDH system in the absence of the substrate without and with application of the magnetic field are shown for comparison, Figure 3B, curves a and c, respectively. It should be noted that the extent of the increase of the noncatalytic current that corresponds to the redox process of the Cyt *c* itself under an applied magnetic field is the same as for both bioelectrocatalytic processes mediated by Cyt *c* (enhancement factor ca. 3.5). This suggests that the magnetic enhancement of the bioelectrocatalytic processes originates from the enhancement of the mass-transport of the Cyt *c* in the primary interfacial redox process and not from the secondary biocatalytic reactions, which include reduction of O_2 or oxidation of lactate in the bulk solution. The bioelectrocatalytic currents increase as the magnetic field strength is elevated. Figure 4 shows the changes of the current density values that correspond to the bioelectrocatalyzed reduction of O_2 (curve a) and to the bioelectrocatalyzed oxidation of lactate (curve b) at different external magnetic field strengths. The log *i* vs log *B* plots of the two processes reveal linear dependencies with identical slopes of ca. 0.31 ± 0.03 as expected by the theoretical model ($i \propto B^{1/3}$).[11]

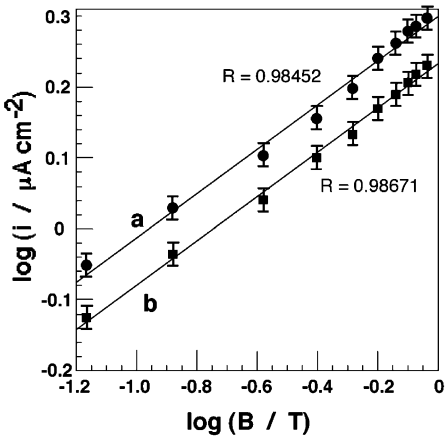

Figure 4. Analysis of the bioelectrocatalytic limiting current densities of the cyclic voltammograms as a function of the strength of the applied magnetic fields for (a) the bioelectrocatalyzed reduction of O_2 by the Cyt c/COx system (at $E = -0.3$ V) and (b) the bioelectrocatalyzed oxidation of lactate by the Cyt c/LDH system (at $E = 0.2$ V).

Conclusions

In conclusion, the present study has demonstrated the novel magnetic field effects on bioelectrochemical transformations occurring at electrodes. The redox process of Cyt *c* at a modified Au electrode and the Cyt *c*-mediated biocatalytic transformations were enhanced by applying a constant magnetic field. The observed phenomena are explained in the terms of the magnetohydrodynamic effect, and the enhancements of the bioelectrochemical reactions are quantitatively correlated to the theoretically predicted dependencies. The studied systems are considered as simplified models for biological electron transport reactions at biomembranes. The discovered phenomenon does not necessarily mean that the magnetic sensitivity observed in many natural biological systems should be explained by the same mechanism, but it shows for the first time that kinetics of simple bioelectrocatalytic transformations occurring at biomembranes could be significantly affected by constant magnetic fields, implying the possibility of the magnetohydrodynamic effect for the interfacial biological electron transfer processes.

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The fact that biological transformations exhibit amplification features suggests that even small effects generated by the natural magnetic field of Earth may be biologically amplified.

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Supporting Information Available: A detailed discussion and comparison between the experimental and calculated limiting current densities are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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