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Magnetic field effects on near-infrared optical properties of cytochrome oxidase

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Abstract

This paper reports the possible effects of intense magnetic fields on the near-infrared optical properties of cytochrome oxidase. Optical measurements performed on both cytochrome aa3 suspension and in vivo showed changes in optical absorbance at 690–830 nm under magnetic field exposure. Magnetic fields of up to 14 T generated by a superconducting current were able to change the oxidation of the oxidized cytochrome aa3 periodically depending on the density of the magnetic flux. Measurements with a cooled CCD system revealed that the absorbance at 830 nm was slightly increased by a magnetic field of 8 T. We performed an animal experiment on the head of a living rabbit, and obtained results showing the enhancement of cytochrome aa3 oxidation in the mitochondria of cells under the magnetic fields. The effects of magnetic fields on the paramagnetic behavior of oxygen, electron transfer in cytochromes, and cell membrane conformation in mitochondria may play a role in increasing the sensitivity to NIR light for detecting cyto-ox oxidation, which is one of the primary indicators of cellular activity.

Keywords: Magnetic field; Near infrared light; Cytochrome oxidase; Cellular activity; Mitochondria

1. Introduction

Near-infrared (NIR) light in the range of 690–900 nm is useful for noninvasive measurements of both hemoglobin oxygenation and cytochrome aa3 oxidation in the living body. Changes in the absorbance of near-infrared light can be associated with neural and cellular activities. Evaluation of living tissue activity can be based on the oxygenation level of hemoglobin in red blood cells and the blood volume in the tissue. Increases in hemoglobin oxygenation and cytochrome aa3 oxidation indicate activation of the tissue near the blood containing the hemoglobin and the cells containing cytochrome aa3. Hemoglobin oxygenation shows a significant change in the spectrum profile between 780 and 830 nm. The high transparency of near-infrared light in a living body makes it possible to non-invasively measure the hemodynamics of the targeted living tissue [1–7].

Similar, to EEG, MEG, MRI, and PET, among others, NIR spectroscopy is recognized as a useful method for investigating human brain functions. Advances in the theory of optical tomography are expected to produce a new technique to visualize a map of human brain functions. At the same time, a multi-channel type of NIR apparatus has enabled the measurement of brain function localization in an ordinary living space.

We became interested in the effects of magnetic fields on the functions of proteins such as hemoglobin, which interacts with oxygen molecules [8]. The biochemical processes involving oxygenation or oxidation were candidates for a system of sensing magnetic fields in a living body because the processes had paramagnetic molecules. In addition, strong magnetic fields of Tesla orders would have a diamagnetic effect such as a conformational modification in a protein.

In the present study, we used two kinds of near-infrared optical measurement systems, and performed experiments on the possible effects of intense magnetic fields on the order of Teslas on the oxidation of cytochrome oxidase.

Compared to hemoglobin, NIR detection of oxidation in cytochrome oxidase (cyto-ox) under a physiological oxygen-gas-pressure was difficult because of the high affinity of oxygen molecules for cyto-ox [1–2]. The oxygenation of hemoglobin provides indirect information on neural cells in the brain, because there is a time delay in the response of blood flow to neural activities. However, the oxidation level of cyto-ox

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indicates direct information in neural cells in real time. Our long-term goal is to provide a new technique for detecting cyto-ox oxidation under physiological oxygen–gas–pressure by exposure to magnetic fields.

2. Experimental

Two superconducting magnet systems, maximum 14 and 8 T (Oxford Inst. plc), were applied.

Near-infrared measurements were performed with either a non-invasive oxygenation monitor (OM-100AS, Shimadzu Co.) or a cooled CCD system (UCD-2000, Unisoku Co.). In the first case, trains of pulsed light at 690, 780, 805, or 830 nm were sequentially emitted from the end of an optical fiber and passed through a tube containing a suspension of cytochrome oxidase (aa3). The scattered optical transmission was introduced into another optical fiber. The optical absorbance for each wavelength was measured. Calculation of the relative change in the oxidation of cytochrome aa3 was based on the principle that oxidized cytochrome aa3 has a maximal peak at 830 nm.

In the second case, the CCD system was assembled with an external optical cell holder, optical fibers, thermal stabilizer (water tubing), halogen lamp, and a superconducting magnet. Fig. 1 shows the set-up of the fiber-optic system, which has a pair of optical fibers, one of which is for the outlet and the other is for the inlet of light (so called optrodes). When the CCD system was used to measure the cytochrome aa3 suspension, the suspension was placed in an optical cuvette that was set vertically and was exposed to a light beam directed perpendicularly to the external magnetic fields (Fig. 1(a)). In the non-invasive oxygenation monitor, a polystyrene tube filled with the suspension was equipped with the optrodes (emitter and detector) and set horizontally. The light beams passed through the tube perpendicularly to the magnetic fields (Fig. 1 (b)). In the in vivo experiments, the optrodes providing light to the head of the rabbit were set in the same manner as with the tube (Fig. 1(c)). The in vivo experiments were conducted based on the guidelines for such experiments at The University of Tokyo.

Powder type cytochrome aa3 was purchased from Biozyme Laboratories Limited (UK). The reagent was suspended in phosphate buffer, and the suspension was set in a quartz type of optical cuvette.

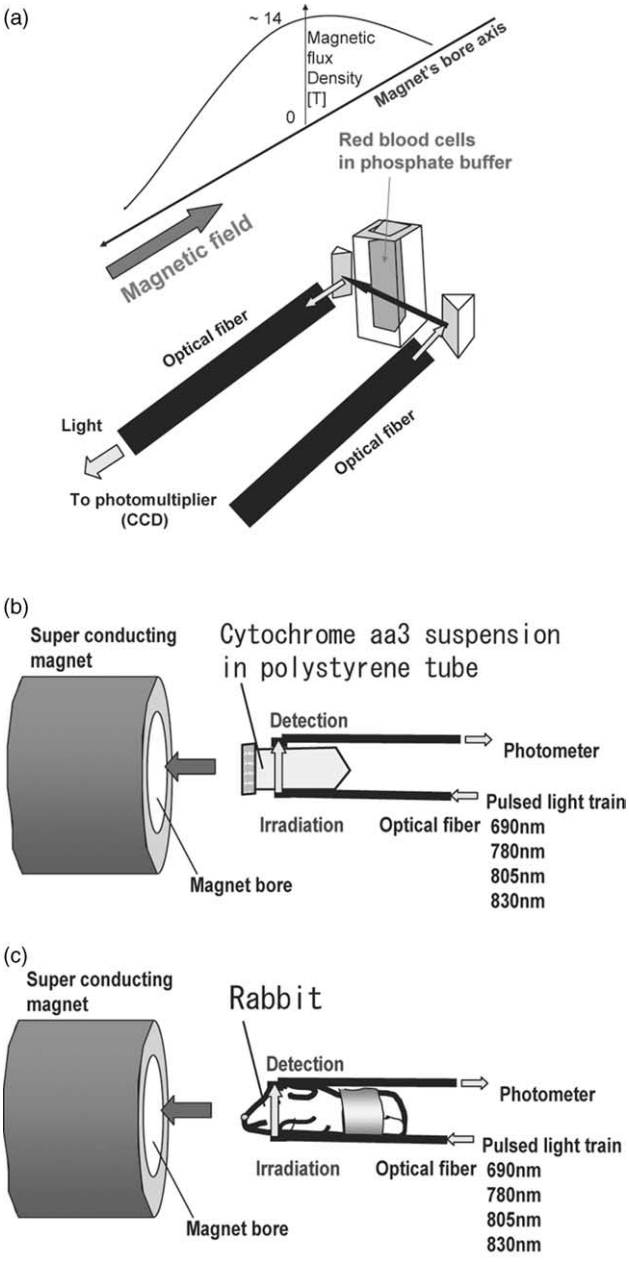

Fig. 1. Set-up of near-infrared optical measurements of cytochrome aa3 in suspension and in an animal. (a) Fiber-attached optical cell system for cooled CCD photo-multiplier, (b) pair of optrodes (emitter and detector) of near-infrared pulsed light trains with a current wave (OM-100AS), and sample tube containing cytochrome aa3 suspension, (c) scheme of the optrodes and the experimental animal, rabbit.

3. Results and discussion

The effects of magnetic fields of up to 14 T on cytochrome aa3 oxidation were obtained as shown in Fig. 2. During magnetic field sweeps between ∼0 and 14 T, the relative change in the oxidation of cytochrome aa3, ΔCyt, changed periodically. The suspension contained 0.25 mg/ml of cyto-chrome aa3 at pH 5.5 and 1% Tween 80. The ΔCyt was calculated using two different formulas, each of which is shown at the bottom of Fig. 2(a) and (b). The methods of calculation are based on the fact that the oxidized cytochrome aa3 has a maximal peak at 830 nm, and were recommended by the manufacturer. The results suggest a possible effect of magnetic fields on the optical absorption processes in cytochrome aa3. Both Fig. 2(a) and (b) show a relative increase in absorbance at 830 nm compared to absorbance at 780 nm, although the time course patterns varied depending on the formulas used for estimating ΔCyt. In Fig. 2(a), the initial level of cyto-ox oxidation was 0.13 (arbitrary unit), and showed a maximum ΔCyt of −0.16 when the magnetic field was increased. Similar effects on cyto-ox oxidation were observed when the magnetic field was decreased. The formulas