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.

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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 oxidation 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
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.

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 cytochrome 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...
used in Fig. 2(b) set the initial level of cyto-ox oxidation to zero, and the magnetic field exposure at 14 T increased the cyto-ox oxidation by 0.08 (arbitrary unit). After the magnetic fields became 0 T, the cyto-ox oxidation was lower than the initial level, probably due to a baseline shift that was caused by the spatial density change of cyto-ox by the gradient magnetic fields. The obtained changes in cyto-ox oxidation by exposure to the magnetic field were significant compared to the baseline shift. The same kinds of effects of 14 T magnetic fields on cyto-ox oxidation were observed in three series of magnetic field sweep-up/down experiments.

The oxidation level of cytochrome aa3 is a direct indicator of cellular activity, and it is an interesting question as to whether the magnetic modulation of cytochrome aa3 oxidation occurs in the cells of living animals. To answer this question, we used the non-invasive oxygenation monitor placed above the head of a rabbit. Fig. 3(a) and (b) shows a time sequence of $\Delta Cyt$ and the changes in oxy/deoxy hemoglobin concentrations. The upper panel (a) is accompanied by raw data. The experiment included one course of sweep-up/-down (0 to 8 T, 8 to 0 T) in (a) and two courses of sweep-up/-down in (b). $\Delta Cyt$, oxy/deoxy hemoglobin concentrations, and total blood changes are shown based on the calculation performed by OM-100AS.

$$\Delta\text{oxyHb} = +2.370 \times \Delta\text{abs780} - 1.800 \times \Delta\text{abs690}$$

$$\Delta\text{deoxyHb} = -1.070 \times \Delta\text{abs780} + 1.470 \times \Delta\text{abs690}$$

Both figures in Fig. 3 show that the $\Delta Cyt$ increased during magnetic field exposure at 8 T. After the magnetic field returned to 0 T, $\Delta Cyt$ reached a level that was lower than the initial level due to a possible redox balance change in the cytochromes.

In the third stage of the study, we used a cooled CCD system to optically measure cytochrome aa3 and observed the near-infrared absorption spectrum in the range of 690–900 nm (Fig. 4). The concentration of the cytochrome aa3 solution at pH 7 was 0.25 mg/ml. The figure shows the spectra at ~0 T (ambient fields) and at 8 T. A slight increase of absorbance occurred at 830 nm under the 8-T magnetic fields. In contrast, absorbance at 690–780 nm was decreased by exposure to the magnetic fields. A slight enhancement of cytochrome aa3 oxidation by the magnetic fields was detected in a neutral pH condition in vitro. However, the effect was not distinct.

With diamagnetic materials, the magnetic energy in a molecule that is directly exposed to an external magnetic field on the order of less than a Tesla is small and, in most cases, the energy does not exceed the thermal energy. Even under magnetic fields of greater than a Tesla, it is difficult for a nano-sized diamagnetic molecule to respond to the magnetic fields. We speculate that the response of cytochrome aa3 to the applied magnetic fields of ~14 T was partially due to
a diamagnetically induced conformational change in the macromolecule and a subsequent increase of the affinity to the oxygen molecules. Other possible mechanisms, for example, are the effect of magnetic fields on the paramagnetic behavior of oxygen, an electron transfer process in cytochrome aa3 and oxygen molecules. Future studies that include the measurement of circular dichroism of cytochromes under strong magnetic fields are needed.

To explain the mechanism for the results of the in vivo experiments, we should consider investigating the effects of magnetic fields on lipid membrane conformation in mitochondria and its effect on the functions of cytochrome aa3.

In summary, the in vitro experimental results (Figs. 2 and 4) showed that a near-infrared spectrum profile of cytochrome aa3 was affected by the magnetic fields of up to 14 T. The characteristic response of the spectrum to the magnetic fields was an increase in optical absorption at 830 nm. In addition, the in vivo study on a living animal supports the speculation that the oxidation of cytochrome aa3 was slightly enhanced by exposures to magnetic fields of up to 8 T.

At present, near-infrared detection of cyto-ox is not utilized for clinical investigation or brain research because of the difficulty in distinguishing signals for cyto-ox. A specific detection method or a new analytical method for cytochrome aa3 oxidation that could be exhibited by switching magnetic fields on/off would support the validation of cyto-ox measurements. In surgery, monitoring the cerebral blood oxygenation levels is quite important. Clinicians are searching for an effective method to measure cellular activities in the brain by non-invasive techniques during surgery.

Recently, magnetic resonance imaging (MRI) has been used for monitoring during surgery. NIR spectroscopy is a conventional blood oxygenation monitoring method and is suitable for use in parallel with MRI. The present work suggested a new application of strong magnetic fields in MRI systems for monitoring cytochrome aa3 oxidation with NIR spectroscopy. Moreover, improvements in cytochrome aa3 oxidation monitoring techniques are beneficial for learning about brain functions in basic research. It is anticipated that the effects of exposure to magnetic fields on cytochrome aa3 oxidation will be applicable in the verification of cytochrome aa3 monitoring by NIR light.

4. Conclusions

We observed the effects of magnetic fields up to 14 T on the near-infrared optical properties of cytochrome oxidase, both in vitro and in vivo. In vitro experiments with cytochrome aa3 suspensions showed a slight increase in the absorbance at 830 nm under the influence of the magnetic field. The calculation of relative changes in the oxidation of cytochrome aa3, ΔCyt, confirmed a periodical change of cytochrome aa3 oxidation during the magnetic field sweeps. Experiments with a live rabbit head under the influence of a magnetic field showed that cytochrome aa3 oxidation was distinctly enhanced compared with the trends in hemoglobin oxygenation, which were changed by physiological changes in the brain.

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