35th Annual International Conference of the IEEE EMBS
Osaka, Japan, 3 - 7 July, 2013

Magnetic field effects on mitochondrion-activity-related optical properties in slime mold and bone forming cells

Yuri Mizukawa, *Member, IEEE* and Masakazu Iwasaka, *Member, IEEE*

Abstract— **In the present study, a cellular level response of Cyto-aa3 oxidation was investigated in real time under both time-varying and strong static magnetic fields of 5 T. Two kinds of cells, a slime mold, *Physarum polycephalum*, and bone forming cells, MC-3T3-E1, were used for the experiments. The oxidation level of the Cyto-aa3 was calculated by optical absorptions at 690nm, 780nm and 830nm. The sample, fiber-optics and an additional optical fiber for light stimulation were set in a solenoidal coil or the bore of a 5-T superconducting magnet. The solenoidal coil for time-varying magnetic fields produced sinusoidal magnetic fields of 6mT. The slime mold showed a periodic change in Cyto-aa3 oxidation, and the oxidation-reduction cycle of Cyto-aa3 was apparently changed when visible-light irradiated the slime mold. Similarly to the case with light, time-varying magnetic stimulations changed the oxidation-reduction cycle during and after the stimulation for 10 minutes. The same phenomena were observed in the MC-3T3-E1 cell assembly, although their cycle rhythm was comparatively random. Finally, magnetic field exposure of up to 5T exhibited a distinct suppression of Cyto-aa3 oscillation in the bone forming cells. Exposure up to 5T was repeated five times, and the change in Cyto-aa3 oxidation reproducibly occurred.**

I. Introduction

For the purpose of detecting the biological real-time response to physical and chemical stresses for quick and simple cellular functional detection, various kinds of physiological and genetic parameters have been investigated in a huge number of works [1]-[4]. In the cases with magnetic field effects on living systems, for a long time, metallo-proteins concerning metabolic systems as a candidate for novel indicators for highly-sensitive monitoring of living systems has been discussed. For example, oxy- and deoxy-hemoglobin are very useful in the measurement of tissue activity by means of functional MRI evaluating oxygen consumption [5]. As well as hemoglobin, cytochrome oxidase (Cyto-aa3) is an important indicator for cellular activity which is strongly connected to oxygen consumption and ATP release.

The bio-energetic enzyme, cytochrome oxidase (Cyto-aa3) causes an oxidation-reduction cycle in mitochondrion in living cells. By utilizing near infrared spectroscopy (NIRS), the intracellular mitochondrion activity can be directly evaluated non-invasively from outside of living systems. Our previous study revealed the effect of strong static magnetic fields of 10 T order on the oxidation level of cytochrome oxidase in vivo and on the enzyme's optical absorption [6]. In the present study, a cellular level response of Cyto-aa3 oxidation was investigated in real time under both time-varying and strong static magnetic fields of 5 T. Two kinds of cellular systems, a slime mold, *Physarum polycephalum*, and bone forming cells MC-3T3-E1, osteoblast were investigated for this purpose. *Physarum polycephalum* is well known for its high responsiveness to environmental, physical and chemical parameters. Therefore, we investigated changes of Cyto-aa3 concentration in *Physarum polycephalum* under three kinds of electromagnetic fields, light, time-varying and static magnetic fields, by comparing its responsiveness with osteoblast.

II. Materials and Method

Two kinds of cells, a slime mold, *Physarum polycephalum,* and bone forming cells, MC-3T3-E1, were used for the experiments. The cells were cultivated in a chamber to form a cell colony larger than 50mm in length, and near-infrared light was emitted and detected by a fiber optic system. The oxidation level of the Cyto-aa3 was calculated by optical absorptions at 690nm, 780nm and at 830nm in an optical path of approximately 35mm, as shown in Figure 1 (a). The calculation for relative change in the oxidation level of Cyto-aa3, ΔCyt, was carried out using the following equation:

$$\Delta Cyt = -14.910 \times \Delta abs780 + 10 \times \Delta abs830 + 4.740 \times \Delta abs690 \quad (1),$$

where Δabs means the relative change in absorbance at each of the three optical wavelengths [7],[8]. The utilized methods for optical irradiation and detection followed the modified Lambert-Beer law [9] where both the emitter and detector direct the sample and the detector collects transmitted light scattering.

Fig. 1 (b) shows the case with additional visual light stimulation for the slime mold. A white light was utilized for the illuminator, and the light was introduced into the sample exposure room with an optical power of 0.03mW at the surface of the slime mold. The probe light didn't interfere with the stimulating fluorescent light, and the experimental result was not influenced by the probe light, because the wavelength range of the probe light was within a longer wavelength range than the wavelength range for the stimulation. The sample, fiber-optics and an additional optical fiber for light stimulation were set in a solenoidal coil, and the solenoidal coil was covered with a shade cloth. The time varying

*Resrach supported by JST, PRESTO.

Y. Mizukawa is with Graduate School of Engineering, Chiba University, Chiba 263-8522 Japan (corresponding author to provide phone: +81-43-290-3499; fax: +81-43-290-3499; e-mail:acxa3788@chiba-u.jp).

M. Iwasaka was with Chiba University, Chiba 263-8522 Japan, and with Japan Science and Technology Agency PRESTO, Saitama 332-0012 Japan (e-mail:iwasaka@faculty.chiba-u.jp).

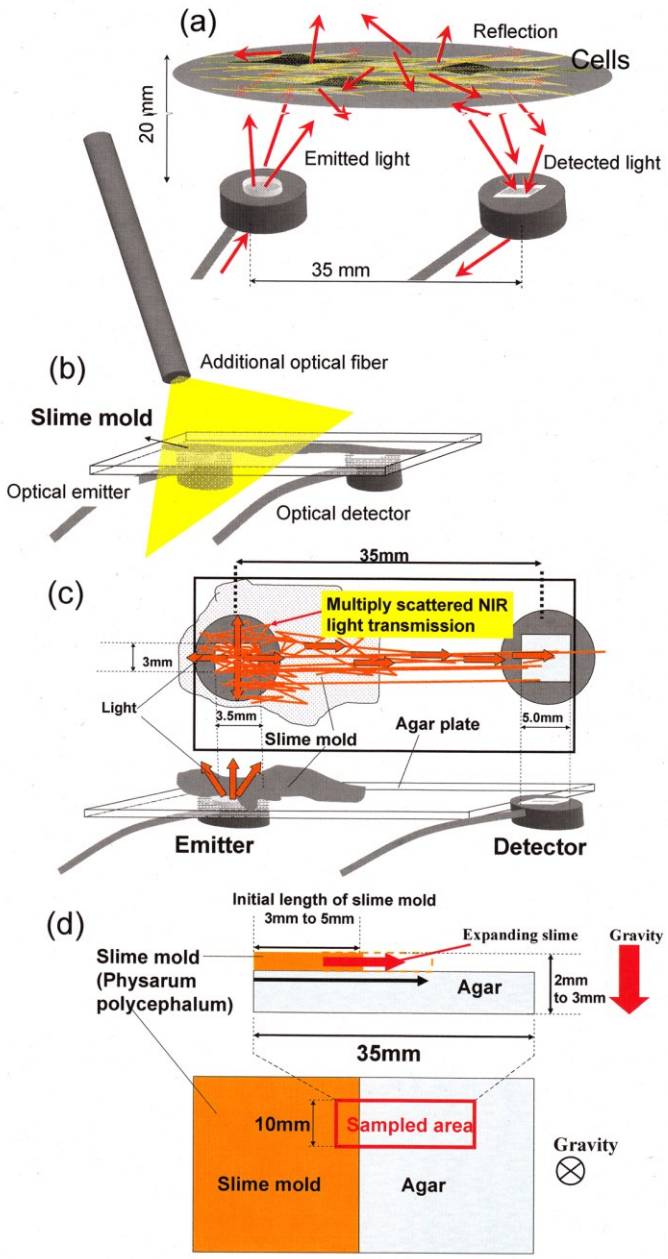

Figure 1. Schematic diagrams for near infrared light measurement of cells. (a) configuration of near infrared light emitter, detector and cell's attaching plate. (b) arrangement of visual light exposure by an additional optical fiber for slime mold on an agarose plate. (c) details of the experimental setup for the optical measurement of the slime mold, *Physarum polycephalum*. (d) size of the samples including both slime mold and agar medium.

magnetic field generators produced sinusoidal magnetic fields of up to 6mT. The sinusoidal magnetic fields at 50 Hz were generated by five circular coils enclosing the observed sample. The diameter of electromagnet bore was 130mm and the length was 240mm.

The near-infrared (NIR) light was applied to the slime mold from an optical emitter, and the light was scattered by objects in the slime mold, *Physarum polycephalum* and agar gel. It is known that NIR light is absorbed less by materials such as water, so the light can be absorbed by materials which have a specific absorption at 690nm~830nm during which the light was scattered and transmitted through. The transpired light was absorbed by a black-out curtain which covered the sample space as shown in Fig 1(c). Fig 1(d) indicates the thickness of the samples, which including both slime mold and agar medium, was about 2mm to 3mm, and thickness of the slime mold was approximately less than 1mm. The slime mold sample having an active protoplasmic streaming was cut from the area at the tip of the slime mold. The length of the slime mold piece was about 35mm.

III. Results and Discussion

Figure 2 shows two examples for Cyto-aa3 oxidation dynamics in the slime mold, *Physarum polycephalum*,

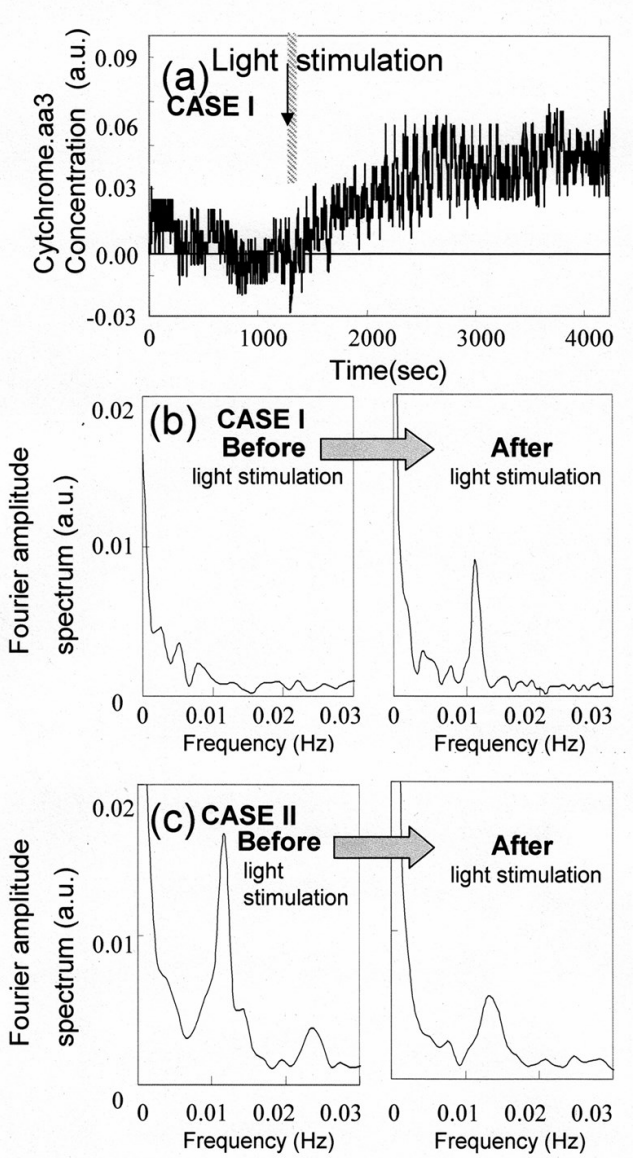

Figure 2. Changes in Cyto-aa3 oxidation dynamics in the slime mold with light irradiation; (a) raw time course of Cyto-aa3 oxidation concentration (a.u.) before and after visual light stimulation. Fourier amplitude spectra of two cases for the slime mold's responses to visual light stimulation. (b) CASE I, oscillation enhancement, (c) CASE II, oscillation suppression.

with light stimulation. Fig. 2 (a) is an example of raw data of the time-course in Cyto-aa3 concentration, and Fig. 2 (b) and (c) show Fourier spectrum in amplitude style. The slime mold showed a periodic change in Cyto-aa3 oxidation, and the oxidation-reduction cycle of Cyto-aa3 was apparently changed when visible-light irradiated the slime mold. In the case-I with light irradiation, which are shown in Fig. 2 (a) and (b), the periodic change in Cyto-aa3 concentration was enhanced and showed a cycle-entrainment after light stimulation. In another case in a different sample of slime mold, which is shown in Fig. 2 (c), the results showed the opposite pattern, a cycle-extinction of oxidation/reduction cycle where the oscillation peak at 0.012 Hz decreased.

In the next, the slime mold, *Phyazarm polycephalum*, provided a clear change in Cyto-aa3 oxidation dynamics under a sinusoidal magnetic field at 1-6 mT and 50Hz.

Figure 3 (a) shows one of the raw data of the time-course in Cyto-aa3concentration, and the periodic cycle in Cyto-aa3 oxidation clearly reduced during and after magnetic stimulation. In addition, Figure 3 (b) represents the Fourier amplitude spectrum before, during and after magnetic exposure. Before stimulation, the oscillation peaked at 0.08 Hz, but during and after stimulation the oscillation peak at 0.08 Hz was clearly reduced. The statistically analyzed data in Fig. 3(b) which is the case with sinusoidal magnetic field stimulation shows that the effect of suppression/enhancement was not significant. However the time-varying magnetic fields induced slight tendencies to show suppression and enhancement depending on the initial intensity of the prime peak.

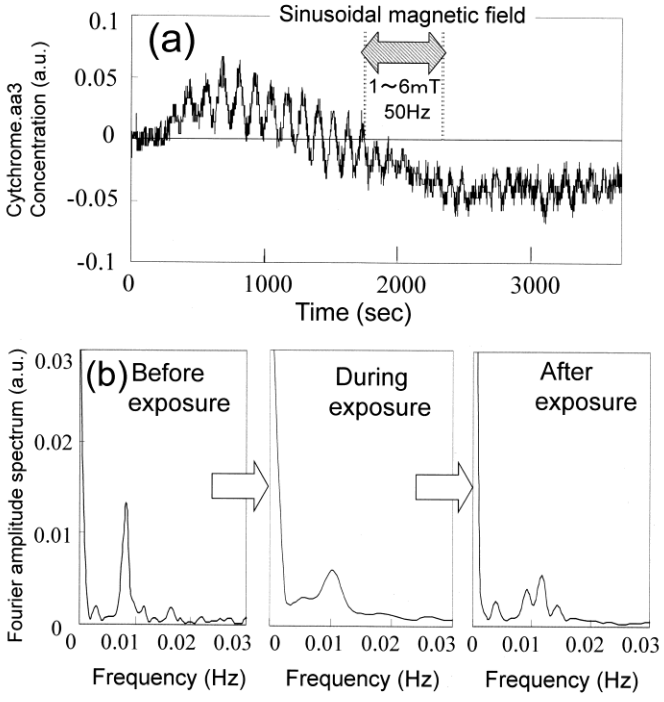

Figure 3. Time course of change in Cyto-aa3 concentration of the slime mold under sinusoidal magnetic field of 1mT - 6 mT at 50Hz; (a) raw data of time-course in Cyto-aa3 oxidation oscillation under magnetic fields, (b) Fourier amplitude spectrum of Cyto-aa3 oscillation before, during and after magnetic exposure.

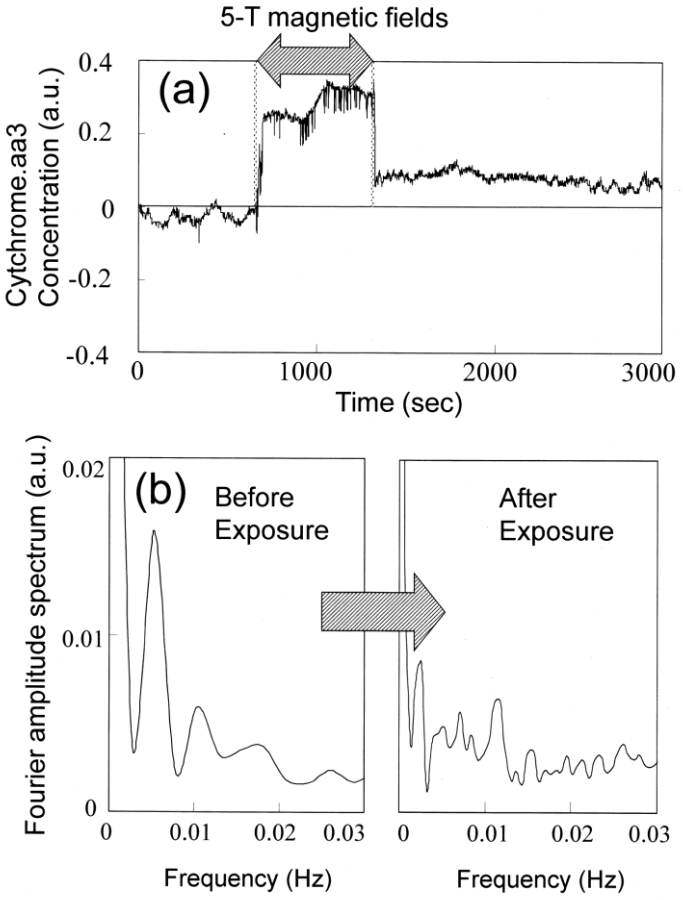

Figure 4. (a) Time course of change in Cyto-aa3 oxidation oscillation in the slime mold under 5-T strong static magnetic fields. (b) shows the comparison of the Fourier amplitude spectrum before and after 5-T strong magnetic field, respectively.

Figure 4 shows the change in Cyto-aa3 concentration in the slime mold under 5-T strong static magnetic fields. The concentrations of Cyto-aa3 oxidation in the slime mold converted the Fourier amplitude spectrum before and after magnetic exposure, as shown in Figure 4 (b). Similarly to the results with time-varying magnetic fields (Fig. 3), the strong static magnetic fields suppressed the oscillation despite of the existence of the sinusoidal oscillation right before magnetic field exposure. In the raw data shown in Fig. 4 (a), the time course shows a sudden increase and decrease at 600sec and 1200sec, respectively, but this is a kind of artifact due to moving samples in the flask during 5-T strong static magnetic exposure, and the artifact did not affect the results shown in Fig. 4 (b). The slime mold, *Physarum polycephalum* apparently responded to the applied light stimulation, but both enhancement and suppression of Cyto-aa3 oxidation oscillation equally occurred.

On the other hand, utilizing time-varying and strong static magnetic fields, a tendency to suppress the oscillation in comparison with light stimulation was discovered. This indicates that the cellular system in the slime mold, *Physarum polycephalum*, responded.

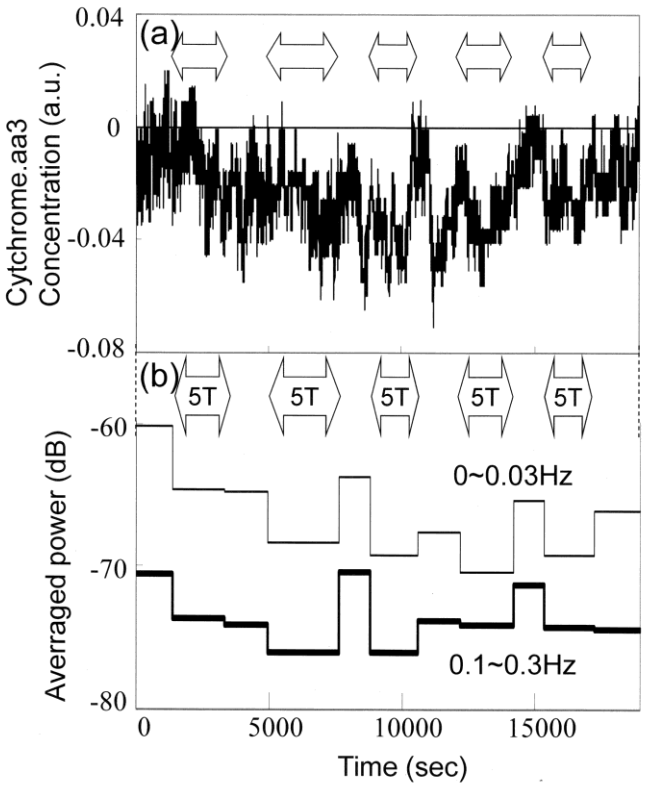

Figure 5. Time course of change in the Cyto-aa3 oxidation concentration of osteoblast, MC-3T3-E1, under magnetic field exposure at 5 T. (a) raw data with arrows showing the 5-T magnetic field exposure periods. (b) time course in the same time scale with (a) of averaged Fourier power spectrum.

How about the Cyto-aa3 oxidation response in the other cell sample, osteoblast MC-3T3-E1? In the cases with the slime mold, the Cyto-aa3 signals were associated with a rhythmic oscillation in protoplasmic streaming, and there is a possibility that the obtained optical oscillation originated from oscillation in the protoplasmic streaming.

The next experiments with osteoblast can provide an answer to the question because osteoblast does not show dynamic motion like a slime mold in the same time-scale. We carried out the same kinds of exposures with osteoblast. In the magnetic field exposure at 5 T, the magnetic field switching ON and OFF was repeated five times, as shown in Figure 5. Fig. 5(b) shows a reproducible suppression of averaged power (dB) in the Fourier power spectrum during the static 5-T magnetic field exposures. The averaged powers in the two bands, 0~0.03Hz and 0.1Hz~0.3Hz, were calculated. The same results were observed in osteoblast under the rectangular type magnetic fields of 10mT with a maximum dB/dt of 500T/sec. The Fourier spectrum in between 0~0.03Hz potentially includes a d.c. offset. However, in comparison with the spectrum at 0.1~0.3Hz with that at 0~0.03Hz, the spectra with different frequencies showed the same pattern changing in the ON/OFF points. The results indicated that the effects were dominated by the extremely low frequency fluctuations in cytochrome aa3 oxidation, but not dominated by the d.c. offset. This final data on osteoblast supported the explanation for the cellular level response of Cyto-aa3 oxidation occurring by two kinds of magnetic field exposures.

The candidates for the mechanism are eddy current by dB/dt, diamagnetic orientation of intracellular molecules, and possible effects of magnetic fields on an electron transfer system in mitochondrion, however, detailed investigation for clarification remains for future study.

IV. Conclusion

A slime mold, *Physarum polycephalum*, and bone forming cells, MC-3T3-E1, were investigated by the near-infrared spectroscopy under magnetic field exposures. Cyto-aa3 oxidation was evaluated by the optical absorptions at 690nm, 780nm and 830nm.

The oxidation-reduction cycle of Cyto-aa3 in slime mold was changed by a visible-light irradiation. Also, time-varying magnetic stimulations changed the oxidation-reduction cycle. The same phenomena were observed in the bone forming cells. 5T magnetic fields exhibit a suppression of Cyto-aa3 oscillation in the bone forming cells.

Acknowledgment

This study was supported by JST, PRESTO, “Creation of Basic Technology for Improved Bioenergy Production through Functional Analysis and Regulation of Algae and Other Aquatic Microorganisms”.

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