Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Journal of Magnetism and Magnetic Materials 293 (2005) 358–364
Journal of magnetism and magnetic materials
www.elsevier.com/locate/jmmm

Cytomagnetometric study of interactions between microfilaments and microtubules by measuring the energy imparted to magnetic particles within the cells

Iku Nemoto[a,*], Kazuhisa Kawamura[b]

[a]*Tokyo Denki University School of Information Environment 2-1200, Muzai-Gakuendai, Inzai, Chiba 270-1382, Japan*
[b]*Tokyo Denki University School of Science and Engineering Hatoyama, Saitama 350-0394, Japan*

Available online 5 March 2005

Abstract

Cytomagnetometric measurements of the energy imparted to intracellular organelles were made to study the relationship between microtubules and microfilaments. Depolymerization of microtubules by colchicine resulted in an increase in the energy suggesting that microtubules in control condition suppress the activity of microfilaments.

Keywords: Cytoskeleton; Microtubules; Microfilaments; Cytomagnetometry; Biomagnetism

1. Introduction

The cytoskeleton is a system of filaments within cells that play a major role in mechanical cellular functions such as cellular motility, phagocytosis, mitosis, intracellular transportation of organelles and molecules, and maintaining the correct cell shape and proper internal structures. There are three types of filamentous structures, namely microtubules, microfilaments and intermediate filaments. Microtubules and microfilaments have been well studied and their functions are quite well defined. However, there remains much to be known as regards their behavior in living cells. Recently, microtubules and microfilaments have been shown to interact with each other in a systematic manner. It is not surprising that their roles are different, simply considering their location within cells. Microtubules run radially from the structure called centroid near the nucleus. They look like the skeleton of an umbrella. Microfilaments are not as clearly distinguishable as microtubules under a fluorescent microscope because they are thinner and distributed quite uniformly near the periphery of the cell. Free mobile cells such as macrophages have numerous thin hair-like pseudopods, which are abundant in microfilaments and keep on changing the shape within the

*Corresponding author. Tel.: +81 476 46 8424; fax: +81 476 46 8449.
E-mail address: nemoto@sie.dendai.ac.jp (I. Nemoto).

0304-8853/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jmmm.2005.02.031

ruffling periphery. Microfilaments are actually actin filaments, which together with myosin filaments, constitute the contracting structure of the muscle cell. The relationship between microfilaments and microtubules has been reviewed and summarized in an article by Mandato et al. [1], where they stress the inhibitory nature of the effects of microtubules on actomyosin contraction. Microtubule-based suppression of actomyosin contraction is assumed to be conveyed through reactions involving rho and rac which are both GTPases affecting myosin-2-based contraction. Waterman-Storer et al. [2] showed that microtubules remodel actomyosin networks through direct (physical) interactions. Therefore, interactions between microtubules and microfilaments can either be indirect and far reaching or direct and of short distance.

Cytomagnetometry and its principle were first proposed by Nemoto [3] and Valberg [4]. It is a macroscopic approach to investigate motility and mechanical properties of the intracellular structures. The specific parameters obtained by the method are the energy E_r responsible for random rotary movements of phagosomes [5] and viscoelastic properties of the tissues surrounding the phagosomes [6,7]. Cytomagnetometry is quite macroscopic but allows ordinary live cells to be studied as long as they have phagocytic ability. Most of the studies to investigate microtubule–microfilament interactions have been performed with special cells like *Xenopus* eggs or their extracts. Cytomagnetometry also allows easy quantification of intracellular motility by such parameter as the energy E_r explained below. The method does not give direct evidence for proposed mechanisms for interactions between cytoskeletons but is useful for obtaining circumstantial evidence with the help of cytoskeletal drugs and fluorescent microscopy. Moreover, the relative ease of the technique is quite convenient for many trial experiments before going into detailed molecular-level experiments.

The purpose of the experiments performed in the present paper for measuring the intracellular energy E_r with J774A.1 macrophage-like cells treated with cytoskeletal drugs such as colchicine and cytochalasin B was to clarify the origin of the energy and to understand how it reflects the interactions between microtubules and microfilaments.

2. Methods

J774A.1 macrophage-like cells (Human Science Center, Osaka, Japan) were incubated with spherical magnetic particles of 1.9 μm diameter (Fe_3O_4), which was a generous gift by Dr. W. Moeller [8]. The culture medium used was Dulbecco Eagle medium supplemented with 5% bovine fetal serum (Gibco), 2% penicillin–streptomycin (SIGMA) and 1% L-glutamine (Nissui, Japan). The pH was adjusted to 7.4 ± 0.05. The magnetic moment of the particle was $0.57 \times 10^{-13}\,\mathrm{Am}^2$. A bottle of 12 mm diameter was used to incubate about 0.5 million cells and about the same number of particles in it. Most of the particles were ingested by the cells overnight and those remaining outside the cells were washed away when the culture medium was changed a few hours before the magnetometric measurement.

The following drugs were used to influence the cytoskeleton. Colchicine, vincristine, and nocodazol were used to depolymerize microtubules. They bind to free tubule units and prevent them from binding to the growing ends of microtubules. Taxol was used to stabilize microtubules. Cytochalasin B was used to depolymerize microfilaments. It binds to the growing ends of microfilaments. Latrunculin A also disrupts microfilaments but by binding to free G actin protein. Acrylamide disrupts the network of intermediate filaments. Y-27632 was used to inhibit the actomyosin system. It interferes with rho-kinase and prevents the phosphorylation of myosin.

The above-mentioned bottles, each containing about half million cells, were used for cytomagnetometry. Fig. 1 shows a schematic drawing of the experimental setup. The magnetic field measurement was done with a flux-gate magnetometer (Foerster, Germany). The bottle was supported by a rod, which rotated at 4 rps in front of two probe coils of the magnetometer. Another pair of probe coils was connected to these measurement coils in

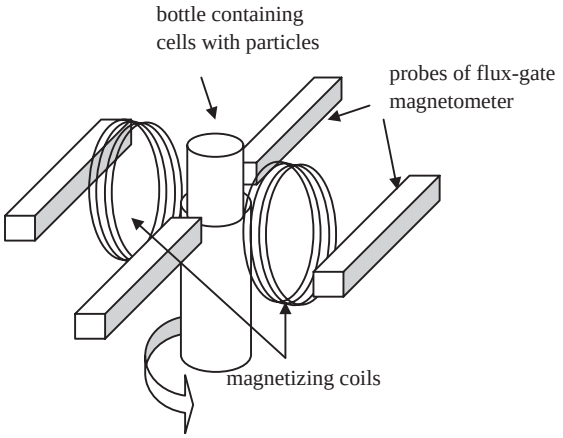

Fig. 1. Schematic drawing of the instrumentation for measurement of the cell field arising from magnetic particles within cells.

such a way that the environmental magnetic noise was canceled out. This system was surrounded by a permalloy magnetic shield. The output of the magnetometer was amplified and band-pass filtered (2–10 Hz) and fed into a computer for synchronous detection to further increase the signal-to-noise ratio. The angle of the rotating rod was measured and used for synchronous detection performed by the computer. The component of the signal varying at 4 Hz synchronously with the rotating rod was thus detected. With this method, the noise amplitude (rms value) was suppressed within around 20–30 pT.

A pair of 10-turn coils was used for magnetizing the particles within cells. The pulse current for driving these coils was produced by discharging a capacitor (1500 μF), which produced a peak field of about 0.3 T. When this pulse field was applied to the cells, the particles were magnetized and the field arising from them measured. We call this field the *cell field*. The cell field decays with a half time of about 1–2 min. The decay of the cell field is called relaxation and is caused by the intracellular movements of the cytoplasm [3–5]. The method we employed for the experiments shown in the present paper was to measure the energy responsible for this relaxation.

We assume that organelles containing particles undergo rotational Brownian motion caused by interactions with the surrounding tissues including filamentous structures such as microtubules and microfilaments. Although the motions of phagosomes are not probably random in the long run but have certain translational directions, this method of cytomagnetometry is completely insensitive to translational movements of particles within cells. When only the directions of the magnetic moments of the organelles (in fact, of the particles) are taken into consideration, the organelles may be considered to be in rotational random motions. Furthermore, it was shown by electron microscopy that particles within phagosomes were quite closely surrounded by the phagosome membrane and not likely to move freely within the phagosomes. Therefore, we can assume that the motion of a phagosome is directly reflected by the field produced by the particle within. The cell field is the vector sum of the fields arising from all the particles. We put the cells in a weak magnetic flux density B to see the degree of alignment of phagosomes in this direction which would depend on the randomizing energy given to the phagosomes from the surrounding tissues. We assume a Boltzmann-like distribution for the direction of the magnetic moment m of the organelle due to the magnetized particle under the magnetic flux density B. It is given by

$$p(x) = \exp\left\{\frac{mB\cos\theta}{E_r}\right\}, \tag{1}$$

where E_r has been substituted for kT in the otherwise true Boltzmann distribution. θ is the angle between the magnetic moment and the direction of the given external flux density B. E_r is an analog of the thermal agitation and thus considered to be the energy responsible for randomizing the direction of the magnetic moments. When there are a great number of magnetic moments distributed according to Eq. (1), the normalized cell field is given by

$$L(a) = \coth a - \frac{1}{a}, \quad a = \frac{mB}{E_r}. \tag{2}$$

The normalization is such that the cell field is one when all the moments are aligned in the direction of the measurement. From the measurement of the cell field under a suitable external magnetic flux density B, E_r can be estimated for known m and B.

We call the magnetization in the weak flux density B, a *secondary magnetization* in contrast to a *primary magnetization* by the pulse field. Primary magnetization is the magnetization of the particle itself without mechanical rotation and the secondary magnetization is a mechanical alignment of the organelle moments without changing the magnetization of the particles itself.

Eqs. (1) and (2) represent the equivalent state, meaning that $p(x)$ does not change with time at least for a short time. We wanted to follow the change of E_r caused by drugs and used the following method to estimate E_r. It was simply to apply B (typically $0.5–1.0 \times 10^{-4}$ T) continuously for an epoch of about 2 h except for short (10 s each) periods with 10–20 min intervals in between. During the short cessations of B, the cell field was measured and used as the equivalent cell field under B. E_r was estimated using Eq. (2). This method is a little different from the ones used in Ref. [5] or in Ref. [6].

3. Results

Fig. 2 shows the change of E_r after treatment with drugs, which depolymerize microtubules. The control curve is the average of all the control experiments performed along with the drug experiments. It shows that it took about 10 min to reach the equilibrium state where the value of E_r stayed at around 0.6×10^{-18} J until 30 min after the start of the measurement. This value of E_r under the control condition is more than 100 times larger than the thermal energy $kT = 4.3 \times 10^{-21}$ J at $T = 310$ K. Its tendency to increase slowly after 30 min through the period of experiment probably reflects difficulty in keeping the control condition for the whole period, which will be discussed later. This control curve is used in all the following results. The figure shows that all of the drugs caused a drastic increase in E_r although their effectiveness varied somewhat. Therefore, it is shown that depolymerization of microtubules increased the energy E_r responsible for rotational random motions of organelles containing the magnetic particles. Fig. 3 shows the change of E_r for various concentrations of colchicine: 1, 2, 5 and 10 μM. It is seen that the rate of increase in E_r was quite sensitive to the concentrations between 1 and 5 μM, although its final value did not depend on the concentration within the range used in this experiment. From this figure onward, error bars representing standard deviations are omitted in the figure to avoid confusion. They are quite similar to the ones shown in Fig. 2.

Fig. 4 shows the results with other cytoskeletal drugs. It is seen that disrupting microfilaments by cytochalasin caused a decrease in E_r to about 50% of the original value. Compared to the control curve, this decrease was quite significant.

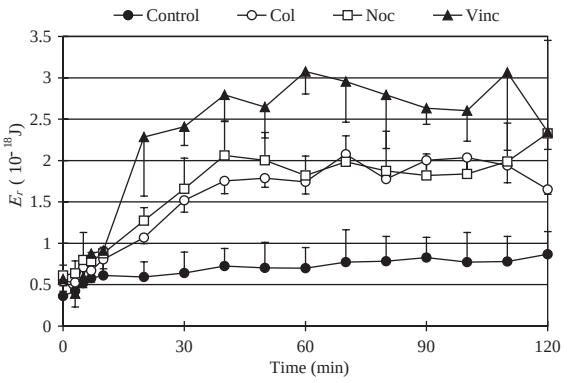

Fig. 2. Time course of the energy E_r after treatment with drugs depolymerizing microtubules. The concentration was 10 μM for all the drugs. Each data point in the graph is the mean of at least four experiments. The error bars (SD) are shown in half to avoid confusion.

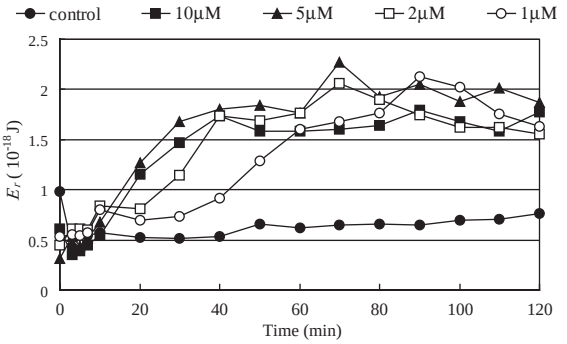

Fig. 3. Time course of the energy E_r after treatment with colchicine of various concentrations. Error bars are omitted to avoid confusion but each data point is the mean of at least four experiments.

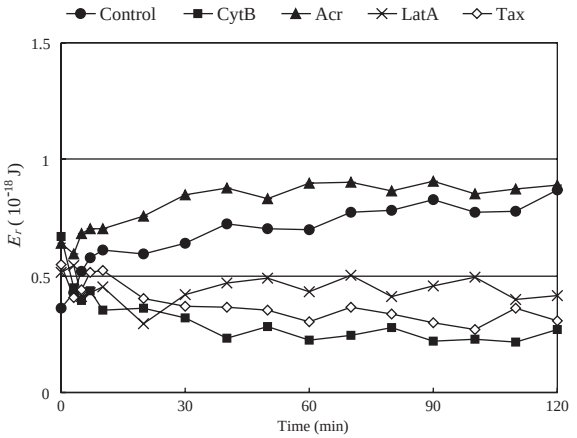

Fig. 4. Time course of the energy E_r after treatment with other cytoskeletal drugs. The concentrations were 2 μM (CytB), 4 mM (Acr), 10 μM (LatA) and 1 mM (Tax). Each data point is the mean of at least four experiments.

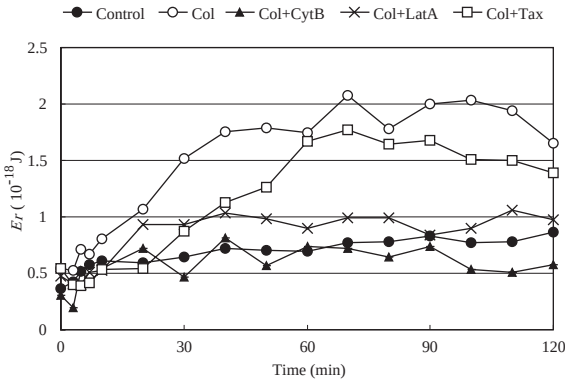

Fig. 5. Result for simultaneous treatment with colchicine and another cytoskeletal drug. The concentrations were 10 μM (Col), 2 μM (CytB), 10 μM (LatA) and 1 mM (Tax). Each data point is the mean of at least four experiments.

Latrunculin A which also disrupts microfilaments did not cause a decrease in E_r. However, it lowered the E_r curve significantly compared to the control curve. Stabilization of microtubules by taxol slightly lowered the E_r curve as well. Acrylamide which disassembles intermediate filament networks did not change the control curve significantly.

Fig. 5 shows the result of combining colchicine with another drug. When either cytochalasin B or latrunculin A was combined with colchicine, it inhibited the colchicine-induced increase in E_r. It was shown then that the increase in E_r caused by depolymerization of microtubules was suppressed by depolymerization of microfilaments. When taxol was combined, the increase was delayed by 20–30 min but was not inhibited all through the measurement.

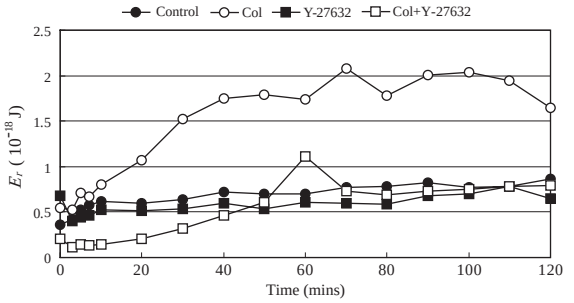

Fig. 6. Result with Y-27632. The concentration was 10 μM for each of the drugs. Each data point is the mean of at least four experiments.

Fig. 6 shows the result with Y-27632. When it was given to the cells, the resulting E_r curve was quite similar to the control curve, but when it was combined with colchicine, it inhibited the colchicine-induced increase in E_r.

4. Discussions

In Ref. [5], the effects of intracellular ATP depletion on E_r up to 30 min after treatment with KCN and/or MIA were investigated. Its purpose was to show that E_r comes from some process using ATP and was not focused on any specific filamentous structure. In Ref. [6], effects of cytoskeletal drugs such as the ones used in the present study on several parameters measured by cytomagnetometry were studied. E_r was one of the parameters and showed similar tendencies to the results shown in the present paper. The present paper focused on E_r alone and its change was measured for a much longer period with the method more suitable for pursuing the slowly changing 'equilibrium' state. More cytoskeletal drugs and their combinations were used in the present study as well.

The increase in E_r after 30 min in the common control curve is probably due to the measured increase in pH from the usual 7.4 to nearly 8 at the end of one experiment lasting 2 h. The temperature of the air within the magnetic shield where the cell-containing bottle was placed and of the culture medium as well, was kept constant at 37 °C but the air did not contain 5% CO_2 as in the incubator, which caused the increase in pH. Therefore, all the data were probably biased by the increased pH, but comparison of each E_r curve with the control curve, such as is done in the present paper, should be justified.

From the result in Fig. 2, the increase in E_r was ascribed to the decrease in microtubules and not to some other effects of specific drugs. The process of increase can then be assumed to correspond to the depolymerization process of microtubules. The rate of change should depend on the concentration of the drug, which indeed was the case as seen in Fig. 3. The rate of increase in E_r changed as the concentration increased from 1 to 5 μM. Within the range of colchicine concentrations used in this investigation, E_r reached the same level after a sufficiently long time. Whether all the microtubules were depolymerized with all the concentrations tested or the depolymerization process stopped at some level cannot be determined by this experiment alone. Immunofluorescent microscopy (not shown in this paper) suggested that some amount of microtubules were present even after treatment with the largest concentration (10 μM) of colchicine. Nevertheless, it is interesting to note that the critical concentration of tubulin (below which microtubules disassemble) in cells in general is 1–10 μM which is the same order of concentration of colchicine used in this experiment. The mechanism of depolymerization of microtubules by colchicine is not simple and much work is still in progress [9]. It may be possible to quantify the amount of microtubules and the rate of their depolymerization in living cells by this cytomagnetometric E_r measurement, taking into consideration the mechanism of depolymerization of microtubules by colchicine.

The question why depolymerization of microtubules resulted in an increase in E_r arises. Canman and Bement [10] and Benink et al. [11] showed that depolymerization of microtubules resulted in faster cortical flow in *Xenopus* oocytes. They estimated that in control condition, microtubules suppress actomyosin-based cortical flow and their depolymerization thus activated the actomyosin system. In Fig. 4, it is seen that depolymerization of actin filaments by cytochalasin B or by latrunculin A lowered the E_r curve significantly compared to the control curve. Acrylamide that disassembles intermediate filament networks slightly raised the E_r curve, if anything, implying that intermediate filaments were not relevant. These results suggest that at least part of the energy E_r comes from microfilaments which actually are actin filaments. Then our results with microtubule-disrupting drugs are in accordance with the above-mentioned result with *Xenopus* oocytes. We may assume that E_r mainly reflects the actomyosin contraction, which causes cortical flow in *Xenopus* oocytes. Depolymerization of microfilaments then naturally results in a decrease in E_r, as was shown in Fig. 4, and depolymerization of microtubules results in the cessation of their suppression of the actomyosin system as suggested by Canman and Bement [10] and in larger E_r and in faster cortical flow in the oocytes. It may also cause an increase in the amount of microfilaments as suggested by Tsai et al. [12] and consequently results in an increase in the value of E_r.

Fig. 5 further supports our view above. When cytochalasin B or latrunculin A was given to the cells with colchicine, depolymerization of microtubules did not activate the actomyosin system because microfilaments were simultaneously disrupted by these drugs. When taxol was added with colchicine, it probably stabilized microtubules for some time and the increase in E_r was delayed but microtubules were eventually depolymerized and activated the actomyosin contraction resulting in the increase in E_r. Fig. 6 is more difficult to interpret. Y-27632 is known to inhibit actomyosin contraction by inhibiting the light chain kinase of myosin. If the energy E_r comes from actomyosin contraction, the drug should lower E_r. However, Fig. 6 shows that the E_r curve is almost identical with the control curve. On the other hand, when it was given to the cells with colchicine, it completely

suppressed the colchicine-induced increase in E_r. This result of course conforms to our view of inhibiting effects of microtubules on actomyosin contraction but it is difficult to understand why Y-27632 itself did not affect the E_r curve. It may be that Y-27632 was not effective enough at this concentration (10 μM) to influence the normal actomyosin contraction but still affected its hyperactivity caused by the depolymerization of microtubules.

5. Conclusion

The results indicate that the energy E_r measured by cytomagnetometry is related to actomyosin contraction or at least to microfilaments. E_r increased when microtubules were disrupted and the increase was suppressed when microfilaments were also depolymerized or actomyosin contraction was inhibited, which implies that depolymerization of microtubules activated the actomyosin system. This is in agreement with the recent view that microfilaments have an inhibitory influence on actomyosin contraction. It has been shown that cytomagnetometry, though a macroscopic method, can be useful for quantitative investigation of the cytoskeleton in living cells. It may especially be useful for trial experiments to find effects of various treatments of the cells before fine scrutiny employing other more microscopic and time-consuming methods.

References

[1] C.A. Mandato, H.A. Benink, W.M. Bement, Cell Motil. Cytoskel. 45 (2002) 87.
[2] C. Waterman-Storer, D.Y. Duey, K.L. Weber, et al., J. Cell Biol. 150 (2000) 361.
[3] I. Nemoto, IEEE Trans. Biomed. Eng. 29 (1982) 745.
[4] P.A. Valberg, D.E. Albertini, J. Cell Biol. 101 (1985) 130.
[5] I. Nemoto, K. Ogura, H. Toyotama, IEEE Trans. Biomed. Eng. 36 (1989) 598.
[6] W. Moeller, I. Nemoto, T. Matsuzaki, et al., Biophys. J. 79 (2000) 720.
[7] I. Nemoto, W. Moeller, IEEE Trans. Biomed. Eng. 47 (2000) 170.
[8] W. Moeller, W. Stahlhofen, C. Roth, J. Aerosol Sci. 21 (1990) S421.
[9] A. Vandecandelaere, S.R. Martin, Y. Engelborghs, Biochem. J. 323 (1997) 189.
[10] J.C. Canman, W.M. Bement, J. Cell Sci. 110 (1997) 1907.
[11] H.A. Benink, C.A. Mandata, W.M. Bement, Mol. Biol. Cell 11 (2000) 2553.
[12] M.A. Tsai, R.E. Waugh, P.C. Keng, Biophys. J. 74 (1998) 3282.