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Article

Effect of electrolyzed water on the orientation of cortical microtubules in *Spirogyra* cells

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Effect of electrolyzed water on the orientation of cortical microtubules (MTs) in *Spirogyra* cells was studied. Acidic electrolyzed water, alkaline electrolyzed water, and tap water affected the orientation of cortical MTs in different ways from each other. About 90% of cells treated in acidic electrolyzed water had random or not repolymerized MTs. All cells treated in alkaline electrolyzed water had transverse MTs. In tap water, many cells were observed to have longitudinal MTs. The orientation of cortical MTs repolymerized in tap water, pH of which was adjusted at about 4.5, was not so different from that in tap water (pH 7.4), which indicates that this pH-adjusted tap water and acidic electrolyzed water affect MT orientation differently. Available chlorine concentration and pH of this pH-adjusted tap water and acidic electrolyzed water were almost similar. Because low concentration of salts changed the effect of available chlorine (NaClO) on MT orientation, these results imply that composition of ions might be important for effect of acidic electrolyzed water on MT orientation in *Spirogyra* cells.

Key words: *Spirogyra*, cortical microtubules, electrolyzed water, available chlorine concentration

Many kinds of functional water are known (1), and are often used without sufficient scientific evidence. We already reported that one kind of functional water treated by far-infrared light could affect the orientation of cortical MTs in *Spirogyra* (a species of algae) cells (2). To further investigate utility of this method, it is necessary that effects of other functional water on the orientation of cortical MTs in *Spirogyra* cells should be studied.

Electrolyzed water, which is made by electrolysis of tap water, is used in wide fields (1). Water electrolysis on the anode and the cathode is as follows.

The abbreviations used are: APM, Amiprophos-methyl; APW, Artificial pond water; MT, microtubule.

$2H_2O \rightarrow O_2 + 4H^+ + 4e^-$ (anode)

$2H_2O + 2e^- \rightarrow H_2 + 2OH^-$ (cathode)

On the anode side, acidic electrolyzed water containing generated H^+ with a bactericidal effect is made. Hypochlorous acid (HClO) is a major chemical having a bactericidal activity. Alkaline electrolyzed water on the cathode side containing generated OH^- has beneficial effect on disorders in digestive tract. Dissolved hydrogen is thought to be one of the effective elements now (3 for review). It seems important to investigate the effect of electrolyzed water on the orientation of cortical MTs in *Spirogyra* cells because electrolyzed water is most studied in functional water.

First, we investigated the effects of (acid and alkaline) electrolyzed water and tap water on the orientation of cortical MTs. In addition, the effect of available chlorine, pH or salts on the orientation of cortical MTs was studied. In this paper, the effects of acidic electrolyzed water, alkaline electrolyzed water and tap water on the orientation of cortical MTs are clearly different, and these differences coincide with the tendency which is generally accepted as effects of electrolyzed water on living organisms.

Materials and Methods

Plant materials and treatments with solutions

The species used (*Spirogyra fluviatilis* Hilse) and the culture method were the same as described elsewhere (2). Cells were used for the experiments within 2 to 7 days (growing stage) after transferring them from the stock culture to the fresh Ichimura medium (4).

Artificial pond water (APW) (0.1mM each of KCl, NaCl, $CaCl_2$ and 5mM Hepes-Tris [pH7.5]) was used as the experimental medium when cortical MTs in *Spirogyra* cells were depolymerized. Amiprophos-methyl (APM) was dissolved in DMSO to a concentration of 5mg/ml as a stock solution. Cells were treated with 3 μg/ml APM in order to depolymerize the cortical MTs for 1 h. After the APM was removed by washing the cells quickly three times with distilled water, cortical MTs were reorganized in tap water, electrolyzed water or some concentration of NaClO solution with or without some salts for 1.5 h.

Preparation of test solution

Tap water (Hirakata Osaka, Japan) was used or was electrolyzed by DX-7000 (Altech).

NaClO solution was prepared by diluting NaClO with distilled water. pH of tap water was adjusted at about 4.5 by 10% HCl.

Immunostaining

MTs were observed using the method of Hogetsu (5) as described in the previous paper (2). Cells were fixed in formalin solution, cut with a razor blade to allow entry of antibodies and treated with detergent, primary antibody, and secondary antibody. Then, MTs were observed with an epifluorescence microscopy.

The orientation of MTs was classified into three categories based on MT angles to the cell axis, longitudinal (L) for 0^0-10^0, oblique (O) for 10^0-80^0, transverse (T) for 80^0-90^0. Cells having other MTs than transverse, oblique or longitudinal (random) or no repolymerized MTs were indicated as others.

Response of cells is slightly different in each experiment. MT orientation of cells treated with control water and sample water was observed at the same time in each experiment.

Assay of available chlorine concentration

According to JIS K 0101 Methods for Examining Industrial Water (6), DPD method was employed.

Results and Discussion

After depolymerization of cortical MTs with APM, they were repolymerized in tap water and acidic electrolyzed water. In tap water, many cells had longitudinal MTs (Fig. 1). In acidic electrolyzed water, 90% of cells had random or no repolymerized MTs (Fig. 2). It is known that, in distilled water or APW, all the cells have transverse MTs in this *Spirogyra* species (7, 8). These results mean that a certain element(s) in tap water affect(s) the orientation of cortical MTs and that cell treated in acidic electrolyzed water could be damaged. HClO in acidic electrolyzed water has strong oxidation potential and may damage cells in this case.

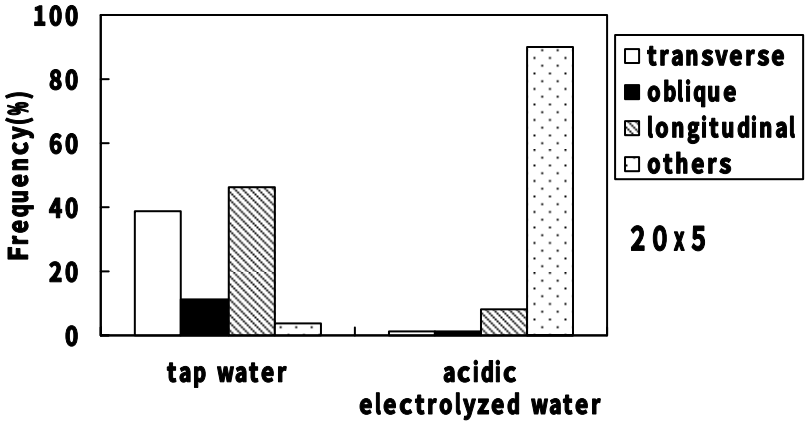

Fig. 1 The orientation of cortical MTs repolymerized in tap water and acidic electrolyzed water. In one experiment, 20 cells were examined and 5 experiments were repeated.

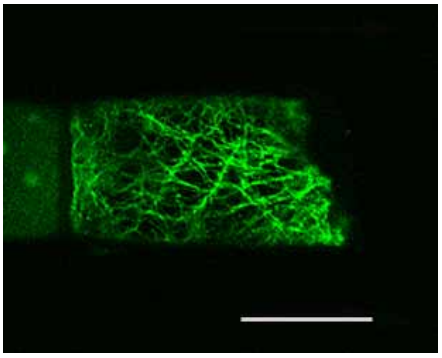

Fig. 2 Photograph of *Spirogyra* cells and immunofluorescence images of random cortical MTs in *Spirogyra* cell. Bar=30 μm.

The orientation of cortical MTs repolymerized in tap water and alkaline electrolyzed water was also examined. Apparent differentiation of MT orientation was observed between in tap water and alkaline electrolyzed water. In alkaline electrolyzed water, all the cells had transverse MTs. On the other hand, about 50% of cells had transverse MTs in tap water (Fig. 3). In normal condition, *Spirogyra* cells have transverse MTs (9). This result implies that *Spirogyra* cells in alkaline electrolyzed water might be nearer to normal condition than those in tap water, which may be related to the fact that alkaline electrolyzed water is known as functional water good for human health, for example, beneficial effect on disorders in digestive tract (10 for review).

As mentioned above, MT orientation in *Spirogyra* cells was sensitively changed in tap water, acidic electrolyzed water and alkaline electrolyzed water. In addition, physiological effects of water on cells could be inferred from the orientation of cortical MTs.

pH and available chlorine concentration were also measured because these factors are important for function of electrolyzed water (table 1). Although available chlorine concentration of tap water was only

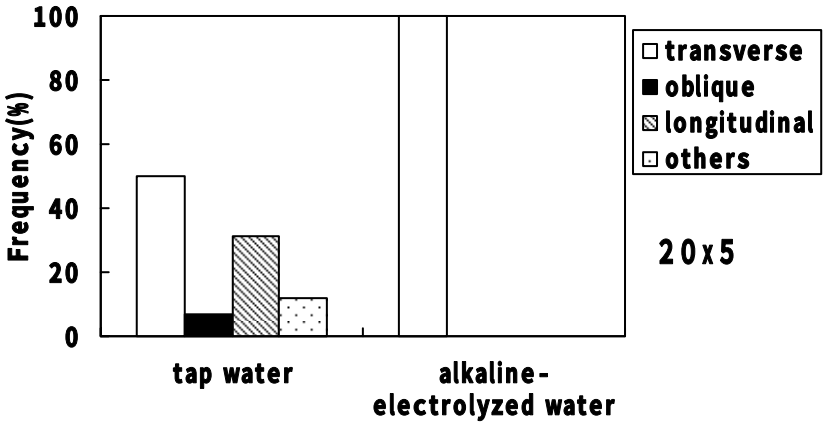

Fig. 3 The orientation of cortical MTs repolymerized in tap water and alkaline electrolyzed water. In one experiment, 20 cells were examined and 5 experiments were repeated.

Table 1 pH and available chlorine concentration of each water

	pH (n=5)	Cl(mg/l) (n=3)
non-electrolyzed water	7.74±0.08	0.31±0.04
acidic electrolyzed water	4.51±0.64	0.28±0.02
alkaline electrolyzed water	10.16±0.13	0.03±0.00

slightly higher than that of acidic electrolyzed water, cells in acidic electrolyzed water seemed to be damaged worse than in tap water. It is known that pH controls the ratio of HClO in available chlorine concentration (11) and that low pH increases the ratio of HClO and effects of available chlorine (1). These are not contradictory to an inference from MT orientation.

Available chlorine concentration of alkaline electrolyzed water was quite low compared to tap water or acidic electrolyzed water. It is probable that difference of MT orientation between alkaline electrolyzed water and tap water might be caused by difference of available chlorine concentration. So, effect of available chlorine on MT orientation was examined.

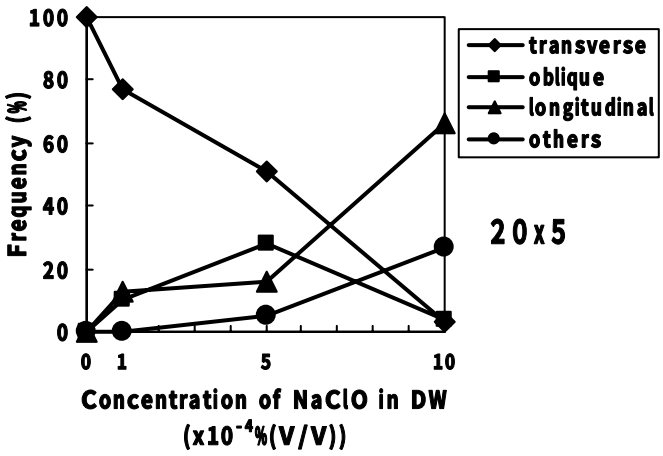

Fig. 4 The orientation of cortical MTs repolymerized in NaClO solution. In one experiment, 20 cells were examined and 5 experiments were repeated.

Table 2 pH and available chlorine concentration of each water

	pH (n=3)	Cl(mg/l) (n=3)
tap water	7.37±0.08	0.26±0.00
acidic water	4.46±0.02	0.24±0.00

NaClO was used as available chlorine. The orientation of cortical MTs was clearly affected by NaClO (Fig. 4). Because effect of NaClO on MT orientation differed quite from that of NaCl (8), effect of NaClO was thought to be caused by ClO^-. As NaClO concentration increased, ratio of cells having transverse MTs decreased. Effect of alkaline electrolyzed water on MT orientation could be explained at least partially by low level of available chlorine concentration. It is interesting that how dissolved hydrogen, putative active element in alkaline electrolyzed water (3), affects the orientation of cortical MTs in *Spirogyra* cells.

MT orientation repolymerized in tap water and in tap water, pH of which was adjusted at 4.5, was also examined. In addition, available chlorine concentration was also assayed.

There was no apparent difference (Fig. 5, Table 2). As already known, when pH is lowered, the ratio of HClO and bactericidal effects increase (11). However, when pH was lowered at the level of acidic electrolyzed water, MT

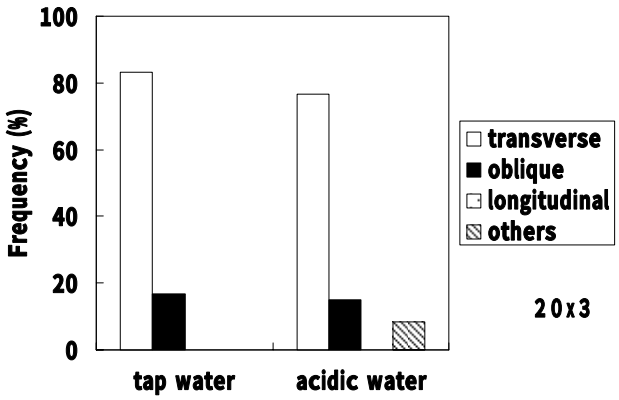

Fig. 5 The orientation of cortical MTs repolymerized in tap water (pH7.4) and acidic water (pH-adjusted tap water (pH4.5)). In one experiment, 20 cells were examined and 3 experiments were repeated.

orientation was not so affected in tap water. These results suggest that something other than available chlorine in tap water affect MT orientation. These also imply that something in acidic electrolyzed water might be important for its effects.

difference of composition of salts in tap water.

Compared with tap water, amount of cation decreases and that of anion increases in acidic electrolyzed water. In alkaline electrolyzed water, reverse changes occur (10). As mentioned above,

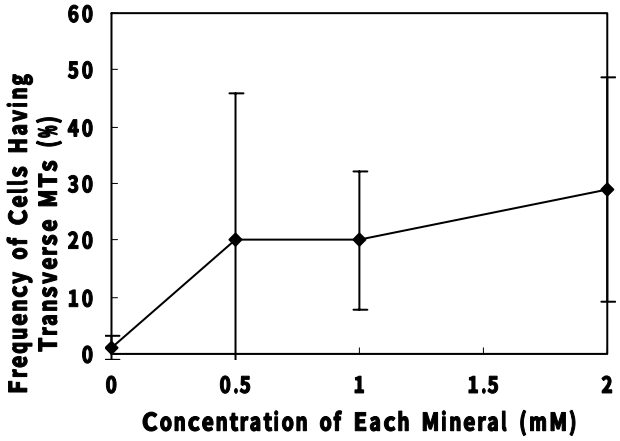

Fig. 6 Frequency of cells having transverse MTs. Cortical MTs repolymerized in 10^{-4}% (V/V) NaClO solution containing the same concentration of NaCl, KCl, $CaCl_2$ and $MgCl_2$. In one experiment, 20 cells were examined and 5 experiments were repeated.

To investigate effects of salts on available chlorine, MTs were repolymerized in 10^{-4}% NaClO solution containing the same concentration of salts (NaCl, KCl, $CaCl_2$ and $MgCl_2$). As concentration of salts increased, frequency of cells having transverse MTs increased (Fig. 6). Some salts surely affect the effect of available chlorine on MT orientation at quite low concentration (1 mM). This result suggests that salts in acidic electrolyzed water might be important for its effects. Difference of effects of tap water on MT orientation in Fig. 3 and Fig. 5 could be explained by

effects of pH-adjusted tap water and acidic electrolyzed water on MT orientation were quite different, though available chlorine concentration and pH were similar. Change of amount of ions in acidic electrolyzed water might be responsible for its effects.

In this paper, we described that effect of electrolyzed water on living organisms could be detected using *Spirogyra* cells.

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