DIELECTRIC BEHAVIOR OF YEAST CELLS TREATED WITH $HgCl_2$ AND CETYL TRIMETHYL AMMONIUM BROMIDE

YOSHIHIKO SUGIURA *and* SHOZO KOGA
From the Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan

ABSTRACT The change in dielectric properties caused by the destruction of the transport barrier of yeast cells has been investigated. Dielectric measurements were made over the frequency range of 1 kc to 2 Mc by using "leaky" yeast cells prepared by treatments with $HgCl_2$ or CTAB (cetyl trimethyl ammonium bromide). The Hg-treated cells were observed to give smaller dielectric constants and lower critical frequencies as compared with that of the intact cells, while the CTAB-treated cells gave no clear-cut dielectric dispersion. These observations are interpreted on the basis of Maxwell-Wagner's theory as indicating the changes in the intracellular conductivity, the membrane capacitance and the membrane conductance.

INTRODUCTION

As described in a preceding paper (1), dielectric studies on yeast and green algal cells in suspension have shown that these intact cells have high apparent dielectric constants of 2100 and 400, respectively. Such high values of dielectric constant have also been observed with, *e.g.*, yeast (2), *E. coli* (3), erythrocytes (4, 5), and pleuropneumonia-like organism (6). The observed high values of apparent dielectric constant have been explained in accordance with Maxwell-Wagner's theory (7) on the assumption that a thin layer of low conductivity is located in the cell envelope (1, 8-10). The thin layer thereby assumed has been suggested to be a plasma membrane which consists of lipids and proteins, making a transport barrier to ions and molecules. The aim of the present work is to reveal the relationship between the thin layer electrically assumed and the plasma membrane as a transport barrier.

Reageants such as heavy metals, surface active compounds, or organic solvents are known to affect the plasma membrane and induce loss of some cytoplasmic constituents. As for yeast cells, a marked loss of potassium ions was observed when

intact cells were left in an aqueous solution of $HgCl_2$ (11) or CTAB (12). Therefore, if the plasma membrane has an important bearing on the dielectric properties of yeast cells, those reagents are likely to induce a considerable change in the dielectric behavior of the cell suspension. According to Maxwell-Wagner's theory, it is expected that a suspension of cells treated with the reagents mentioned above will give no dielectric dispersion or a smaller dielectric constant and a lower critical frequency than those of intact cells. The dielectric dispersion of erythrocytes in suspension was observed to disappear upon the treatment with saponin (4), which little is known about the dielectric behavior of microbial cells treated with various reagents.

The present paper deals with the dielectric behavior of the suspension of "leaky" yeast cells prepared by treating with $HgCl_2$ or CTAB.

MATERIALS AND METHODS

Impedance study was made by using baker's yeast (*Saccharomyces cerevisiae*) obtained from Oriental Yeast Co., Tokyo. The yeast cells were treated for 2 hours at room temperature with an aqueous solution of $HgCl_2$ or CTAB. The concentration used was so high that all the cells were attacked and became "leaky" cells. The treated cells were washed several times with distilled water and resuspended in a 10^{-4}M NaCl solution. Dielectric measurements were made after an hour of standing at room temperature. A microscopic examination showed that the Hg-treated cells remained in their intact shape, while a small amount of microscopic cell inclusions appeared to be released from the CTAB-treated cells. No change in cell population was observed in both cases.

Dielectric constant and conductivity measurements on suspensions of the treated cells were made at a controlled temperature over the frequency range of 1 kc to 2 Mc by the use of low and radio frequency bridge circuits (13) which permitted to measure the equivalent parallel resistance (in ohms) and the equivalent parallel capacitance (in $\mu\mu$F) of the cell suspensions. A standard bridge substitution method (14) for measuring impedance was employed to eliminate some errors in the bridge circuits, such as inequality of the ratio arms, inductance effects and errors due to stray capacitance. The electric cell had a pair of parallel platinum electrodes of 1.5×1.5 cm^2, 1cm apart, which were coated with platinum black to decrease the polarization capacity. The cell was calibrated with water and several potassium chloride solutions. The errors caused by the inductance of the electric cell and leads was negligibly small in the frequency region used. Precautions were taken to minimize the stray field effect and the electric cell was always filled with the same volume of sample solutions. On account of instability of the sample properties and their electrical characteristics, the precision in the measurements was limited by the nature of the sample rather than by the bridge circuit characteristics. The over-all experimental errors were estimated in most cases to be $\pm$ 1 per cent and $\pm$ 5 per cent for resistance and capacitance, respectively. When necessary, the conductivity of the external media was measured after centrifuging at 2000 *g* for 5 minutes.

Volume fractions of the suspended cells were calculated, with the error of 10 per cent, from the cell number per unit volume of the suspension and diameter of the cells measured under the microscope, respectively.

RESULTS

1. *Frequency Dependence of Dielectric Constant.* Values of dielectric constant ε observed on suspensions of the Hg- and CTAB-treated cells are shown in Fig. 1 as a function of frequency. A dielectric constant curve for intact cells (1) is also presented for reference. These suspensions were prepared in approximately equal volume fractions by using a medium of the same composition.

As for intact and Hg-treated cells, observed data fitted well Maxwell-Wagner's theoretical curve with a single relaxation time at frequencies above 20 kc, where electrode polarization capacity was negligibly small. The arrows in Fig. 1 indicate the positions of the critical frequency f_c. The value of f_c for the Hg-treated cells (62 kc) was found to be much lower than that of intact cells (350 kc).

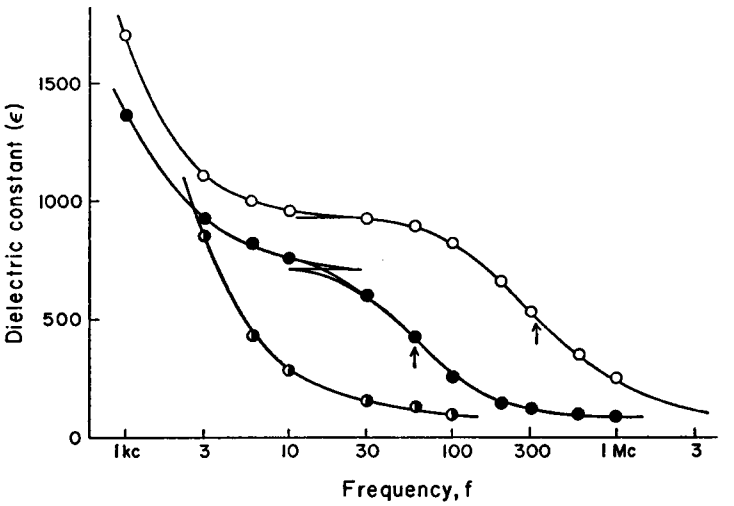

FIGURE 1 Frequency dependence of dielectric constant ε of yeast suspensions. $T = 15.0°C$. (○): intact cell ($\phi = 0.34$, $\kappa_a = 2.09 \times 10^{-4}$ mho cm^{-1}); (●): Hg-treated cell (0.34, 2.26×10^{-4}); (◑): CTAB-treated cell (0.32, 2.15×10^{-4}).

With suspensions of the CTAB-treated cells, no dispersion could be found. Although over the frequency range used there was a remarkable decrease in dielectric constant with increasing frequency, the true dispersion curve could not be obtained because of uncertainties in eliminating the electrode polarization effect. Hence, evaluation of the critical frequency was not made for the CTAB-treated cells.

2. *Relation Between Dielectric Constant and Volume Fraction.* Dielectric constant of the Hg-treated cells in suspension was measured at various volume fractions, and the zero-frequency dielectric constant, ε_0, was obtained as an extrapolated value on the ordinate axis in a plot of ε against $f^{-1.5}$ (1, 14), where f is the frequency.

The values of ε_0 are shown in Fig. 2 as a function of volume fraction of the suspended cells, where open circles represent the intact cells for reference and closed circles the Hg-treated cells. The curve II denotes the theoretical values calculated as will be described later. The dielectric constant of the Hg-treated cells was found to be smaller than that of the intact cells.

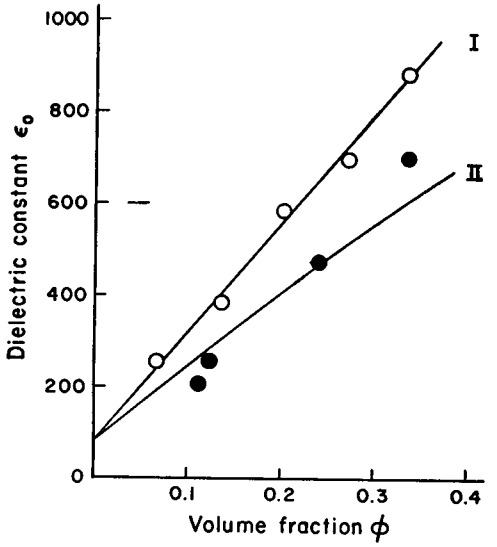

FIGURE 2 Volume fraction dependence of dielectric constant ϵ_0 of yeast suspensions. (○): intact cell (curve I); (●): Hg-treated cell (curve II).

3. *Conductivity of "Leaky" Cell Suspensions.* Conductivity measurements were made at 5 kc on suspensions of the cells treated with $HgCl_2$ or CTAB, and on their corresponding media. The results are shown in Fig. 3 where the ordinate represents $\kappa_a/\bar{\kappa}$ and the abscissa κ_a. The apparent conductivity of the treated cell, $\bar{\kappa}$, was calculated from

$$\frac{\kappa - \kappa_a}{\kappa + 2\kappa_a} = \phi \frac{\bar{\kappa} - \kappa_a}{\bar{\kappa} + 2\kappa_a}, \tag{1}$$

where κ and κ_a are the conductivity of a suspension and of a suspending medium, respectively, and ϕ the volume fraction of suspended cells.

The straight solid line represents the theoretical values calculated from equation 4 (see Discussion) with $(R/d)\kappa_s = 1.1 \times 10^{-4}$ mho·cm^{-1} derived experimentally for the intact yeast (1). Fig. 3 shows that the observed data on the Hg-treated cells fall approximately on the solid line, while the data on the CTAB-treated cells fall below the solid line. The results indicate that the membrane conductance of the cells was almost unaffected by the Hg treatment and markedly increased by the CTAB treatment.

DISCUSSION

The reagents used above, mercury and CTAB, are known to affect the plasma

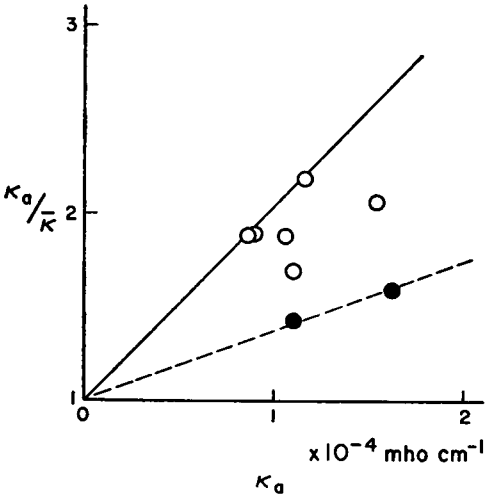

FIGURE 3 Values of $\kappa_a/\bar{\kappa}$ as a function of κ_a. (○): Hg-treated cell; (●): CTAB-treated cell.

membrane of yeast cells, resulting in a marked loss of intracellular potassium ions (11, 12). Accordingly, in interpreting the dielectric behavior of the treated cells, two effects—the loss of intracellular potassium ions and the disorganization of the membrane part—should be both taken into consideration.

The potassium content of the baker's yeast is relatively high, about 0.15M per Kg of cells and, after the period of the treatments (2 hours), a large percentage of the intact potassium content was found by flame photometry to have been released to the external medium. Since potassium is considered to be a main charge carrier inside the intact cell, the loss of potassium might cause the decrease in the conductivity of the cell interior.

Let us assume, therefore, that as a limiting case the conductivity of the interior of the "leaky" cell, κ_i, is equal to that of the external suspending medium, κ_a. Then, the following equations are obtained on the basis of Maxwell-Wagner's theory applied to the two phase model (1, 10, 15) where a cell is assumed to be a spherical particle surrounded with a thin layer of low conductivity.

$$f_c = \frac{1}{2\pi\epsilon_r} \frac{3R/d \cdot \kappa_s + 2(1 + \frac{1}{2}\cdot\phi)\kappa_a}{3R/d \cdot \epsilon_s} \tag{2}^1$$

$$\epsilon_0 = \epsilon_a + \frac{9}{4} \frac{\phi R/d \cdot \epsilon_s}{\{(1 + \frac{1}{2}\cdot\phi) + \frac{3}{2}\cdot R/d \cdot \kappa_s/\kappa_a\}^2} \tag{3}^1$$

$$\kappa_a/\bar{\kappa} = 1 + (d/R \cdot 1/\kappa_s) \cdot \kappa_a \tag{4}$$

where

ϵ_a = the dielectric constant of the suspending medium,

[1] Equations (2) and (3) are derived from equations (14) and (10) of reference (1), respectively, by letting $\kappa_i = \kappa_a$. Equations (2) and (3) become identical with the Schwan equations (15), $T = RC_M(\kappa_i + 2\kappa_a)/2\kappa_i\kappa_a + RG_M(\kappa_i + 2\kappa_a)$ and $\epsilon_s = 9/4\epsilon_r \phi RC_M/[1 + RG_M(1/\kappa_i + 1/2\kappa_a)]^2$, respectively, when $\kappa_i = \kappa_a$ and $\phi \ll 1$.

ε_s = the dielectric constant of the thin layer,
κ_s = the conductivity of the thin layer,
R = the radius of the particle,
d = the thickness of the thin layer,
ε_r = $1/\pi \cdot 9 \cdot 10^{11}$ farad cm^{-1}, the conductivity is expressed in who cm^{-1}.

Moreover, since as shown in Fig. 3 the membrane conductance of yeast cells was not remarkedly affected by the Hg treatment, it seems reasonable to assume that the dielectric constant as well as conductance of the membrane be unaffected by the treatment. Under the assumptions mentioned above, the zero-frequency dielectric constant, ε_o, of the Hg-treated cells can be theoretically obtained for various values of ϕ by the use of equation (3) with the values of the membrane capacitance and conductance for the intact cells, namely, $\varepsilon_s/d = 1.2 \times 10^7$ cm^{-1}, $\kappa_s/d = 0.35$ mho cm^{-2} and $\kappa_a = 2.2 \times 10^{-4}$ mho cm^{-1}. The theoretical curve thus obtained is shown as curve II in Fig. 2. The experimental data (closed circle) fit approximately the theoretical curve, showing that the membrane parameters are not markedly affected by the Hg treatment.

The critical frequency, f_c, of the Hg-treated cells can be calculated as 140 kc in the same way from equation (2) with $\varepsilon_s/d = 1.2 \times 10^7$ cm^{-1}, $\kappa_s/d = 0.35$ mho cm^{-2}, $\kappa_a = 2.2 \times 10^{-4}$ mho cm^{-1} and $\phi_e = 0.22$ where ϕ_e denotes an effective volume fraction obtained from ϕ after correction for the cell wall space (1).

The observed value of f_c was found to depend upon the time interval between the treatment and the measurement, shifting toward lower frequency regions with increasing stock time. The experimental value shown in Fig. 1, where the measurement was made after an hour of standing, was 62 kc, being small as compared with a calculated value of 140 kc. The discrepancy appears to be, at least partially, due to the time variation of f_c which might reflect a slow change in membrane parameters and others. The time variation of ε_o was not so marked as that of f_c.

Hence, although the interpretation is not conclusive, the shift of the critical frequency and the decrease of the zero-frequency dielectric constant of the Hg-treated cells are able to explain to a first approximation by considering the decrease of the intracellular conductivity caused by the treatment.

The CTAB-treated cells showed no clear-cut dispersion in the observed frequency range and gave a dielectric constant smaller than that of the Hg-treated cells. These facts cannot be accounted for by only assuming the decrease in the intracellular conductivity, suggesting that the membrane might be affected to a further extent in the CTAB treatment than in the Hg treatment. In this connection the CTAB treatment really gave rise to an increase in the membrane conductance as shown in Fig. 3.

The mechanism of the disorganization of the plasma membrane caused by the reagents used is not fully understood. However, it seems that mercury first attacks the SH-groups of the protein part in the plasma membrane (11) and surface active

compounds act directly on its lipid components in the same way as observed with the protoplast membrane of *Micrococcus lysodiekticus* (16). If so, the disappearance of the dielectric dispersion upon the CTAB treatment is due to the destruction of the lipid part in the plasma membrane, indicating that the lipid part corresponds to the thin layer electrically assumed for interpreting the dielectric behavior of the intact microbial cell suspensions (1).

The present impedence analyses of the "leaky" yeast cells seem to confirm the validity of the suggestion that the electrically assumed thin layer might correspond to the lipid part in the plasma membrane of the microbe.

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