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Dielectric Properties of Yeast Cells

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Summary. Dielectric measurements were made on suspensions of intact yeast cells over a frequency range of 10 kHz to 100 MHz. The suspensions showed typical dielectric dispersions, which are considered to be caused by the presence of cytoplasmic membranes with sufficiently low conductivity. Since the conductivity of the cell wall was found to be of nearly the same value as that of the suspending medium, composed of KCl solutions in a range from 10 to 80 mM, the cell wall may be ignored in establishing an electrical model of the cells suspended in such media. An analysis of the dielectric data was carried out by use of Pauly and Schwan's theory. The membrane capacitance was estimated to be $1.1 \pm 0.1\ \mu F/cm^2$, which is compared with values reported so far for most biological membranes. The conductivity of the cell interior was almost unchanged with varying KCl concentrations and showed low values owing to the presence of less conducting particles, presumably intracellular organelles. The relatively low dielectric constant of about 50 obtained for the cell interior, in comparison with values of aqueous solutions, may be attributed also to the presence of intracellular organelles and proteins.

So far little has been known about the electrical properties of cytoplasmic membrane and cytoplasm of microorganisms which are too small to be investigated with microelectrode techniques. Electrical properties of such small biological cells can be obtained from an analysis of dielectric behavior of suspensions. Generally, suspensions of biological cells and intracellular organelles covered with poorly conducting surface membranes show dielectric dispersions due to the Maxwell-Wagner mechanism. Fricke and his collaborators [4–6] carried out dielectric measurements of erythrocytes, yeasts and *E. coli* in suspensions, and calculated their membrane capacitances by use of Fricke's approximate equation which was derived from the potential theory on cell suspensions. The capacitance values estimated for the various biological membranes were found to be close to $1\ \mu F/cm^2$. Fricke's theory was, however, insufficient to determine electrical parameters of the inner phase of the cells.

Recently, Sugiura, Koga and Akabori [16] reported dielectric dispersions of yeast suspensions over a frequency range from 10 kHz to

several MHz and confirmed that the dispersions can be interpreted in terms of the Maxwell-Wagner mechanism by assuming the cell membrane to be less conductive. Unfortunately, electrical phase parameters (except for membrane capacitance) were not obtained in their study.

With a view to carrying out closer analysis of dielectric behavior of biological cell suspensions, Pauly and Schwan [13] developed a dielectric theory for a suspension of spherical particles covered with shells. They derived approximate equations usable for numerical analysis of the data [11, 12]. Membrane capacitance and conductance of the inner phase of mitochondria were successfully determined by the application of their approximate equation to dielectric data of mitochondrial suspensions.

Since the approximate equations are not acceptable for a practical system in certain cases, Hanai, Koizumi and Irimajiri [8], on the basis of a general equation of Pauly and Schwan's theory, proposed a systematic procedure to determine three electrical phase parameters of suspended cells: membrane capacitance, dielectric constant, and conductivity of an inner phase of the cells.

In this study, dielectric behavior of yeast cell suspensions is observed over a frequency range of 10 kHz to 100 MHz and at different KCl concentrations of the suspending medium. It is found that an electrical model of single-shell spheres may effectively be applied to the yeast cells, though the cells are possessed of two shells: a cytoplasmic membrane and a cell wall. Finally electrical phase parameters of the cells are determined from the observed data on dielectric dispersions by means of the procedure proposed by Hanai et al. [8].

Materials and Methods

Yeast cells *(Saccharomyces cerevisiae)* were grown in shaken cultures at 27 °C in a medium containing 10 g yeast extract, 10 g polypepton and 20 g D-glucose per liter. The cells were collected at their early stationary phase and washed twice with distilled water, then suspended in the KCl solutions in concentrations ranging from 10 to 80 mM. The suspension was incubated for at least one hour at room temperature so as to attain an ionic equilibrium between the inner and the outer phase. After the incubation the collected yeast cells were resuspended in a KCl solution containing 0.1 % agar. The addition of agar was effective in avoiding sedimentation of the cells during dielectric measurements and had no influence on the dielectric constant and conductivity of the medium.

Measurements of capacitance and conductance were carried out with a TR-1C Transformer Ratio-Arm Bridge of Ando Electric Co., Ltd., and with a 250A RX-Meter of the Boonton Radio Corporation over a frequency range of 10 kHz to 3 MHz and of 1 to 100 MHz, respectively. The measuring cell is a parallel plate capacitor consisting of two platinized platinum plates and a lucite spacer, the cell constant being 0.03 pF. Above several tens of MHz, the bridge readings of the capacitance and the conductance are seriously affected by residual inductance arising from the terminal leads and the measuring cell. Correction for

the residual inductance was made following Schwan's method [14].

Diameters of yeast cells were measured under an optical microscope. Yeast cells were spheroidal, ranging from 2 to 5 μm in minor diameter and 3 to 6 μm in major diameter. The mean diameter of the cells was calculated to be 4.3 μm by assuming a sphere of the same volume as that of the spheroid. The mean diameter inside the cell wall was assumed to be 3.8 μm by using a value of 0.25 μm for the thickness of the cell wall reported by Agar and Douglas [1]. The number of cells in suspension was counted by a Micro-Cell Counter Model CC-1002 of Toa Medical Electronic Co., Ltd.

Symbols

ε	dielectric constant
κ	electrical conductivity
ε_0	dielectric constant of free space
ε^*	complex dielectric constant given by $\varepsilon^* = \varepsilon + \frac{\kappa}{j2\pi f \varepsilon_0}$
f	measuring frequency
f_P	characteristic frequency of the P-dispersion
D	inner diameter of cell
d, t	thickness of cell membrane and cell wall
C_M	specific membrane capacitance
Φ	volume fraction of cells including cell wall in suspension
p	volume fraction of the spheres consisting of the cell membrane and its interior

Subscripts

a	outer phase
i	inner phase of cell
m, w	membrane phase and wall phase
l	limiting value at low frequencies
h	limiting value at high frequencies

Results and Discussion

Dielectric Behavior of Yeast Cell Suspensions

Fig. 1 illustrates results of dielectric measurements for the suspensions of yeast cells in various volume fractions. The dilution of each suspension is the following: Specimen 1-*A*, dilution 1; 1-*B*, 3/5; 1-*C*, 2/5; 1-*D*, 1/5. The dielectric constant ε and electrical conductivity κ of the suspensions showed remarkable dependence on frequency. These dielectric dispersions are assigned to the *P*-dispersion [8] or the β-dispersion following a nomenclature by Schwan [14]. The complex plane plots of the dielectric data shown in Fig. 1 are found to be circular arcs as seen in Fig. 2. In Table 1 are summarized the dielectric parameters of the dielectric dispersions illustrated in Figs. 1 and 2. The values of ε_l and κ_l are remarkably

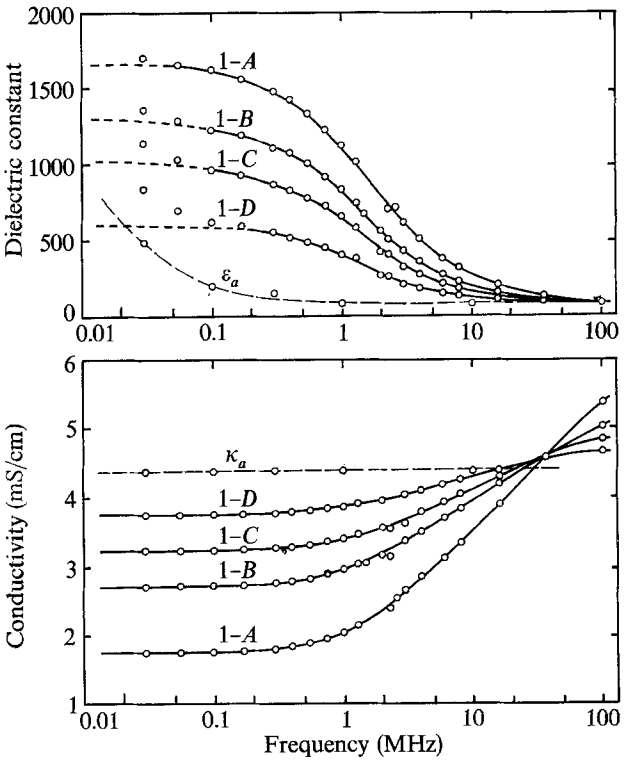

Fig. 1. Frequency dependence of dielectric constant and conductivity of yeast cell suspensions in various volume fractions. The suspending medium is 40 mM KCl solution. Dielectric measurements were made at 14 °C. Volume fractions were changed by diluting a specimen as: Specimen 1-*A*, dilution 1; 1-*B*, 3/5; 1-*C*, 2/5; 1-*D*, 1/5

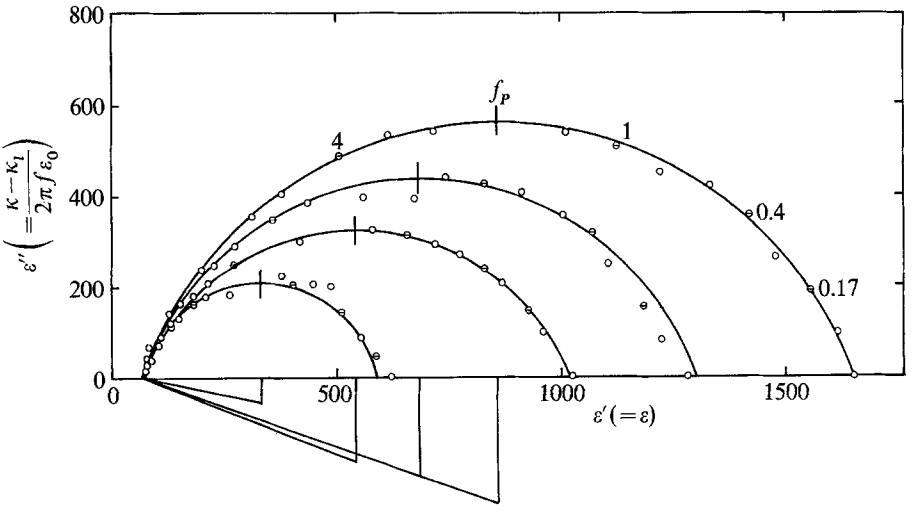

Fig. 2. Complex plane plots of the dielectric data shown in Fig. 1. Numbers beside the measured points are frequency in MHz

Table 1. Dielectric parameters of yeast suspensions in dilution series and estimated phase parameters of yeast cells

Specimen	Dilution	Dielectric parameters				Phase parameters				
		κ_l mS/cm	ε_l	ε_h	f_P MHz	p	C_M μF/cm^2	ε_m	ε_i	κ_i mS/cm
1-*A*	1	1.7	1659	64	1.9	0.50	1.05	5.9	51	2.6
1-*B*	3/5	2.7	1305	71	1.5	0.29	1.17	6.6	51	2.5
1-*C*	2/5	3.2	1017	74	1.4	0.19	1.24	6.9	49	2.5
1-*D*	1/5	3.7	592	77	1.4	0.10	1.20	6.8	52	2.5

$\kappa_a = 4.4$ mS/cm, $\varepsilon_a = 80$, $d = 50$ Å, $D = 3.8$ μm, measuring temperature = 14 °C.

dependent on volume fraction. The values of ε_h are slightly lower than that of the suspending medium, showing a tendency to decrease with the increase in volume fraction. The values of f_P remain constant except for Specimen 1-*A*, as expected from the results of numerical estimation from Pauly and Schwan's theory [8]. The deviation of f_P for Specimen 1-*A* can probably be explained by the interaction among the cells, because the volume fraction of Specimen 1-*A* may be high beyond the applicable range of the theory.

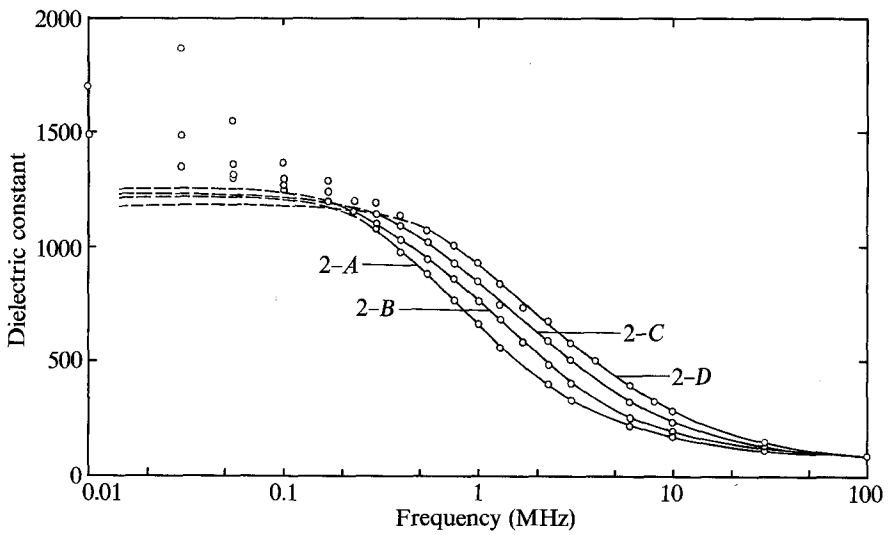

Fig. 3. Frequency dependence of dielectric constant of yeast cells suspended in various KCl concentrations. The KCl concentration of specimens: Specimen 2-*A*, 10 mM; 2-*B*, 20 mM; 2-*C*, 40 mM; 2-*D*, 80 mM. Osmotic moles of the medium were kept constant (150 mosm) by addition of sorbitol. Volume fractions are in the range of 0.26 to 0.31. Dielectric measurements were made at 28 °C

Fig. 3 shows the frequency dependence of dielectric constant for yeast cell suspensions in varying KCl concentrations of the suspending medium, each of the suspensions having approximately the same volume fraction. Osmotic moles of the suspending medium were kept constant to prevent the cells from volume change by addition of sorbitol, which is hardly metabolized by the strain. The dielectric parameters obtained are listed in Table 2. The values of ε_l and ε_h are almost independent of KCl concentration of the suspending medium, while f_P shifts markedly to higher frequencies with increasing conductivity of the suspending medium. A steep rise of dielectric constant at frequencies below 0.1 MHz, as seen in Figs. 1 and 3, is due to electrode polarization, shifting to higher frequencies with the increase in specimen conductivity.

Sugiura *et al.* [16] reported similar results for yeast cell suspensions and suggested that the dielectric dispersions in question are explained in terms of the Maxwell-Wagner mechanism by regarding a yeast cell as a conducting particle covered with a less conducting shell. In addition, they [17] concluded that the less conducting shell corresponds to a cell membrane and not to a cell wall, because marked reduction of the dielectric dispersions was observed by treating the cells with cetyl trimethyl ammonium bromide, which acts directly on lipid components in the cell membranes. In the present study, similar results were also obtained by treatment with dodecyl dimethyl benzyl ammonium chloride and sodium dodecyl sulfonate. The question still remains as to whether the presence of cell wall gives no influence on the dielectric dispersions at all, even though the dielectric dispersions may result dominantly from the cell membranes. We shall next consider whether or not the cell wall may be ignored for the electrical model of the yeast cells.

Table. 2. Dielectric parameters of yeast suspensions in varying KCl concentrations of suspending medium and estimated phase parameters of yeast cells

Specimen	KCl concentration mM	Dielectric parameters					Phase parameters				
		κ_a mS/cm	κ_l mS/cm	ε_l	ε_h	f_P MHz	p	C_M μF/cm²	ε_m	ε_i	κ_i mS/cm
2-*A*	10	1.5	0.98	1237	74	1.0	0.26	1.22	6.9	49	2.2
2-*B*	20	2.9	1.8	1251	72	1.4	0.28	1.14	6.4	54	2.5
2-*C*	40	5.6	3.3	1240	77	1.9	0.31	1.07	6.0	52	2.9
2-*D*	80	11.0	6.5	1230	67	2.5	0.31	1.04	5.9	42	3.5

Osmotic mole of suspending medium = 150 mosM, $\varepsilon_a = 80$, $d = 50$ Å, $D = 3.8$ μm, measuring temperature = 28 °C.

Electrical Model for Yeast Cells

A cell of yeast has usually an ellipsoidal shape, and the protoplasm is enclosed with a cytoplasmic membrane and a cell wall. The cell wall is possessed of comparatively narrow pores which are impermeable to molecules such as dextran with a moleculer weight higher than about 4500 [7]. The pores are large enough to permeate small molecules such as inorganic ions, amino acids and sugars. The barrier of permeation to such small molecules is the cytoplasmic membrane. As an electrical model of yeast cells, we assume a conducting particle covered with two shells corresponding to the cytoplasmic membrane and the cell wall: the former is less conductive, the latter more conductive. The model is schematically shown in Fig. 4*a*. If the cell wall has nearly the same conductivity and dielectric constant as those of the suspending medium, the electrical model of the cells may be simplified to a conducting particle covered with a less conducting shell as shown in Fig. 4*b*. In such a case the present data on dielectric dispersion can be analyzed by means of the procedure which was proposed by Hanai *et al.* [8] for a single-shell sphere.

As reported by Carstensen, Cox, Mercer and Natale [2] for *E. coli* and *M. lysodeikticus*, conductivities of the cell wall κ_w show behavior similar to that of ion-exchange resin membranes, of which the conductivity is increased in proportion to that of the outer medium κ_a at higher salt concentration of the medium. In order to examine the relationship of κ_w to κ_a in the present yeast suspensions, we shall derive a relation among κ_w, κ_a and low frequency-limiting conductivity κ_l. If the discussion is confined to characteristics at low frequencies, the effective model of the

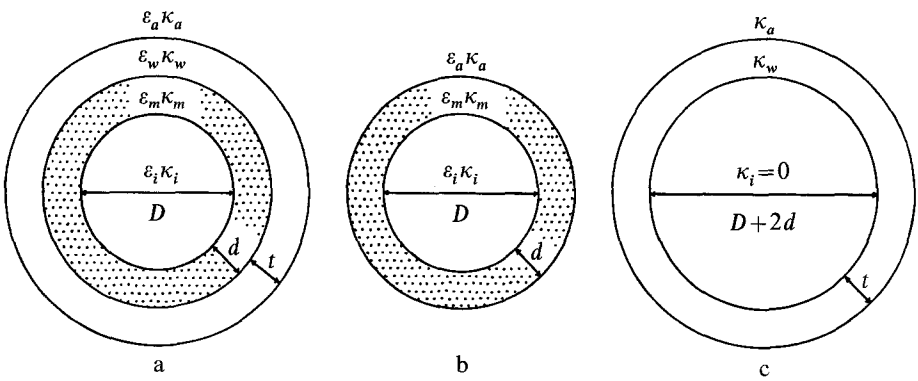

Fig. 4. *a.* An electrical model of yeast cells taking into consideration both cell membrane and cell wall. *b.* An electrical model of the cell having no regard for cell wall. *c.* An effective electrical model of the cell on conductivity at low frequencies

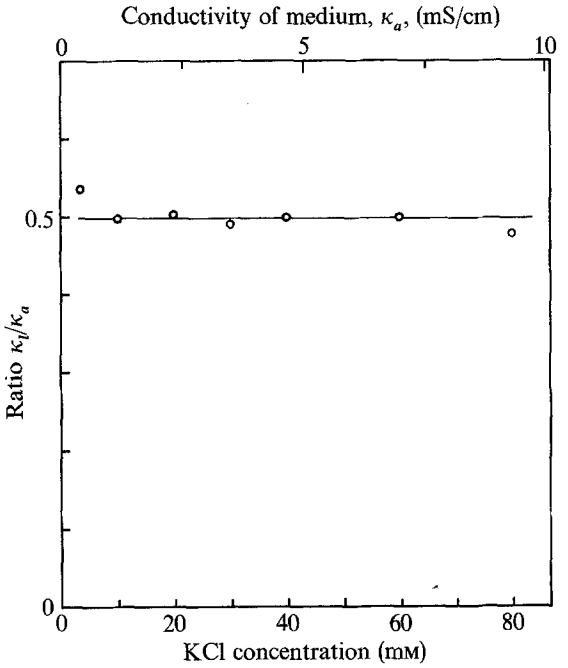

Fig. 5. Plots of κ_l/κ_a versus KCl concentration and conductivity of suspending medium. Osmotic moles are kept constant (150 mosM) by addition of sorbitol. Volume fractions are kept constant ($p \approx 0.4$)

whole cell (with respect to conductivity at low frequencies) is considered to be composed of a nonconducting sphere covered with a conducting shell (Fig. 4*c*). For this model, the ratio κ_l/κ_a is given by the following Eq. (1), which is derived from Eq. (2) (shown in a succeeding section) by substituting $\kappa_i = 0$ and replacing κ_w by κ_m.

$$\frac{\kappa_l}{\kappa_a} = \frac{2(1+2\Phi)(1-V)\kappa_w/\kappa_a + 2(1-\Phi)(2+V)}{2(1-\Phi)(1-V)\kappa_w/\kappa_a + (2+\Phi)(2+V)} \tag{1}$$

where

$$V = \left(\frac{D+2d}{D+2d+2t}\right)^3.$$

When Φ and v are kept unchanged in Eq. (1), a change in κ_l/κ_a reflects only that in κ_w/κ_a. Fig. 5 shows the ratio κ_l/κ_a of suspensions at the same volume fraction plotted as a function of KCl concentration of the suspending medium. In the KCl concentrations ranging from 10 to 80 mM, the ratio κ_l/κ_a appears to be constant irrespective of κ_a. Thus, the ratio κ_w/κ_a

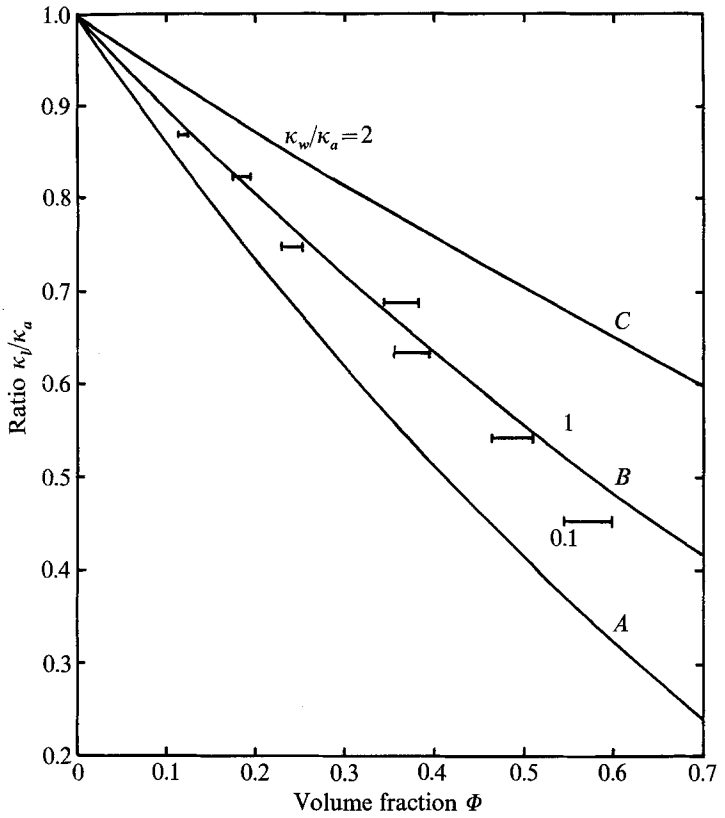

Fig. 6. Plots of κ_l/κ_a versus Φ. For each suspension, the volume fraction Φ was calculated from average cell volume and cell number, which was obtained by photomicroscopic observation and by Cell Counter, respectively. The curves are calculated from Eq. (1) with $\kappa_w/\kappa_a=0.1$ for Curve *A*, 1 for Curve *B* and 2 for Curve *C*. The suspending medium is 40 mM KCl solution

can be regarded as constant throughout the concentration range studied, κ_w being proportional to κ_a.

Next we observed the dependence of κ_l/κ_a on volume fraction Φ at a fixed κ_a (40 mM KCl solution), the results being shown in Fig. 6. The observed values fit, within experimental errors, to a curve which is drawn according to Eq. (1) for a case where $\kappa_w/\kappa_a=1$. It may be concluded from these results that κ_w is nearly the same value as κ_a at the KCl concentrations ranging from 10 to 80 mM. The model of the single-shell sphere shown in Fig. 4*b* can be accepted, therefore, in such a range of KCl concentration as far as ε_w is the same order of magnitude as ε_a or ε_m.

Electrical Phase Parameters of Yeast Cells

Since yeast cells suspended in KCl solutions of 10 to 80 mM may be regarded as a sphere covered with a nonconducting shell, as discussed in

the preceding section, the present dielectric data of yeast suspensions may be treated with the procedure [8] based on a general expression of Pauly and Schwan's theory given by

$$\frac{\varepsilon_a^* - \varepsilon^*}{2\varepsilon_a^* + \varepsilon^*} = \frac{(\varepsilon_a^* - \varepsilon_m^*)(2\varepsilon_m^* + \varepsilon_i^*) + (\varepsilon_a^* + 2\varepsilon_m^*)(\varepsilon_m^* - \varepsilon_i^*)(1 + 2d/D)^{-3}}{(2\varepsilon_a^* + \varepsilon_m^*)(2\varepsilon_m^* + \varepsilon_i^*) + 2(\varepsilon_a^* - \varepsilon_m^*)(\varepsilon_m^* - \varepsilon_i^*)(1 + 2d/D)^{-3}} p. \quad (2)$$

The phase parameters, C_M, ε_m, ε_i and κ_i, obtained by the analysis of the data in Figs. 1 and 3 are listed in Tables 1 and 2 respectively. Morphological parameters of yeast cells employed in this analysis are described in *Materials and Methods*. A value of 50 Å was assumed as the thickness of the cytoplasmic membranes. The membrane capacitance C_M was calculated from ε_m and d by means of the following equation, which was derived on the assumption that $d/D \ll 1$:

$$C_M = \frac{\varepsilon_0 \varepsilon_m}{d}. \quad (3)$$

As pointed out by Hanai *et al.* [8], the values of C_M were not affected seriously by varying the value of d, while ε_m was directly dependent on d.

From Table 1 it is readily seen that C_M, ε_m, and κ_i of yeast cells remain unchanged regardless of dilution and change in volume fraction of the suspension, except for the value of C_M in Specimen 1-*A*. These results seem to be reasonable, because these parameters are supposed to be intrinsic in the cell itself and independent of volume fraction. For the variation of salt concentration in the outer medium, κ_i shows a tendency to increase slightly with increasing conductivity of the medium, while C_M, ε_m and ε_i are kept unchanged.

A mean value 1.1 ± 0.1 μF/cm^2 estimated for the specific membrane capacitance of yeast cells is consistent with those of most biological cells so far reported with values of about 1 μF/cm^2. The only available values of membrane thickness of yeast cells are obtained by electronmicroscopic studies. According to Vitols *et al.* [18], cytoplasmic membranes of yeast cells are composed of two dense layers 20 to 25 Å thick, separated by a less dense layer of the same thickness. Probably the three layers may be regarded as a hydrophilic-hydrophobic-hydrophilic sandwich structure of the membrane. Provided that the thickness of the hydrophobic layer corresponds to dielectric thickness of the membrane, dielectric constants of the membranes are estimated to be 2.6 to 3.2 by using the values of 20 to 25 Å for the less dense layer in the membrane of yeast cells. This value of dielectric constant is consistent with dielectric constant 2–3 estimated for hydrocarbon.

Fettiplace, Andrews and Haydon [3] discussed the difference between the capacitances of biological cell membranes ($\sim 1\ \mu F/cm^2$) and those of bilayer lipid membranes (BLM) ($0.4–0.6\ \mu F/cm^2$). In order to explain the thin dielectric thickness of biological membranes, they proposed a model of the stretched leaflet, in which hydrocarbon chains interact with non-polar residues of protein. Another possible explanation for the difference is the increase in effective dielectric constant by the presence of proteins immersed in the hydrocarbon region of membranes.

Electrical conductivity of an aqueous phase inside the cells can not be discussed directly from κ_i, because yeast cells contain intracellular organelles. For the specimens listed in Tables 1 and 2, a remarkable increase in conductivity of the suspending medium was observed by treating the yeast cells with heat, detergents and repetition of freezing and thawing. This increase apparently resulted from ion leakage from the interior of cells to the outer medium. Hence the ionic concentration of the aqueous phase in the cell is considered to be higher than that of the suspending medium. The measured κ_i is nevertheless lower than κ_a except for Specimen 2–*A* in Table 2. These results imply that ionic mobility in the aqueous phase inside the cells is considerably low as compared with that of the outer medium, and/or that the cells contain some regions which are not available for the ionic movements.

No change in κ_i is observed with the variation of salt concentration of the outer medium. The ionic concentration in the aqueous phase inside the cells, therefore, seems to be kept unchanged throughout this experimental condition. Similar results were reported for living cells such as pleuropneumonia-like organisms [15], chlorella [9] and lymphoma cells (A. Irimajiri, *unpublished observations*). On the other hand, for isolated subcellular organelles such as mitochondria [11, 12], and synaptosomes [10], κ_i varied in proportion to κ_a.

The dielectric constant of an inner phase of the yeast cells is always lower than the suspending medium. This fact implies that the parts having low dielectric constant are included in the cell. These parts are probably membranous organelles, lipid granules and proteins.

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