DIELECTRIC ANALYSIS OF *ESCHERICHIA COLI* SUSPENSIONS IN THE LIGHT OF THE THEORY OF INTERFACIAL POLARIZATION

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ABSTRACT Dielectric measurements of *Escherichia coli* suspensions were carried out over a frequency range from 10 kHz to 100 MHz, and marked dielectric dispersions having characteristic frequency of ~1 MHz were observed. On the basis of the cell model that a spheroid is covered with two confocal shells, a dielectric theory was developed to determine accurately four electrical parameters for *E. coli* cells such as the conductivity of the cell wall, the dielectric constant of the cell membrane, and the dielectric constant and the conductivity of the protoplasm. The observed data were analyzed by means of the procedure based on the dielectric theory to yield a set of plausible electrical parameters for the cells. By taking account of the size distribution of the cells and a dielectric relaxation of the protoplasm, the observed dispersion curves were successfully reconstituted by the present theory.

INTRODUCTION

Dielectric measurements on suspensions of biological cells have provided valuable information about passive electrical properties of compartments of the cells, such as capacitances of cellular membranes, dielectric constants and conductivities of interiors of cells, and conductivities of cell walls (1, 2, 3). To obtain reliable values for compartments of cells, it is necessary to develop a more elaborate dielectric theory based on a realistic cell model.

For a dielectric model consisting of spheres covered with shells, Pauly and Schwan (4) derived a complete expression to give frequency dependence of the dielectric constant and the conductivity of the suspensions from the equations first quoted by Maxwell (5). On the basis of the general equation of Pauly and Schwan, Hanai et al. (6) proposed a systematic procedure to analyze the observed dielectric data by means of computer calculation. Application of this analysis to the suspensions of synaptosomes (7) and yeast cells (8) have provided highly reliable values for the membranes and the interiors.

For spheroidal cells (e.g., human erythrocyte and *Escherichia coli*), Fricke (9, 10) derived equations of the conductivity and the capacity of the cell suspensions at low frequencies and later extended these equations to include the general case of ellipsoid (11). Thus, it is possible to calculate membrane capacitances of suspended cells in ellipsoidal shape. However, Fricke's equations were insufficient to estimate conductivities and dielectric constants of interiors of cells and to predict the frequency dependence of conductivities and dielectric constants for cell suspensions. In a previous paper (12), a dielectric theory for the suspension of ellipsoids

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covered with a single shell has been developed, being free from the limitations in Fricke's equations.

Dielectric measurements of *E. coli* suspensions were first made by Fricke and collaborators (13). They examined the dielectric dispersions at frequencies between 0.1 and 100 MHz, and suggested that the cell be regarded as a conducting ellipsoid covered with a less conductive surface membrane. According to their cell model, the capacitance of the membrane was estimated to be 0.7 $\mu F/cm^2$ by using the dielectric theory derived by Fricke (11).

Carstensen et al. (14) improved the electrical cell model for bacteria proposed by Fricke et al. to explain the conductivity of *E. coli* and *Micrococcus lysodeikticus* suspensions at low frequencies. In the cell model, the conductive shell corresponding to the cell wall was attached outside the less conductive surface membrane. It seems, therefore, reasonable to adopt an ellipsoid covered with two confocal shells as an electrical cell model of *E. coli.*

In this paper, the theory derived previously (12) is extended to the suspension of the ellipsoids covered with two confocal shells. By the use of the extended theory, the frequency dependence of dielectric constant and conductivity measured for *E. coli* suspensions is examined in detail and four electrical parameters of compartments of the cell (i.e., the conductivity of the cell wall, the dielectric constant of the membrane, and the dielectric constant and the conductivity of the protoplasm) are estimated.

MATERIALS AND METHODS

Cells

E. coli (K 38) were grown in shaken cultures at 38°C. The culture medium containing 10 g polypepton, 1 g yeast extract, and 8 g NaCl per liter was adjusted to pH 7.5. The harvested cells were washed once with the suspending medium, which contained 10–50 mM NaCl and 1 mM $MgCl_2$. Osmolarity of the suspending medium was adjusted to ~0.3 osM by addition of sorbitol. The cell suspensions were then allowed to stand for 1 h so as to attain an equilibrium between cell interior and exterior. Before dielectric measurements the cells were again washed with the same suspending medium.

Size Distribution of Cells

Cell size was determined with ~250 cells using a phase contrast microscope. The cells appeared to be rods or long prolate spheroids ranging from 1.2 to 5.2 μm in length and from 0.6 to 0.7 μm in diameter. The cells showed a distribution of length as shown in Fig. 1, while the diameter was almost uniform. The mean length and diameter were calculated to be 2.79 and 0.68 μm, respectively.

Determination of Volume Fraction

The volume fraction of the cells in suspension was determined by using very large molecules which were impenetrable to the cell wall. The method used was essentially the same as described by Carstensen et al. (14). Ficoll (Parmacia Fine Chemicals, Uppsala, Sweden; mol wt, 4×10^5) was chosen for the large tracer molecule, because Ficoll is harmless to biological cells and has no ionized groups. Ficoll concentration was determined by a polarimeter (Perkin-Elmer Model 241 MC, Perkin-Elmer Corp., Instrument Div., Norwalk, Conn.).

Dielectric Measurements

Dielectric constant and conductivity were measured with a TR-1C Transformer Ratio-Arm Bridge of Ando Electric Co., Ltd., and a 250-A RX-Meter of Boonton Electronics Corp., Parsippany, N.J., over a frequency range of 0.01–3 MHz and of 1–100 MHz, respectively. The measuring cell is a parallel plate

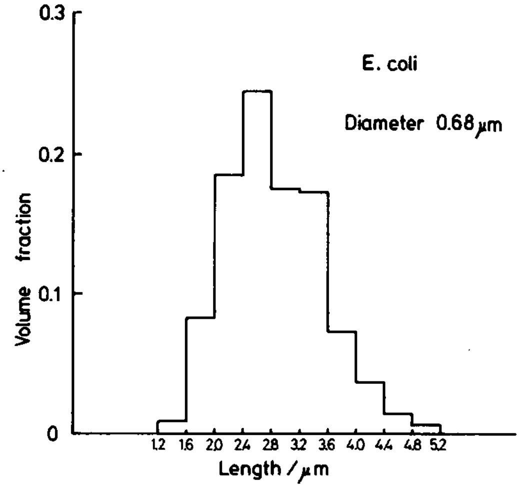

FIGURE 1 Size distribution of *E. coli* cells.

condenser consisting of Pt-coated Pt disk electrodes and a Lucite spacer, the cell constant being ~0.03 pF. The correction for the residual inductance arising from the measuring cell were made by Schwan's method (15).

Analysis of Dielectric Data

To analyze the dielectric behavior of *E. coli* suspensions the cell model of *E. coli* was assumed to be an ellipsoid (ϵ_p^*) covered with two confocal shells corresponding to the plasma membrane (ϵ_m^*) and the cell wall (ϵ_w^*) as shown in Fig. 2. The dielectric theory developed in a previous paper (12) for ellipsoids covered with a single shell can readily be extended to include the case of the cell model for *E. coli*. The derivation of the complex dielectric constant ϵ^* of the cell suspension is outlined in Appendix A. In addition, we take account of the heterogeneity of cell size. According to the principle of superposition, the equations shown in Appendix A are extended to allow for a system having n sizes of ellipsoids, so that the following equations were obtained. The symbols with subscript i refer to the ellipsoid of the i-th size.

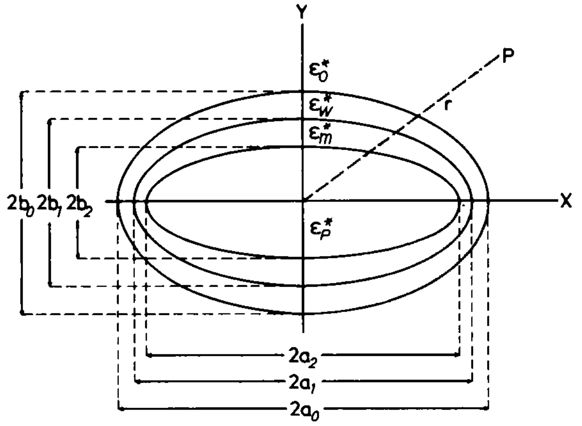

FIGURE 2 Cross section of an electrical cell model represented by three confocal ellipsoids in the x–y plane.

$$\frac{\epsilon^* - \epsilon_o^*}{\epsilon^* + 2\epsilon_o^*} = \frac{1}{9}\sum_i^n \Phi_i \sum_{k=x,y,z} \frac{\bar{\epsilon}_{1ki}^* - \epsilon_o^*}{\epsilon_o^* + (\bar{\epsilon}_{1ki}^* - \epsilon_o^*)A_{0ki}}, \tag{1}$$

$$\bar{\epsilon}_{1ki}^* = \epsilon_w^* \frac{\epsilon_w^* + (\bar{\epsilon}_{2ki}^* - \epsilon_w^*)A_{1ki} + \lambda_i(\bar{\epsilon}_{2ki}^* - \epsilon_w^*)(1 - A_{0ki})}{\epsilon_w^* + (\bar{\epsilon}_{2ki}^* - \epsilon_w^*)A_{1ki} - \lambda_i(\bar{\epsilon}_{2ki}^* - \epsilon_w^*)A_{0ki}}, \tag{2}$$

and

$$\bar{\epsilon}_{2ki}^* = \epsilon_m^* \frac{\epsilon_m^* + (\epsilon_p^* - \epsilon_m^*)A_{2ki} + \mu_i(\epsilon_p^* - \epsilon_m^*)(1 - A_{1ki})}{\epsilon_m^* + (\epsilon_p^* - \epsilon_m^*)A_{2ki} - \mu_i(\epsilon_p^* - \epsilon_m^*)A_{1ki}}, \tag{3}$$

$$(k = x, y, z),$$

with

$$\lambda_i = \frac{a_{1i}b_{1i}c_{1i}}{a_{0i}b_{0i}c_{0i}}, \tag{4}$$

$$\mu_i = \frac{a_{2i}b_{2i}c_{2i}}{a_{1i}b_{1i}c_{1i}}, \tag{5}$$

$$A_{ski} = \frac{a_{si}b_{si}c_{si}}{2}\int_0^\infty \frac{d\xi}{(t_{si} + \xi)D_{si}}, \tag{6}$$

$$D_{si} = \{(\xi + a_{si}^2)(\xi + b_{si}^2)(\xi + c_{si}^2)\}^{1/2}, \tag{7}$$

($t_{si} = a_{si}$, b_{si}, and c_{si} for $k = x, y$, and z, respectively; and $s = 0, 1, 2$).

The numerical calculation of these equations was carried out by using the following assumptions: (*a*) Thickness of the plasma membrane and the cell wall is negligibly small compared with the cell diameter and length, so that the axial ratios q_{1i} $(=a_{1i}/b_{1i})$ and $q_{2i}(=a_{2i}/b_{2i})$ are nearly equal to $q_{0i}(= a_{0i}/b_{0i})$. The depolarization factors, A_{0ki}, A_{1ki}, and A_{2ki}, which are functions of axial ratios only, nearly equal each other. (*b*) Since *E. coli* is a prolate spheroid with semiaxes $a_{0i} > b_{0i} = c_{0i}$, the depolarization factors A_{0xi} and A_{0yi} are given by

$$A_{0xi} = -\frac{1}{q_{0i}^2 - 1} + \frac{q_{0i}}{(q_{0i}^2 - 1)^{3/2}}\ln\{q_{0i} + (q_{0i}^2 - 1)^{1/2}\}, \tag{8}$$

$$A_{0yi} = A_{0zi} = \frac{1}{2}(1 - A_{0xi}). \tag{9}$$

(*c*) Although, in the present cell model, the shells represented by layers between two different surfaces of confocal ellipsoids have nonuniform thickness all over the cell surfaces, the values of λ_i and μ_i are approximately calculated from the following equations instead of Eqs. 4 and 5.

$$\lambda_i = \frac{(a_{0i} - d_w)(b_{0i} - d_w)^2}{a_{0i}b_{0i}^2}, \tag{10}$$

$$\mu_i = \frac{(a_{0i} - d_m - d_w)(b_{0i} - d_m - d_w)^2}{(a_{0i} - d_w)(b_{0i} - d_w)^2}, \tag{11}$$

where d_m and d_w are the thickness of plasma membrane and cell wall, respectively.

The electrical phase parameters of the cell can be evaluated by the analysis of the dielectric dispersion curve observed on the cell suspension. In practice, the phase parameters, κ_w, ϵ_m, ϵ_p and κ_p, are determined

TABLE I

MORPHOLOGICAL AND ELECTRICAL PARAMETERS OF CELLS EMPLOYED IN THE NUMERICAL ANALYSIS OF DIELECTRIC DATA OF *E. COLI* SUSPENSIONS

Morphological				Electrical	
Cellular diameter	Length*	Membrane thickness‡	Wall thickness‡	Membrane conductivity§	Wall dielectric‖ constant
$2a_\alpha$	$2b_\alpha$	d_m	d_w	κ_m	ϵ_w
μm	μm	Å	Å	mScm^{-1}	
0.68	1.2–5.2	50	200	5×10^{-7}	60

*The size distribution shown in Fig. 1.

‡Assumed values.

§This value corresponds to a specific membrane resistance of 1 $\mathrm{k\Omega cm}^2$ for a membrane of 50 Å in thickness.

‖A choice of ϵ_w between 80 and 20 made no discernible difference in the determination of the phase parameters.

by fitting the theoretical curve to the observed dispersion data with respect to the four dielectric parameters, κ_l, ϵ_l, ϵ_h and $f_{1/2}$, which are characteristic of the dispersion curve. The procedure for "curve fitting" is described in Appendix B. The morphological and the electrical parameters employed in the numerical analysis are listed in Table I. The values of κ_o and ϵ_o were determined with the supernate obtained by centrifuging after the dielectric measurements of the suspension.

SYMBOLS

Phase Parameters of the Cell Model

ϵ_o^* complex dielectric constant of outer phase, $\epsilon_o^* = \epsilon_o - j\dfrac{\kappa_o}{2\pi f\epsilon_v}$
- ϵ_o dielectric constant of outer phase
- κ_o conductivity of outer phase

ϵ_w^* complex dielectric constant of wall phase, $\epsilon_w^* = \epsilon_w - j\dfrac{\kappa_w}{2\pi f\epsilon_v}$
- ϵ_w dielectric constant of wall phase
- κ_w conductivity of wall phase

ϵ_m^* complex dielectric constant of membrane phase, $\epsilon_m^* = \epsilon_m - j\dfrac{\kappa_m}{2\pi f\epsilon_v}$
- ϵ_m dielectric constant of membrane phase
- κ_m conductivity of membrane phase

ϵ_p^* complex dielectric constant of protoplasm phase, $\epsilon_p^* = \epsilon_p - j\dfrac{\kappa_p}{2\pi f\epsilon_v}$
- ϵ_p dielectric constant of protoplasm phase
- κ_p conductivity of protoplasm phase

ϵ^* complex dielectric constant of suspension, $\epsilon^* = \epsilon - j\dfrac{\kappa}{2\pi f\epsilon_v}$
- ϵ dielectric constant of suspension
- κ conductivity of suspension

ϵ_v dielectric constant of free space
f frequency of applied a.c. field
j $(-1)^{1/2}$
Φ volume fraction of a cell suspension

Morphological Parameters of the Cell Model

a_0, b_0, c_0	semiaxes of ellipsoid outside cell wall
a_1, b_1, c_1	semiaxes of ellipsoid outside plasma membrane
a_2, b_2, c_2	semiaxes of ellipsoid inside plasma membrane
d_m	thickness of plasma membrane
d_w	thickness of cell wall

Dielectric Parameters of the Cell Suspension

ϵ_l	limiting value of dielectric constant at low frequencies
κ_l	limiting value of conductivity at low frequencies
ϵ_h	limiting value of dielectric constant at high frequencies
κ_h	limiting value of conductivity at high frequencies
$f_{1/2}$	characteristic frequency at which the dielectric constant assumes a value $(\epsilon_l + \epsilon_h)/2$

RESULTS

Fig. 3 *a* shows the frequency dependence of the dielectric constant and conductivity obtained with *E. coli* suspended in a medium containing 10 mM NaCl, 1 mM $MgCl_2$, and 300 mM sorbitol. The remarkable dielectric dispersion found above 0.1 MHz is similar to that reported by Fricke et al. (13), being assigned to the Maxwell-Wagner dispersion. The increase in dielectric constant below 0.1 MHz, which is not discussed here, is due to electrode polarization and/or α-dispersion (1, 16). By application of the analysis described above to the observed dielectric data, a set of plausible phase parameters (κ_w, ϵ_m, ϵ_p, and κ_p) of the cells and the theoretical dispersion curves were obtained. The solid curves were calculated from Eqs. 1, 2, and 3 by taking account of the distribution of the cell size shown in Fig. 1. The agreement of the theoretical curve with the experimental data is quite satisfactory except at higher frequencies.

Fig. 3 *b* shows a set of dielectric dispersion curves obtained with a suspension in a medium containing 50 mM NaCl, 1 mM $MgCl_2$, and 220 mM sorbitol. Volume fraction of the cells was similar to that of the specimen shown in Fig. 3 *a*. The solid curves calculated from the

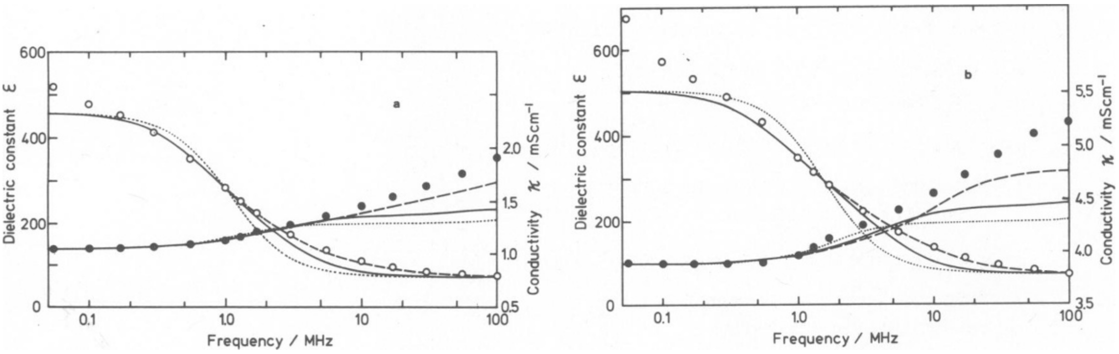

FIGURE 3 Frequency dependence of dielectric constant (○) and conductivity (●) for suspensions of *E. coli* in different mediums: (*a*) 10 mM NaCl, 1 mM $MgCl_2$, and 300 mM sorbitol; (*b*) 50 mM NaCl, 1 mM $MgCl_2$, and 220 mM sorbitol. Solid lines are the best-fit calculations of Eq. 1. Dashed lines are calculated by taking account of frequency dependence of ϵ_p and κ_p. Dotted lines show the best-fit dispersion curves for the spherical shell model.

TABLE II

DIELECTRIC PARAMETERS OF *E. COLI* SUSPENSIONS IN DILUTION SERIES AND ESTIMATED PHASE PARAMETERS OF *E. COLI* CELLS

Specimen	Dilution	ϵ_o	κ_o	κ_l	ϵ_l	ϵ_h	$f_{1/2}$	Φ	κ_w	ϵ_m	ϵ_p	κ_p	C_M
			$(mScm^{-1})$	$(mScm^{-1})$			(MHz)		$(mScm^{-1})$			$(mScm^{-1})$	(μFcm^{-2})
1A	1	74	2.56	1.91	525	71	1.4	0.26	7.3	9.5	67	1.7	1.68
									(7.5)	(11.9)	(66)	(1.2)	(2.10)
1B	3/4	74	2.51	2.06	420	71	1.4	0.20	7.9	9.4	63	1.7	1.66
									(8.3)	(11.8)	(61)	(1.2)	(2.09)
1C	2/4	74	2.40	2.19	320	71	1.3	0.13	12.7	10.6	58	1.9	1.88
									(14.2)	(14.1)	(58)	(1.2)	(2.49)
1D	1/4	74	2.34	2.20	215	73	1.2	0.07	10.2	10.4	67	1.8	1.84
									(11.2)	(13.5)	(66)	(1.2)	(2.39)

Membrane capacitances C_Ms were calculated from $C_M - \epsilon_m \epsilon_v/d_m$. The values in parentheses were determined on the basis of the spherical shell model.

theory showed deviations from the data observed in the high frequency range to an extent similar to the result shown in Fig. 3 *a*.

The data summarized in Tables II and III were obtained by systematic changes in NaCl concentration of the suspending medium and cell concentration of the suspensions. The limiting values of dielectric constant ϵ_l and conductivity κ_l at low frequencies were strongly dependent on the cell concentration or the volume fraction of the suspensions. The values of ϵ_l were estimated by the complex plane plots extrapolated to low frequencies. The limiting values of dielectric constant ϵ_h at high frequencies were slightly low compared with the dielectric constant of the suspending medium. The value of characteristic frequency $f_{1/2}$ increased with the increase in NaCl concentration of the medium.

From Table II, it is readily seen that the estimated values of ϵ_m, ϵ_p, κ_p, and C_M remained unchanged regardless of dilution and change in volume fraction of the suspensions. The values of κ_w showed some scatter, but were not systematically dependent on the volume fraction of the suspension. This result seems to be reasonable, because these phase parameters are supposed to be inherent in the cell itself and independent of the volume fraction. It should be noted in Table III that the change in NaCl concentration from 10 to 50 mM of the outer

TABLE III

DIELECTRIC PARAMETERS OF *E. COLI* SUSPENSIONS IN VARYING NACL CONCENTRATIONS OF SUSPENDING MEDIUM AND ESTIMATED PHASE PARAMETERS OF *E. COLI* CELLS

Specimen	NaCl concentration	ϵ_o	κ_o	κ_l	ϵ_l	ϵ_h	$f_{1/2}$	Φ	κ_w	ϵ_m	ϵ_p	κ_p	C_M
	(mM)		$(mScm^{-1})$	$(mScm^{-1})$			(MHz)		$(mScm^{-1})$			$(mScm^{-1})$	(μFcm^{-2})
2A	10	74	1.21	1.06	460	71	1.1	0.21	6.8	10.8	61	1.9	1.91
									(7.6)	(14.4)	(61)	(1.3)	(2.55)
2B	30	74	3.15	2.56	490	72	1.4	0.19	9.3	11.4	67	2.1	2.02
									(9.6)	(14.3)	(65)	(1.4)	(2.53)
2C	50	73	5.00	3.89	505	72	1.6	0.20	9.6	11.1	65	2.1	1.97
									(8.9)	(13.5)	(64)	(1.5)	(2.39)

Membrane capacitances C_Ms were calculated from $C_M - \epsilon_m \epsilon_v/d_m$. The values in parentheses were determined on the basis of the spherical shell model.

medium exerted no significant influence on the values of the protoplasm (ϵ_p, κ_p) and the membrane (ϵ_m).

To judge the validity of the present analysis based on the ellipsoidal shell model including size distribution of the cells, it seems helpful to compare the results of this analysis to those obtained with a spherical shell model of a single cell size. Numerical analysis for the spherical shell model was also based on the present theory. Substituting $n = 1$ and $a_s = b_s = c_s$ in Eqs. 1, 2, and 3, yields the general equations so far proposed for the spherical cell model with two shells (17, 18). The phase parameters were obtained by means of the curve fitting procedure shown in Appendix B. The used diameter of the spherical cell is calculated to be 1.09 μm from the mean cell volume observed for *E. coli.* The other morphological parameters and the electrical parameters are the same as those shown in Table I. The resulting phase parameters are listed in parentheses in Tables II and III. Remarkable differences are found between the two models with respect to the values of κ_w, ϵ_m, κ_p, and C_M as seen in the Tables. The best-fit dispersion curves for the spherical shell model are depicted by the dotted lines in Fig. 3, being close to dispersion curves of the Debye type. It is clear that the ellipsoidal shell model provides a better representation of the observed dispersion curves than the spherical shell model.

DISCUSSION

On analyzing the dielectric behavior of *E. coli* suspensions, the present dielectric theory has the following advantages over Fricke's theory (9–11): (*a*) A realistic cell model for *E. coli* is adopted, i.e., an ellipsoid covered with two shells corresponding to the cell membrane and the cell wall. (*b*) Frequency dependence of dielectric constant and conductivity can be predicted for the cell suspensions. (*c*) Heterogeneity in size of the cells in the suspension can be taken into account in the present theory. These advantages enable quantitative analysis of the whole profile of the dispersion curves, so that electrical parameters of the cell compartments can be determined with accuracy.

The observed dielectric dispersion curves for *E. coli* suspensions, which extended over a wider range of frequency than those of a simple Debye type, were simulated satisfactorily with the present theory by taking account of the size distribution of the cells. The broadening of the dispersion curves results from two kinds of distributions of relaxation times: one is inherent in the ellipsoidal cell model and the other is due to size distribution of the cells.

Next, we shall consider the remaining deviation of the calculated values of dielectric constant and conductivity from the observed at the higher frequency. The dielectric theory employed in the analysis of data of *E. coli* suspension was constructed on the assumption that the phase parameters of cells are independent of frequency. An attempt is made here to reexamine this assumption.

Einolf et al. (16) reported dielectric measurements with protoplasts of *M. lysodeikticus* together with intact cells, suggesting that the removal of the cell wall had hardly any influence on the dielectric dispersion in the radio-frequency range except to decrease the conductivity of the suspension at low frequencies. It is inferred from their result that there is little possibility of frequency dependence of ϵ_w and κ_w in the frequency range in question.

It appears that present knowledge of frequency dependence of membrane capacitance is still limited. Cole (2) derived a concept of membranes with a frequency-independent electrical phase angle from the evidence that impedance loci of cell suspension and tissues show circular arcs. Recently Takashima and Schwan (19) measured transmembrane admittance with squid axon using an internal electrode and found a dielectric dispersion of membrane itself in the frequency range of 0.5–10 kHz. However, we have no reliable information on the dielectric dispersion in the high frequency range (1–100 MHz) in question. We reserve mention of frequency dependence of membrane capacitance as well as cell wall.

In the high frequency region, the dispersion curves calculated from Eqs. 1, 2, and 3 are susceptible to changes in the values of ϵ_p and κ_p. Irimajiri et al. (20) made it possible to reproduce the dielectric dispersion curves obtained with lymphoma cells by considering Maxwell-Wagner dispersion due to a large nucleus in the cell interior. On the other hand, the protoplasm of *E. coli* contains neither nucleus nor any other membranous organelles,[21] being regarded as a concentrated solution of proteins. In general, the frequency range of dielectric dispersion of proteins is between 100 kHz and 50 MHz, though most of the measurements were carried out with dilute protein solutions containing traces of salts. Shaw et al. (22) investigated the dielectric properties of β-lactoglobulin at high concentrations comparable to the protein concentration in protoplasm of *E. coli,* describing marked dielectric dispersion with a magnitude >100 in dielectric unit. Therefore, an attempt should be made to take account of the frequency dependence of ϵ_p and κ_p.

If the dielectric dispersion due to proteins is assumed to be of the Debye type, the complex dielectric constant of protoplasm is rewritten as

$$\epsilon_p^* = \epsilon_{ph} + \frac{\epsilon_{pl} - \epsilon_{ph}}{1 + j(f/f_{p0})} + \frac{\kappa_{pl}}{j2\pi f \epsilon_v}, \tag{12}$$

where ϵ_{ph} is the limiting value of ϵ_p at high frequencies, ϵ_{pl} and κ_{pl} are the limiting values of ϵ_p and κ_p at low frequencies, f_{p0} is the characteristic frequency of the dielectric dispersion. By the substitution of Eq. 12 for ϵ_p^* in Eq. 3, the dielectric dispersion curves calculated before are modified by varying the values of the dielectric parameters ϵ_{ph}, ϵ_{pl}, κ_{pl}, and f_{p0}. For the values of ϵ_{ph} and κ_{pl}, we use the values of ϵ_p and κ_p estimated on the assumption of frequency-independent phase parameters of cells, because, in the analysis of the observed dispersion curves, ϵ_p and κ_p were determined for the limiting values of ϵ at high frequencies ($\simeq$100 MHz) and for the values of ϵ at $f_{1/2}$ ($\simeq$1 MHz), respectively. The values of $\Delta\epsilon_p(= \epsilon_{pl} - \epsilon_{ph})$ and f_{p0} were chosen for best fit of the observed dispersion curve for ϵ. The broken curves shown in Fig. 3 were obtained with $\Delta\epsilon_p$ of 300 and f_{p0} of 10 MHz. It is found that the agreement with experimental data for ϵ is quite satisfactory and that the difference between the theoretical and the experimental values of κ is fairly reduced. The estimated value of $\Delta\epsilon_p$ is plausible in view of the protein concentration of ~15% in the protoplasm of *E. coli,* and the estimated value of f_{p0} is in the range of characteristic frequencies for the dielectric dispersion of protein solutions. Therefore, it seems reasonable to assume that the frequency dependence of ϵ_p and κ_p is due to the dielectric dispersion of proteins.

Assuming the relaxation of ϵ_p^* to be of the Debye type, the limiting value of κ_p at high frequencies, κ_{ph}, for *E. coli* is calculated from Eq. 12 by using $\Delta\epsilon_p$ of 300 and f_{p0} of 10 MHz and mean value 1.9 mS/cm estimated for $\kappa_p(= \kappa_{pl})$. The obtained value of κ_{ph} is 3.6 mS/cm. Carstensen (17) carried out dielectric measurements for the suspensions of *E. coli* Cl (spherical cell) and estimated the conductivity of the protoplasm to be 3.1 mS/cm from the value of κ_h. This value which corresponds to κ_{ph} is compared with the values of κ_{ph} calculated from our data.

The value of κ_p listed in Table III remained unchanged in the variation of NaCl concentration of the suspending medium from 10 to 50 mM. Similar results were reported for living cells such as pleuropneumonia-like organisms (23), lymphoma cells (20), and yeast (8).

The estimated value of ϵ_p shown in Tables I and II, which corresponds to ϵ_{ph}, always shows a low dielectric constant compared with the suspending medium. This finding is in accord with the fact that the presence of protein decreases dielectric constants of the solution at high frequencies.

The obtained values of κ_w were always higher than those of κ_o despite variation of NaCl concentration in the suspending medium from 10 to 50 mM. The behavior of κ_w is similar to that reported by Carstensen et al. (14). They suggested that the cell walls were highly conductive owing to the presence of mobile counterions for the fixed charges in the wall phase.

As discussed in the previous paper (12), the value of the membrane capacitance C_M may be estimated to be larger than those of actual plasma membranes, because the shells of the present cell model have nonuniform thickness all over the cell surfaces. Thus, for accurate estimation of the dielectric constant of the plasma membrane, it is desirable to develop a theory for the cell model with a shell of uniform thickness.

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APPENDIX A

The cell model as shown in Fig. 2 may be approximated by three confocal ellipsoids given by:

$$\frac{x^2}{a_0^2+\xi}+\frac{y^2}{b_0^2+\xi}+\frac{z^2}{c_0^2+\xi}=1, \tag{A1}$$

where ξ is a parameter representing a family of confocal ellipsoids. When $\xi = 0$, Eq. A1 describes the standard ellipsoid with semiaxes a_0, b_0, and c_0.

We shall consider the electric potential ψ in the system that the shelled ellipsoid is placed in an external homogeneous field $E(E_x, E_y, E_z)$. The potential is given by a solution of Laplace's equation with the following boundary conditions: At a point remote infinitely from the shelled ellipsoid, the potential is equal to that of the original field. At the surfaces of the three confocal ellipsoids the continuity of both the potential and the normal component of the electric displacement is held. The potential ψ_o at a point P outside the shelled ellipsoid is given by

$$\begin{aligned}\psi_o = &- E_x x\left[1-\frac{\bar{\epsilon}_{1x}^*-\epsilon_o^*}{\epsilon_o^*+(\bar{\epsilon}_{1x}^*-\epsilon_o^*)A_{0x}}A'_{0x}\right]\\ &- E_y y\left[1-\frac{\bar{\epsilon}_{1y}^*-\epsilon_o^*}{\epsilon_o^*+(\bar{\epsilon}_{1y}^*-\epsilon_o^*)A_{0y}}A'_{0y}\right]\\ &- E_z z\left[1-\frac{\bar{\epsilon}_{1z}^*-\epsilon_o^*}{\epsilon_o^*+(\bar{\epsilon}_{1z}^*-\epsilon_o^*)A_{0z}}A'_{0z}\right],\end{aligned} \tag{A2}$$

where $\bar{\epsilon}^*_{1k}$ is the effective, homogeneous, complex conductivity of the shelled ellipsoid along the $k(=x, y, z)$ axis:

$$\bar{\epsilon}^*_{1k} = \epsilon^*_w \frac{\epsilon^*_w + (\bar{\epsilon}^*_{2k} - \epsilon^*_w)A_{1k} + \lambda(\bar{\epsilon}^*_{2k} - \epsilon^*_w)(1 - A_{0k})}{\epsilon^*_w + (\bar{\epsilon}^*_{2k} - \epsilon^*_w)A_{1k} - \lambda(\bar{\epsilon}^*_{2k} - \epsilon^*_w)A_{0k}}, \tag{A3}$$

and

$$\bar{\epsilon}^*_{2k} = \epsilon^*_m \frac{\epsilon^*_m + (\epsilon^*_p - \epsilon^*_m)A_{2k} + \mu(\epsilon^*_p - \epsilon^*_m)(1 - A_{1k})}{\epsilon^*_m + (\epsilon^*_p - \epsilon^*_m)A_{2k} - \mu(\epsilon^*_p - \epsilon^*_m)A_{1k}}, \tag{A4}$$

with

$$\lambda = \frac{a_1 b_1 c_1}{a_0 b_0 c_0}, \tag{A5}$$

and

$$\mu = \frac{a_2 b_2 c_2}{a_1 b_1 c_1}. \tag{A6}$$

Here, A_{0k}, A_{1k}, and A_{2k} are the depolarization factors along the $k(=x, y, z)$ axis, i.e.,

$$A_{sk} = \frac{a_s b_s c_s}{2} \int_0^\infty \frac{\mathrm{d}\xi}{(t_s^2 + \xi)D_s}, \tag{A7}$$

where subscript $s = 0, 1, 2$; $t_s = a_s$, b_s, and c_s for $k = x$, y, and z, respectively; and $D_s = [(\xi + a_s^2)(\xi + b_s^2)(\xi + c_s^2)]^{1/2}$.

The magnitude A'_{0k} in Eq. A2 is a function of the coordinate ξ and is determined by the equation:

$$A'_{0k} = \frac{a_0 b_0 c_0}{2} \int_\xi^\infty \frac{d\xi}{(t_0^2 + \xi)D_s}. \tag{A8}$$

When the distance r between the point P and the origin of the coordinates is much greater than the length of each semiaxis of the ellipsoid (i.e., $r \gg a_0, b_0, c_0$), A'_{0k} is reduced in the following form:

$$A'_{0k} \approx \frac{a_0 b_0 c_0}{3} \frac{1}{r^3}. \tag{A9}$$

Next we shall derive the equation of the complex dielectric constant ϵ^* for the homogeneous suspension of the shelled ellipsoids oriented at random. Let us consider a wide spherical region with radius R containing N shelled ellipsoids. To obtain the potential due to N ellipsoids oriented at random, we calculate the average potential $\langle\psi_o\rangle$ of the potential ψ_o over all possible orientations.

$$\langle\psi_o\rangle = -E\zeta + \frac{1}{3}\frac{a_0 b_0 c_0}{3}\frac{1}{r^3} \sum_{k=x,y,z} \frac{\bar{\epsilon}^*_{1k} - \epsilon^*_o}{\epsilon^*_o + (\bar{\epsilon}^*_{1k} - \epsilon^*_o)A_{0k}} E\zeta, \tag{A11}$$

where ζ-axis is in parallel to the electric field E. By assuming that the interaction between the suspended ellipsoids is negligible, the potential due to the N shelled ellipsoids inside the wide spherical region is given by

$$\langle\psi_o\rangle_N = -E\zeta + \frac{1}{3} N \frac{a_0 b_0 c_0}{3}\frac{1}{r^3} \sum_{k=x,y,z} \frac{\bar{\epsilon}^*_{1k} - \epsilon^*_o}{\epsilon^*_o + (\bar{\epsilon}^*_{1k} - \epsilon^*_o)A_{0k}} E\zeta. \tag{A12}$$

If the spherical region containing N particles has a homogenous complex dielectric constant ϵ^*, the potential $\psi_{o\epsilon^*}$ due to the spherical region is given by Wagner (16) as follows:

$$\psi_{o\epsilon^*} = -E\zeta + \frac{R^3}{r^3}\frac{\epsilon^* - \epsilon_o^*}{\epsilon^* + 2\epsilon_o^*}E\zeta. \tag{A13}$$

Equating Eq. A12 to Eq. A13, we have an equivalent complex dielectric constant ϵ^* of the suspension as:

$$\frac{\epsilon^* - \epsilon_o^*}{\epsilon^* + 2\epsilon_o^*} = \frac{1}{9}\Phi \sum_{k=x,y,z} \frac{\bar{\epsilon}_{1k}^* - \epsilon_o^*}{\epsilon_o^* + (\bar{\epsilon}_{1k}^* - \epsilon_o^*)A_{0k}}, \tag{A14}$$

where

$$\Phi = \frac{Na_0b_0c_0}{R^3}. \tag{A15}$$

APPENDIX B

To establish the procedure to evaluate the electrical phase parameters of suspended cells from the observed dispersion curves on the basis of the ellipsoidal cell model, it seems helpful to examine the behavior of the characteristic dielectric parameters caused by individual changes in phase parameters according to the present theory. Computation was made, for simplicity, on a disperse system consisting of uniform spheroidal cells. Table IV shows the relative variation in the dielectric parameters from the reference state when one of the phase parameters decreases by 50%. The parameters comprising the reference state have been chosen from the specimens in Tables II and III obtained in Results. Table IV indicates the following characteristics which are available for finding a procedure of determination of the phase parameters: (*a*) The values of ϵ_w and κ_m have little influence on all the dielectric parameters. (*b*) The six terms enclosed by broken line are vanishingly small.

Further calculation confirms that these characteristics are found over wide ranges of the phase

TABLE IV
RESPONSE OF CHARACTERISTIC DIELECTRIC PARAMETERS TO INDIVIDUAL CHANGES (−50%) IN PHASE PARAMETERS

Case	Phase parameter	Variation from values for the reference state,* dielectric parameter			
		κ_l	ϵ_l	ϵ_h	$f_{1/2}$
		(%)	(%)	(%)	(%)
1	κ_w	−5.23	6.16	−0.00	2.29
2	ϵ_m	0.00	−44.60	−0.91	91.61
3	ϵ_p	0.00	0.00	−9.56	1.99
4	κ_p	0.00	−0.01	0.00	−45.34
5	ϵ_w	−0.00	−0.11	−1.13	0.10
6	κ_m	0.00	0.01	0.00	0.00

*The reference state is specified: $\epsilon_o = 80$, $\kappa_o = 2.5$ mS/cm, $\epsilon_w = 60$, $\kappa_w = 10.0$ mS/cm, $\epsilon_m = 10$, $\kappa_m = 5 \times 10^{-7}$ mS/cm (membrane resistance of 1 kΩcm^2), $\epsilon_p = 70$, $\kappa_p = 2.0$ mS/cm, $d_m = 50$ Å, $d_w = 200$ Å, $a_0 = 1.5$ μm, $b_0 = c_0 = 0.5$ μm, $\Phi = 0.2$.

parameters: $\kappa_o = \kappa_w = \kappa_p = 0.1\text{–}100$ mScm^{-1}, $\epsilon_w = 10\text{–}80$, $\epsilon_m = 3\text{–}80$, $\epsilon_p = 3\text{–}500$, $\kappa_m < \kappa_o \times 10^{-5}$, $d_m =$ 50–200 Å, $d_w =$ 50–1000 Å. Item *a* suggests that the estimation of ϵ_w and κ_m is difficult and that a choice of ϵ_w between 10 and 80 and κ_m between 0 and $\kappa_o \times 10^{-5}$ makes no discernible difference in the determination of the other phase parameters. Thus, the determination procedure is restricted to the phase parameters except for ϵ_w and κ_m. The item (*b*) makes it possible to determine a unique set of values for κ_w, ϵ_m, ϵ_p, and κ_p through the following five steps, because the step in operation has less influence on the results of the preceding steps.

Step 1. To put temporarily phase parameters as:

$$\kappa_w = \kappa_o, \epsilon_m = 3, \epsilon_p = \epsilon_o, \kappa_p = \kappa_o, \epsilon_w = 60, \kappa_m \simeq 0.$$

Step 2. To start computation and search out a proper value of κ_w so that the calculated value of κ_l may fit in with the observed value of κ_l.

Step 3. To search out ϵ_m so that the calculated ϵ_l may fit in with the observed ϵ_l.

Step 4. To search out ϵ_p so that the calculated ϵ_h may fit in with the observed ϵ_h.

Step 5. To search out κ_p so that the calculated $f_{1/2}$ may fit in with the observed $f_{1/2}$.

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