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Dielectric Properties of Yeast Cells: Effect of Some Ionic Detergents on the Plasma Membranes

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Summary. Dielectric measurements were made on suspensions of yeast cells treated with two homologous series of sodium alkyl (C_8, C_{10}, C_{12}, C_{14}) sulfonates and alkyl (C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}) benzyl dimethyl ammonium chlorides over a frequency range of 10 kHz to 100 MHz. Dielectric dispersions observed for the suspensions of intact yeast cells are found to be reduced by treatment with these detergents, the reduction being accompanied by a decrease in packed volume of the cells and by a leakage of intracellular compounds. The reduction of dielectric dispersions is considered to be caused by a decrease in volume of the cells in suspensions and an increase in conductivity of the cell membranes. An effect of the alkyl chain length of the detergents on the reduction of dielectric dispersions is also examined for these ionic detergents. The reducing effect shows the maximum at the alkyl chain C_{14} for sodium alkyl sulfonates and at C_{16} for alkyl benzyl dimethyl ammonium chlorides. These results are consistent with hemolysis and bactericidal activity.

It is known from many examples [8] that suspensions of biological cells show marked dielectric dispersions due to the Maxwell-Wagner mechanism, because the cells are covered with poorly conducting surface membranes. An increase in electrical conductivity of cell membranes gives rise to a reduction of the dielectric dispersions, so that the dielectric approach is useful to detect the change in membrane conductivity and the destruction of membranes caused by treatments with detergents, organic solvents, heat, freezing and thawing, and so on. However, little work has so far been reported on the effects of such treatments on the dielectric dispersions. The dielectric approach is particularly effective for cells covered with cell walls, since changes in dielectric properties of cell membranes can readily be examined without removing cell walls owing to little contribution of the cell walls to the dielectric dispersion of cell suspensions.

In a previous paper [1], the authors reported typical dielectric dispersions of intact yeast cell suspensions and elucidated dielectric properties of walls, membranes and inner phases of the cells. In the present study, dielectric behavior of suspensions of yeast cells treated with two homologous series of ionic detergents is observed over a frequency range of 10 kHz to 100 MHz. It is found that a reduction of dielectric dispersions is accompanied by a volume change of the cells and an outflow of intracellular compounds. An effect of the alkyl chain length of the detergents on the dielectric dispersion is discussed in connection with hemolysis and bactericidal activity.

Materials and Methods

Yeast cells (*Saccharomyces cerevisiae*) of the same strain as studied previously [1] were grown in nutrient broth by shaken culture at 27 °C. The cells harvested at an early stationary phase were washed twice with distilled water. The collected cells were suspended in various detergent solutions, care being taken to adjust the cell concentration to $5\text{–}6 \times 10^8$ cells/ml. The suspensions were incubated for 30 min at 30 °C, then centrifuged. The pellets were washed twice with distilled water and resuspended in a 20 mM KCl solution. The suspensions were kept at 4 °C till dielectric measurements were carried out in order to protect the treated cells from autolysis. Before the measurements, the cells were washed again with a 20 mM KCl solution which was kept at measuring temperature, and then resuspended in a 20 mM KCl solution containing 0.1% agar. The addition of agar prevents the cells from sedimenting during dielectric measurements, giving no effect on the dielectric constant and conductivity of the medium. In each set of measurements, cell concentrations of specimens were kept constant. The series of sodium alkyl (C_8, C_{10}, C_{12}, C_{14}) sulfonates were obtained from Tokyokasei Co., Ltd. The series of alkyl (C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}) benzyl dimethyl ammonium chlorides were kindly supplied by Mr. Tuji at Kao Soap Co., Ltd.

Dielectric measurements were carried out with a TR-1C Transformer Ratio-Arm Bridge of Ando Electric Co., Ltd. and with a 250A RX-Meter of Boonton Radio Corporation. The measuring cell and the correction for the residual inductance arising from the measuring cells were described in the previous paper [1].

Compounds flowed out of the cells were measured with a spectrophotometer. Volumes of the cells packed by centrifugation ($1000 \times g$ for 10 min) were measured with a hematocrit.

Results

Dielectric Behavior for Suspensions of Yeast Cells Treated with Ionic Detergents in Various Concentrations

Fig. 1 shows frequency dependence of dielectric constant ε and conductivity κ for suspensions of yeast cells treated with sodium dodecyl sulfonate (SDSO) and dodecyl benzyl dimethyl ammonium chloride (DBDAC). As indicated in Fig. 1, the concentration of SDSO and

DBDAC used in the treatments was varied from 2 to 10 mM and from 0.1 to 1 mM, respectively. In each set of measurements the same number of cells was suspended in a definite volume of 20 mM KCl solution. As shown in the previous paper [1], the intact yeast cell suspensions showed typical dielectric dispersions which are considered to be caused by the presence of cytoplasmic membrane with sufficiently low conductivity. A steep rise in dielectric constant at frequencies below 0.1 MHz is due to electrode polarization. The treatment with both kinds of ionic detergents reduces dielectric dispersion in a similar way. The values of limiting dielectric constant ε_l at low frequencies decrease gradually with the increase in concentration of the detergents below 4 and 0.2 mM for SDSO and DBDAC, respectively, and are remarkably diminished by 5 mM of SDSO and 0.3 mM of DBDAC, while the values of limiting dielectric constant ε_h at high frequencies are almost unchanged. The values of limiting conductivity κ_l at low frequencies increase with increasing concentration of the detergents but remain lower than that of suspending medium at higher concentrations of the detergents. Dielectric dispersions disappear completely at about 10 and 1 mM for SDSO and DBDAC, respectively. Since the reducing effects of the detergents on the dielectric dispersion were observed for DBDAC and SDSO below their critical micelle concentration as shown in Fig. 1, it is considered from the results that the detergent in a monomer form affects the membrane structure and causes the increase in membrane conductivity.

Change in Packed Volume of Yeast Cells and Leakage of Compounds

Intracellular metabolites such as amino acids, purines, pyrimidines and pentose are known to be released from certain bacteria treated with anionic and cationic detergents as a result of breakdown of permeability barrier [7]. In the present study, it is found that the yeast cells treated with SDSO and DBDAC release some compounds showing absorption maximum at about 260 nm, probably a mixture of purines, pyrimidines, nucleosides and mononucleotides. In Fig. 2 are shown changes in optical density at 260 nm (OD_{260}) of suspending medium with which the cells were treated, together with changes in packed volume of the treated cells and changes in relative magnitude of the dielectric dispersion $(\varepsilon_{0.1}-\varepsilon_{100})/(\varepsilon_{0.1}-\varepsilon_{100})_{\text{intact}}$, where the values of $\varepsilon_{0.1}$ and ε_{100} are dielectric constant at 0.1 and 100 MHz, respectively.

In the case of SDSO-treated cells as seen in Fig. 2*A*, the reduction of dielectric dispersion accompanied by only the decrease in packed

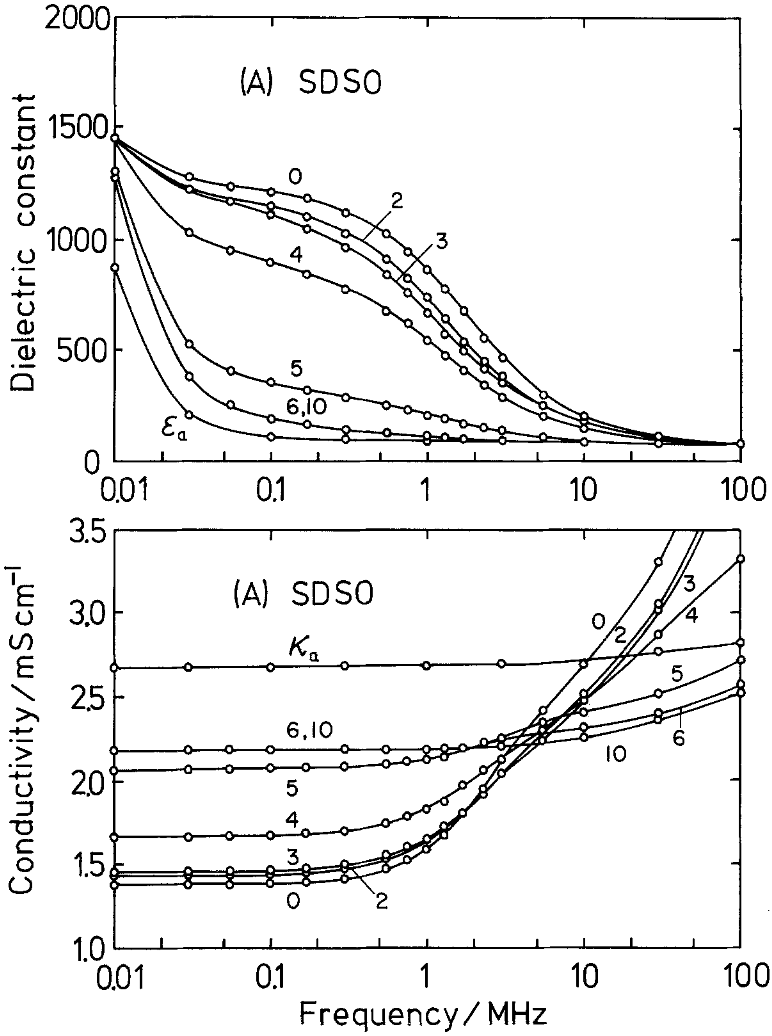

Fig. 1. Frequency dependence of dielectric constant and conductivity for suspensions of yeast cells treated with (*A*) sodium dodecyl sulfonate (SDSO) and (*B*) dodecyl benzyl dimethyl ammonium chloride (DBDAC) solutions in various concentrations. Numbers beside the curves indicate the concentration of SDSO and DBDAC in mM. The cell concentrations of specimens are kept constant in each series. The final suspending medium is 20 mM KCl solution. Dielectric measurements were made at (*A*) 23 °C and (*B*) 20 °C

volumes of the cells is observed at concentrations lower than 3 mM where no appreciable increase is found in the OD_{260} value. At concentrations higher than 5 mM, the reduction of dielectric dispersion is still going on with the concomitant increase in the OD_{260} value and without further decrease in packed volume.

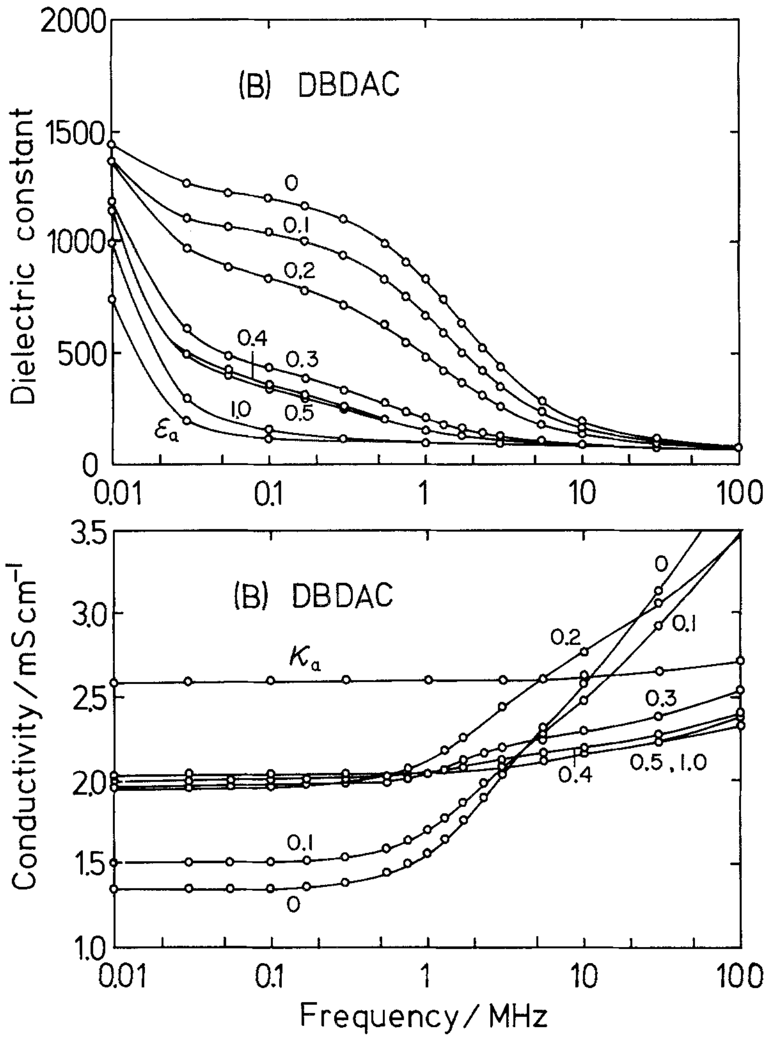

Fig. 1B

In contrast with the behavior of SDSO-treated cells, the dielectric dispersion for DBDAC-treated cells (Fig. 2*B*) shows a remarkable decrease at concentration as low as 0.1 mM with both changes in packed volume and in OD_{260}. At concentrations higher than 0.3 mM, the reduction of dielectric dispersion is going on with further increase in OD_{260}, whereas the packed volume of the cells remains at its lower limiting value.

Effect of Alkyl Chain Length of the Detergents on the Yeast Cells

In general, bactericidal activity and hemolysis are known to be dependent upon alkyl chain length of homologous series of ionic detergents.

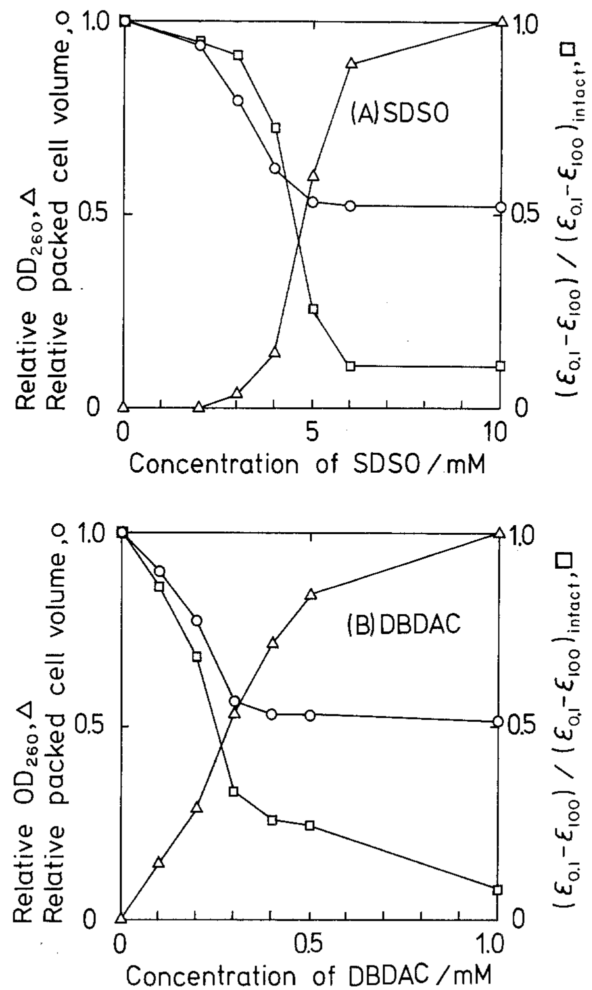

Fig. 2. Effects of (*A*) SDSO and (*B*) DBDAC on packed volume of cells, leakage of compounds showing absorption maximum at about 260 nm and relative magnitude of dielectric dispersion

A similar effect of the chain length of detergents on reduction of dielectric dispersions were observed for the present series of detergents, sodium alkyl sulfonates (SASO) and alkyl benzyl dimethyl ammonium chlorides (ABDAC). Fig. 3 shows the frequency dependence of the dielectric constant of the yeast cell suspensions treated with the solutions of these detergents. As readily seen in the Figures, the magnitude of the dielectric dispersion of the suspensions is markedly dependent on the alkyl chain length of the detergents. The effect of the detergents on the reduction of dielectric dispersions is in order of $C_{14} > C_{12} > C_{10}$, C_8 for SASO's

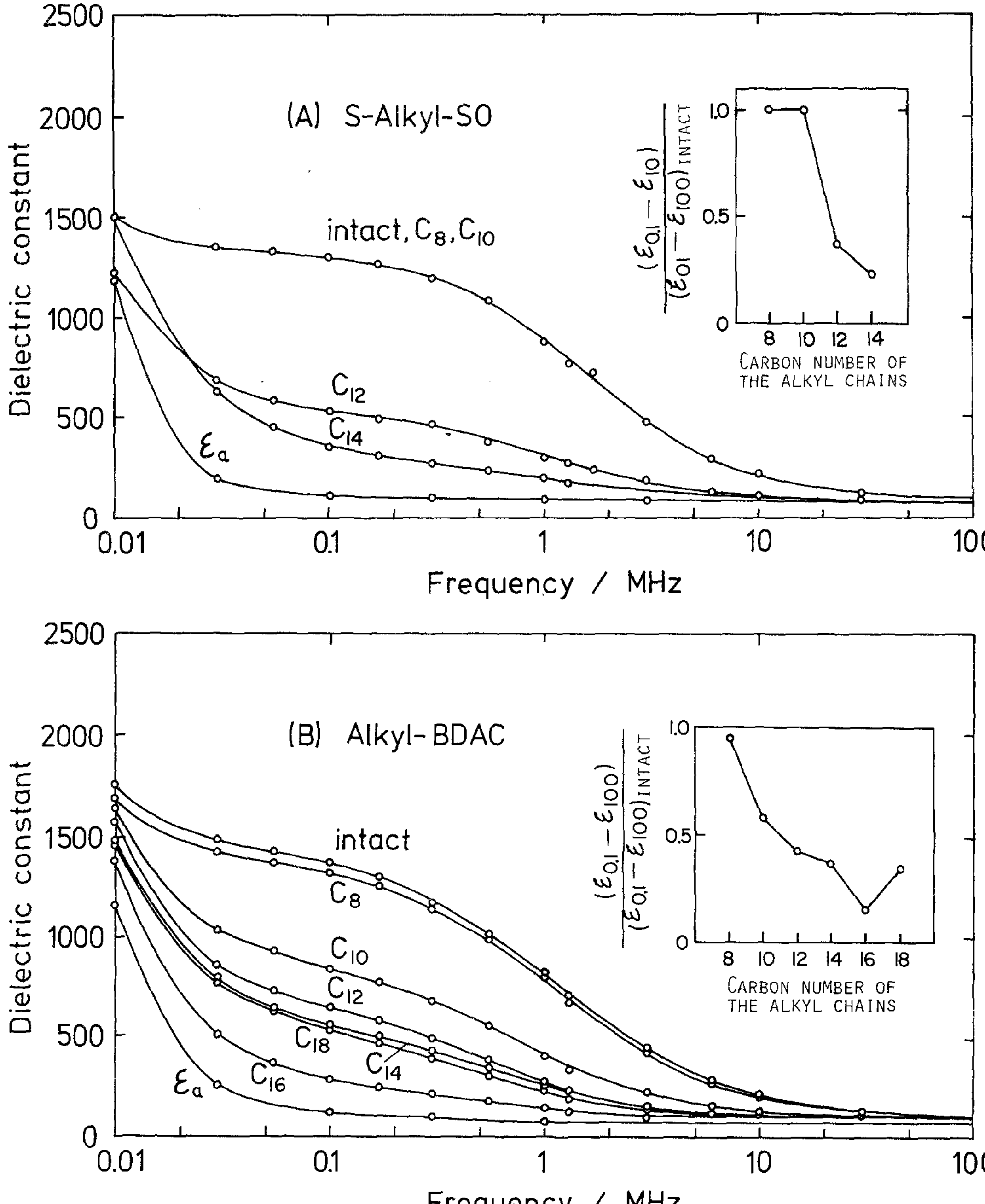

Fig. 3. Effects of alkyl chain length of (*A*) sodium alkyl sulfonates (SASO's) and (*B*) alkyl benzyl dimethyl ammonium chlorides (ABDAC's) on dielectric dispersion. The concentrations of SASO's and ABDAC's in the treatments are 5 and 0.5 mM, respectively. The cell concentrations of specimens are kept constant in each series. The suspending medium is 20 mM KCl solution. Dielectric measurements were made at (*A*) 20 °C and (*B*) 28 °C. Figures' C_n's beside the curves indicate alkyl chain of the detergents used. The relative magnitude $(\varepsilon_{0.1}-\varepsilon_{100})/(\varepsilon_{0.1}-\varepsilon_{100})_{\text{intact}}$ of the dielectric dispersion versus carbon number of the alkyl chains is also depicted in the Figures

and $C_{16} > C_{18}$, $C_{14} > C_{12} > C_{10} > C_8$ for ABDAC's. The higher members of SASO's such as C_{16} and C_{18} were not examined owing to their low solubility in water. The reverse order observed for C_{16} and C_{18} in ABDAC's might be due to the low effective concentrations of monomer form of C_{18} compared with C_{16}. The circumstances could be made clear by a series of experiments at concentrations lower than the critical micelle concentration 7.1×10^{-6} M of C_{18} [5], though the experiments turn out inaccurate owing to such extremely low concentrations.

Discussion

Reduction of Dielectric Dispersion by Ionic Detergents

Dielectric behavior of biological cell suspensions can be discussed in terms of a dielectric theory which was developed by Pauly and Schwan [4] for a suspension of spherical particles covered with shells. It is expected from the theory that the reduction of dielectric dispersion results from the decrease in volume fraction of the cells in suspensions and/or the increase in conductivity of cytoplasmic membranes κ_m.

In view of the present result that the packed volume of the cells in suspensions was decreased markedly by treatment with the detergents, the reduction of dielectric dispersion of the yeast cell suspensions is attributable to the decrease in volume fraction due to the shrinkage of the cells. The values of ε_l and κ_l of dielectric dispersion in varying cell diameter were calculated from Pauly-Schwan's equation by using the phase parameters which were estimated for intact yeast cells [1], the results being shown in Fig. 4. As seen in the Figure, the decrease in cell diameter corresponding to the shrinkage causes the decrease in ε_l and increase in κ_l, leading to the reduction of dielectric dispersion.

Provided that the relative packed volume roughly corresponds to the relative cell volume, it is expected from the results shown in Fig. 2 that the relative cell volume after the shrinkage is about 0.5, where a certain dielectric dispersion still remains as shown in Fig. 4. To understand the complete disappearance of the dielectric dispersions, we have to take into consideration the increase in the membrane conductivity κ_m in addition to the shrinkage of the cells. Numerical estimation of ε_l and κ_l for varying κ_m were made with Pauly-Schwan's equation by use of the same values of phase parameters of intact cells as used in

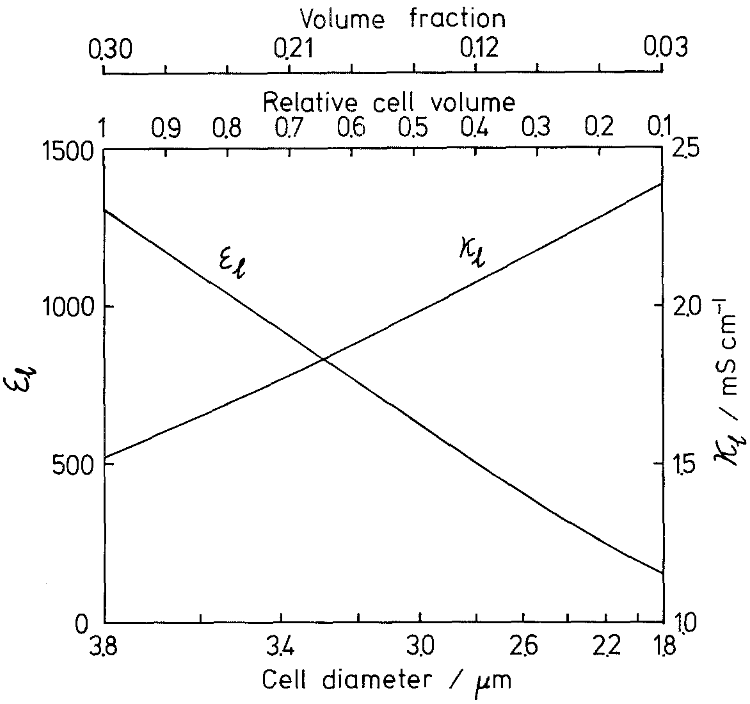

Fig. 4. Effect of change in cell diameter on limiting dielectric constant at low frequencies ε_l and limiting conductivity at low frequencies κ_l. The curves are numerically calculated from Pauly-Schwan's equation with phase parameters as: $\varepsilon_a=80$; $\kappa_a=2.5$ mS/cm; $\kappa_m=0$ mS/cm; $\varepsilon_i=50$; $\kappa_i=2.5$ mS/cm; membrane thickness = 50 Å. The subscripts of symbols: *a*, outer phase; *m*, membrane phase; *i*, inner phase of cells. The cell diameter is varied from 3.8 to 1.8 μm. The volume fraction is calculated from the cell diameter under a constant cell number. Relative cell volume is the volume of a cell relative to that for 3.8 μm

Fig. 4, the results being shown in Fig. 5. As readily seen in the Figure, the dielectric dispersion begins to be reduced at $\kappa_m/\kappa_a \approx 10^{-5}$, and almost disappears at $\kappa_m/\kappa_a \gtrsim 10^{-2}$.

Since the changes in packed volume of cells and in the OD_{260} value reflect qualitatively changes in cell volume and membrane permeability, the results shown in Fig. 2 suggest that reduction of dielectric dispersion in question is attributable to two different processes: the shrinkage of cells and the increase in conductivity of cytoplasmic membranes. In this instance, the decrease in cell volume causes mainly the reduction of dielectric dispersion at relatively low concentration of the detergents, while the increase in membrane conductivity is dominant at the higher concentrations as obviously shown in the case of SDSO (Fig. 2*A*). When

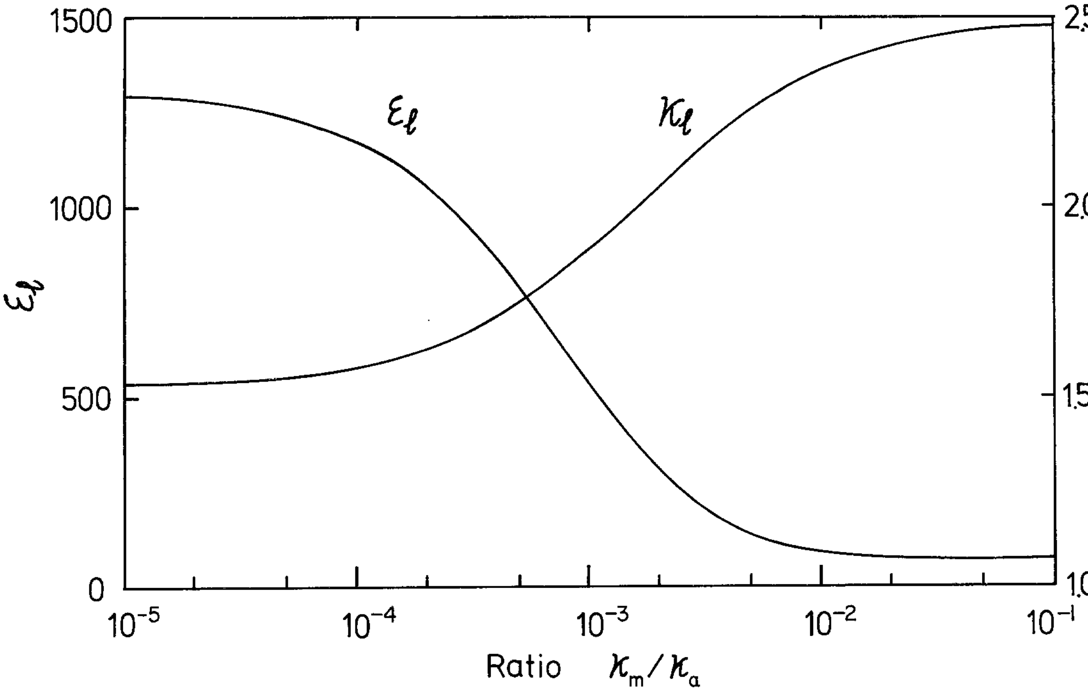

Fig. 5. Effect of change in membrane conductivity κ_m on limiting dielectric constant at low frequencies ε_l and limiting conductivity at low frequencies κ_l. The calculation is carried out by Pauly-Schwan's equation. Volume fraction and diameter of cells are 0.3 and 3.8 μm, respectively, throughout the calculation. Other parameters are the same as those in Fig. 4

the dielectric dispersions completely disappear, the values of κ_l always remain lower than the conductivity of suspending medium κ_a. This result implies that the interior of the cells keeps lower conductivity compared with κ_a, even after the destruction of the cytoplasmic membrane. The low conductivity of the inner phase of the cells may be attributed to the presence of intracellular organelles, suggesting that membranes of the intracellular organelles probably are not appreciably damaged in contrast to cytoplasmic membranes.

Comparison of the Reduction of Dielectric Dispersion of Yeast Cell Suspensions with the Effect of Detergents on Bacteria and Red Blood Cells

Salton [7] reported the difference between anionic and cationic detergents in lytic action on protoplast of *B. megaterium* by the observation of the lysed protoplast with phase contrast microscopy. Protoplast membranes were completely dissolved by sodium dodecyl sulfate, while lysis of protoplast by cetyl trimethyl ammonium bromide was not accompanied by such kind of dissolution of the protoplast membranes. This difference, however, was unable to be observed by the present dielectric measurements, because this method can make no distinction between the presence of membranes with higher conductivity ($\kappa_m / \kappa_a \gtrsim 10^{-2}$) and the absence of membranes.

According to the present results, cationic detergent (DBDAC) is more effective for reducing the dielectric dispersion of yeast cell suspensions compared with anionic detergent (SDSO). Gilby and Few [3] reported the similar sequence for lytic action on protoplast of *M. lysodeicticus* and bactericidal activity as $-NH_3^+ > -N^+(CH_3)_3 > -SO_4^- > -SO_3^-$. In contrast, the opposite order was obtained on hemolytic activity by Ross and Silverstein [6], that is $-SO_4^- > -C_6H_5CH_2N^+(CH_3)_2 > -N^+(CH_3)_3$.

The effect of alkyl chain length of cationic and anionic detergents on the reduction of dielectric dispersion is consistent with the trend of hemolytic and bactericidal activity. The maximum action of hemolysis occurs at C_{14} for sodium alkyl sulfonate and C_{16} for alkyl benzyl dimethyl ammonium chloride [6]. The most effective alkyl chain length on bactericidal activity is in the vicinity of C_{16}, depending upon the test organisms and detergents. The optimum alkyl chain lengths reported are C_{18} on *E. coli* and C_{16} on *M. aureus* for alkyl trimethyl ammonium chloride [2] and C_{14} on *M. pyogenes var. aureus* and *S. typhosa* for alkyl benzyl dimethyl ammonium chloride [5].

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