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On the dielectrically observable consequences of the diffusional motions of lipids and proteins in membranes

2. Experiments with microbial cells, protoplasts and membrane vesicles

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Abstract. *1.* The dielectric properties of suspensions of intact cells of *Methylophilus methylotrophus, Paracoccus denitrificans* and *Bacillus subtilis* have been measured in the frequency range 1 kHz to 13 MHz. All possess a pronounced dispersion corresponding in magnitude and relaxation time to the "β-dispersion" in a terminology defined by Schwan [Adv. Biol. Med. Phys. 5: 147–209 (1957)]. The latter two strains, but not *M. methylotrophus,* also possess a substantial α-dispersion. The relaxation time of the β-dispersion of *B. subtilis* is significantly lower than that of the other two strains, due to the higher internal K^+ content of this Gram-positive organism.

2. Treatment of *P. denitrificans* or *B. subtilis* with lysozyme greatly reduces the magnitude of the α-dispersion; in the latter case it is virtually abolished.

3. The magnitude of both the α- and β-dispersions of protoplasts of these organisms is significantly decreased by treatment with the cross-linking reagent glutaraldehyde, indicating that diffusional motions of the lipids and/or proteins in the protoplast membranes contribute to the dielectric relaxations observed in this frequency range. Such motions cannot be unrestricted, as in the "fluid mosaic" model, since the relaxation times of the lipids and proteins, if restricted by hydrodynamic forces alone, should then correspond, in protoplasts of this radius (0.4–0.5 μm), to approximately 10 Hz.

4. Even after treatment of the (spherical) protoplasts with glutaraldehyde, the breadth of the remaining β-dispersion is still significantly greater than (a) that of a pure Debye dispersion and (b) that to be expected solely from a classical Maxwell-Wagner-type mechanism.

5. It is recognised that the surfaces of the protein complexes in such membranes extend significantly beyond the membrane surface as delineated by the phospholipid head-groups; such molecular granularity can in principle account for the broadened dielectric relaxations in the frequency range above 1 kHz, in terms of the impediment to genuinely tangential counterion relaxation caused by the protruding proteins themselves.

6. The relaxation time of a previously observed, novel, low-frequency, glutaraldehyde-sensitive (μ-) dispersion in bacterial chromatophore suspensions, as well as that of their α-dispersion, is significantly increased by increasing the aqueous viscosity with glycerol. This finding is consistent with the view that, from a dielectric standpoint, the motions of charged proteins (and lipids) in biological membranes are rather tightly coupled to those of the adjacent ions and dipoles in the electric double layer.

7. Membrane vesicles of *P. denitrificans* do not possess a μ-dispersion as marked as that of chromatophores. As with chromatophores, their α-dispersion is somewhat decreased by glutaraldehyde treatment. The relative lack of a μ-dispersion in these vesicles may be related to their different polarity relative to that of bacterial chromatophores; alternatively, and perhaps additionally, the long-range lateral mobility of lipids and proteins in this system may be even more restricted than in chromatophores.

8. Overall, our results draw attention to the fact that the motions of proteins, lipids and double-layer species can contribute to the audio- and radio-frequency dielectric properties of microbial cell, protoplast and vesicle suspensions, and indicate that both the magnitude and the rate of such relaxations depend rather finely on the intimate molecular structure and organisation of the bacterial cytoplasmic membrane and its superincumbent double layers.

Key words: Dielectric spectroscopy, lateral diffusion, microbial membranes, lateral electrophoresis, electroosmotic forces

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Introduction

The audio- and radio-frequency dielectric properties of suspensions of microorganisms (and of other cells) have been studied by a number of workers (e.g. Fricke et al. 1956; Schwan 1957, 1959, 1977, 1981 a, b, 1983 a, b; Pauly 1962; Cole 1972; Einolf and Carstensen 1973; Carstensen and Marquis 1975; Grant et al. 1978; Schanne and Ceretti 1978; Pethig 1979; Asami et al. 1980; Pilla 1980; Schwan and Foster 1980; Adey 1981; Stoy et al. 1982; Zimmermann 1982; Harris and Kell 1983; Harris et al. 1984; Pethig 1984). In most cases, two relatively broad, but clearly (if imperfectly) separable dispersions are observed. The α-dispersion, centred in the audio-frequency range, is mainly ascribed to the tangential relaxation of ions bound in the diffuse double layers surrounding the cell wall and cytoplasmic membrane. The meshlike and extended structure of the former means that the effective surface area is much greater than that of the cytoplasmic membrane, so that the α-dispersion is substantially reduced, although by no means eliminated, upon the removal of the cell wall with lysozyme (Einolf and Carstensen 1969; Harris et al. 1984). The β-dispersion, centred in the radio-frequency range, is largely ascribed to the occurrence of a Maxwell-Wagner effect at the interface between the poorly-conducting cytoplasmic membrane and the aqueous phases which it serves to separate.

Since modern views of membrane structure conceive a substantial amount of both lateral and rotational diffusional mobility of the lipids and proteins in the cytoplasmic membrane of microorganisms (e.g. Houslay and Stanley 1982), we have had cause to consider the possibility that such motions might also contribute to the dielectric properties of microbial cell and vesicle suspensions (Kell 1983, 1984b; Harris et al. 1984; Kell and Harris 1985 a, b).

Complementarily, it then follows that dielectric spectroscopy can in principle offer a unique possibility for assessing the rate and extent (randomness) of such motions (Kell 1983, 1984b; Kell and Westerhoff 1985; Kell and Harris 1985). Thus, the major purpose of this article is to present experiments designed to address the question of the contribution which such processes might make to the dielectric spectra of microbial membrane systems. To this end, we have studied a variety of Gram-positive and -negative organisms, as well as protoplasts and membrane vesicle preparations derived therefrom, so as to gain a broad overview of the dielectric properties of membrane-bounded vesicle systems. In particular, we have exploited the idea that chemical cross-linking agents may be used to assess the contributions of the motions of the membrane components to the dielectric spectra in the audio- and radio-frequency range. Such considerations form the subject of the present paper, whilst a more theoretical and heuristic overview is presented in an accompanying article (Kell and Harris 1985 a).

Experimental

Methylophilus methylotrophus AS 1 (strain G 37-517) was maintained, and grown aerobically in shake-flasks, as described by Windass et al. (1980), except that the methanol concentration was 1% *v/v*. The cells were taken from mid-exponential phase, washed once in 0.2 *M* sorbitol containing 0.25 m*M* disodium EDTA, then once in 0.2 *M* sorbitol, and resuspended in 0.2 *M* sorbitol at ca. 150 mg dry weight/ml.

Bacillus subtilis (strain 34.1 (*pheA12 spoOA34*)) was grown aerobically in shake-flasks, and maintained as described (Donnellan et al. 1964; Ramaley and Burden 1970). The cells were harvested no earlier than 1 h into stationary phase. The cells were washed once in 0.5 *M* sucrose containing 0.5 m*M* disodium EDTA, then twice in 0.5 *M* sucrose, the third washing being necessary to attain a suitably low conductance in the final suspension, and resuspended in 0.5 *M* sucrose at a concentration of approximately 100 mg dry weight/ml.

Protoplasts of *B. subtilis* were prepared in the same way as protoplasts of *P. denitrificans* (see below).

Paracoccus denitrificans NCIB 8944 was grown anaerobically and maintained on a succinate-nitrate medium as described by Kell et al. (1978b) and by McCarthy et al. (1981). The dielectric studies of the intact cells were performed on cells taken from mid-exponential phase, which were washed once in 0.5 *M* sucrose containing 0.5 m*M* disodium EDTA, then once in 0.5 *M* sucrose and resuspended in 0.5 *M* sucrose at ca. 150 mg dry weight/ml. In cases noted in the legends to the figures the sucrose was in some cases replaced by sorbitol.

Paracoccus denitrificans protoplasts were made from cells taken from mid-exponential phase, which were washed once in 0.5 *M* sucrose containing 0.5 m*M* disodium EDTA and then suspended in 0.5 *M* sucrose, so that a 0.1 ml sample of the suspension diluted to 2.5 ml with water had an absorbance at 550 nm of 0.3 when read in a Bausch and Lomb Spectronic 70 spectrophotometer. Lysozyme was added to a concentration of 250 mg/l, and the suspension was then incubated at 30 °C until the O.D. at 550 nm of a 0.1 ml sample diluted to 2.5 ml with water had dropped from 0.3 to approximately 0.06. After treatment with lysozyme, 2 m*M* $MgSO_4$

and a crystal of DNAase I were added to the suspension, and the protoplasts were sedimented then washed in 0.5 *M* sucrose. The washed protoplasts were then resuspended in 0.5 *M* sucrose at ca. 150 mg dry weight/ml.

Membrane vesicles were also prepared from *P. denitrificans*, all procedures except the lysozyme treatment being carried out at 4 °C. The protoplasts (preparation described above) obtained from 4 l of *P. denitrificans* cells were sedimented at 40,000 × *g* for 10 min, then resuspended in 40 ml of 100 m*M Tris* acetate, pH 7.3. This suspension was added to 260 ml of distilled water to disrupt the protoplast membranes by osmolysis. The suspension was left for 20 min, before adding a trace of DNAase I and 2 m*M* magnesium acetate. This suspension was shaken gently, and then left for 20 min or until its viscosity was lowered sufficiently. The suspension was then centrifuged at 40,000 × *g* for 40 min to yield a double-layered pellet and a clear supernatant, which was discarded. The upper, red, layer of the pellet was resuspended in 0.1 *M* sorbitol containing 1 m*M Tris* HCl at pH 8. The lower, white, layer (of poly-β-hydroxybutyrate, a storage polymer in this organism (Scholes and Smith 1968)), was discarded. The suspension was centrifuged at 40,000 × *g* for 40 min and the resulting pellet washed once again before finally being resuspending in 0.1 *M* sorbitol/1 m*M Tris* HCl at pH 8, to give a final protein concentration of ca. 10 mg dry weight/ml as determined by the Lowry method (Lowry et al. 1951).

Rhodopseudomonas capsulata chromatophores were made (Hitchens and Kell 1982) and washed (Kell 1983) as previously described; their bacteriochlorophyll content was assessed spectrophotometrically (Hitchens and Kell 1982).

Dielectric measurements

All dielectric measurements were made using a Hewlett-Packard 4192A impedance analyser controlled by an HP 85 microcomputer as described previously (Harris and Kell 1983, and see also Kosterich et al. 1983; Asami and Irimajiri 1984). Automatic correction of measured data for simple strays and residuals was performed on an empty cell using the "zero open" adjustment at 10 MHz. The cell constant was obtained using solutions of known conductivity and permittivity, such measurements also providing a check on the extent of electrode polarisation. That this extent was of negligible significance to the present considerations was ensured (1) by using media of very low conductivity, (2) by using high volume fractions of the suspended phase, and (3) by regular and heavy platinisation of the electrodes; no corrections were therefore made in this regard to the data obtained. In the present work, an oscillator level of 300 mV was employed and the temperature maintained at 25 °C for chromatophores, *B. subtilis* cells and protoplasts, 30 °C for *P. denitrificans* cells, protoplasts and membrane vesicle preparations and 37 °C for *M. methylotrophus*, by means of a circulating water jacket. Pt electrodes were of the pin-type (8 mm × 1 mm), and plated with Pt black, as described (Kell 1983).

Chemicals

Chemicals were obtained from previously described sources (Harris and Kell 1983), except that the glutaraldehyde ("50% *w/v* solution") was from BDH Chemicals, Poole, Dorset. Water was doubly distilled in an all-glass apparatus.

Results

Methylophilus methylotrophus

Figure 1 shows a dielectric spectrum of *M. methylotrophus* cells in the frequency range 1 kHz to 13 MHz. This Gram-negative, pseudomonad organism has aroused interest because of its use in single-cell protein production (Smith 1980; Windass et al. 1980; Vasey and Powell 1984). The frequency dependence of the RF conductivity is especially evident, indicating the existence of a substantial β-dispersion, in the terminology of Schwan (1957), as also observed for many other microorganisms (e.g. Einolf and Car-

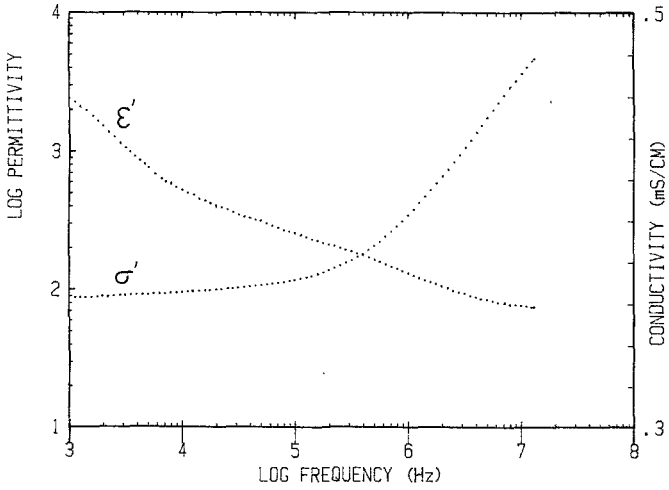

Fig. 1. Dielectric properties of intact cells of *M. methylotrophus*. The cell suspension (102 mg dw/ml) was prepared, resuspended, and its dielectric properties assessed, as described in the Experimental section

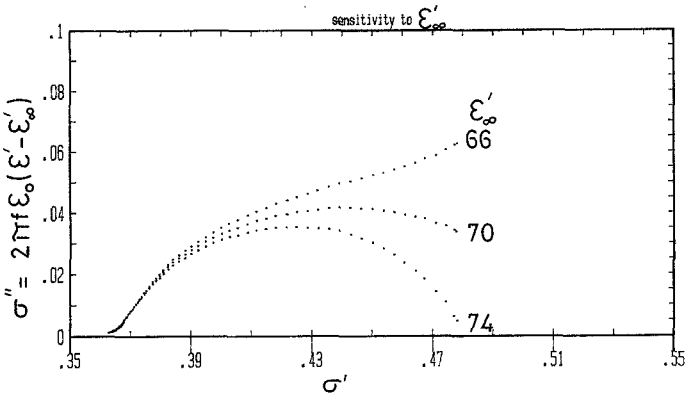

Fig. 2. Effect of "high-frequency" permittivity on the complex conductivity of the intact cells of *M. methylotrophus*. The complex conductivity is plotted, for indicated values of the "high-frequency" permittivity (ε'_∞), using the data of Fig. 1. It is evident that the α-dispersion is virtually absent

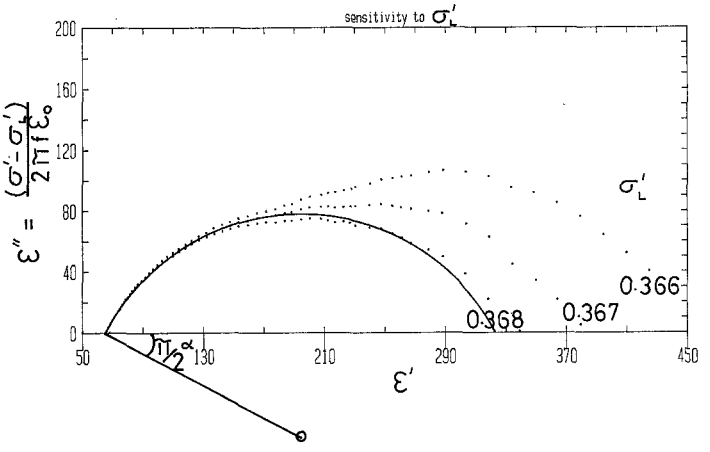

Fig. 3. Effect of "low-frequency" conductivity on the β-dispersion of intact cells of *M. methylotrophus*. The complex permittivity is plotted, for the indicated values of the "low-frequency" conductivity (σ'_L), using the data of Fig. 1. The Cole-Cole α value (Cole and Cole 1941) is 0.31, indicating that the breadth of the dispersion is rather substantial

stensen 1969; Carstensen and Marquis 1975; Asami et al. 1980). The α-dispersion, although just about discernible, is far less pronounced than in Gram-positive organisms (Einolf and Carstensen 1969, 1973; and see later), and much of the permittivity increase below 10 kHz is in this case due to electrode polarisation. The data in Fig. 1 are plotted as a complex conductivity diagram in Fig. 2, which shows how, as discussed elsewhere (Grant 1958; Stoy et al. 1980; Harris and Kell 1983), the high-, but not the low-frequency values of σ'' are extremely sensitive to the value chosen for the "high-frequency" permittivity. Similarly, for the α-dispersion, in the Cole-Cole (complex permittivity) plot of these data (Fig. 3), the "low-frequency" permittivity and hence the calculated dielectric increment, is very sensitive to the "low-frequency" conductivity chosen. Since the high-frequency data are uncontaminated either by electrode polarisation or by any α-dispersion, the appropriate value of the "low-frequency" conductivity (σ'_L) is taken to be the lowest value with which a single semi-circle may still be used to fit the data (Harris and Kell 1983). For the data of Fig. 3, the centre of this semi-circle is also shown; it may be seen that the Cole-Cole α value (Cole and Cole 1941) derived from this analysis possesses a rather large value. This is usually taken to indicate (e.g. Pauly et al. 1960; Pauly and Packer 1960) that there is a substantial distribution of relaxation times underlying the dispersion of interest.

If the β-dispersion is caused *solely* by a Maxwell-Wagner effect then the "low-frequency" permittivity (ε'_L) is given, for spherical particles, by (Schwan 1957):

$$\varepsilon'_L = \varepsilon'_\infty + \frac{9\,P\,r\,C_m}{4\,\varepsilon_0}\left[\frac{1}{1 + r\,G_m\left(\frac{1}{\sigma_i} + \frac{1}{2\,\sigma_o}\right)^2}\right], \qquad (1)$$

where P is the volume fraction, r the cell radius, C_m the membrane capacitance per cm^2, G_m the membrane conductance per cm^2, σ_i and σ_o the internal and external conductivities, ε'_∞ the "high-frequency" permittivity and ε_0 the permittivity of free space ($= 8.854 \times 10^{-14}$ F/cm). If the membrane conductance is negligible, Eq. (1) reduces to:

$$\varepsilon'_L = \frac{9\,P\,r\,C_m}{4\,\varepsilon_0} \qquad (2)$$

whilst if the volume fraction exceeds approximately 0.2, the following equation was derived (Schwan et al. 1970):

$$\varepsilon'_L = \frac{\varepsilon'_{sol}(1-P)}{(1+(P/2))} + \left(\frac{9\,r\,C_m}{4\,\varepsilon_0}\right)\left(\frac{P}{(1+(P/2))^2}\right), \qquad (3)$$

where ε'_{sol} is the permittivity of the suspending medium.

Since there has been some confusion in the literature as to what values of membrane conductance may be considered negligible in this context, Fig. 4 displays a plot of Eq. (1). For conditions pertaining to a typical microbial cell suspension, a membrane conductivity of a magnitude as great as 1 S/cm^2 causes an insignificant decrease in ε'_L. Since the ionic conductivities of typical energy coupling membranes as isolated do not exceed 2×10^{-4} S/cm^2 (e.g. Harold 1977; Junge 1982), and concentrations of protonophorous types of uncoupler necessary fully to uncouple mitochondria induce conductivities only of this type of magnitude (McLaughlin and Dilger

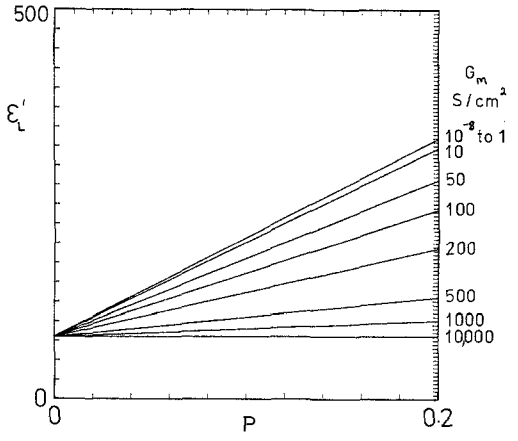

Fig. 4. Effect of the transmembrane conductance on the "low-frequency" permittivity of a suspension of spherical cells displaying a Maxwell-Wagner-type dispersion. Equation 1 is plotted using the following values: cell radius = 5×10^{-5} cm (0.5 μm), internal conductivity = 20 mS/cm, external conductivity = 10 mS/cm, membrane capacitance = 1 μF/cm². The membrane conductance (in S/cm²), and the enclosed volume fraction P are varied as indicated

1980), it should by clear that, from a dielectric standpoint, bioenergetically competent cells are quite indistinguishable from those possessing *zero* transmembrane conductance.

Other equations which may be used to characterise a dispersion conforming solely to a Maxwell-Wagner type of mechanism in spherical shell particles are (see e.g. Schwan 1957; Schwan and Foster 1980):

$$\sigma'_L = \sigma'_o(1-P)/(1+(P/2)) \tag{4}$$

$$\sigma'_\infty = \sigma'_o\,[(1+3P)(\sigma'_i+\sigma'_o)/(\sigma'_i+2\sigma'_o)], \tag{5}$$

$$\tau = r\,C_m\left(\frac{1}{\sigma'_i}+\frac{1}{2\sigma'_o}\right), \tag{6}$$

and, in general,

$$\tau = (\varepsilon'_L-\varepsilon'_\infty)\,\varepsilon_0/(\sigma'_\infty-\sigma'_L), \tag{7}$$

where $\tau(=1/2\pi f_c)$ is the relaxation time. More complicated equations have been applied to bacillary or ellipsoidal membrane systems (Pauly and Schwan 1959; Asami et al. 1980), sometimes taken to consist of confocal shells. However, if we treat the dielectric properties of a bacillus as being broadened (in terms of dielectric increment and relaxation time) so as to encompass those of a sphere of radius equal either to that of the major or minor axes of the ellipsoid of revolution, little error in the analyses will result (Kell and Harris 1985b). That this is so is signified by the fact that the "Coulter counter" and other such electronic particle counters and sizers perceive bacillary organisms as spheres of the equivalent size (e.g. Kubitschek 1969; Curds et al. 1978).

Since the specific enclosed volume V_{sp} of the cells (in ml/g dry weight) is related to their volume fraction P and their concentration C in mg dry weight/ml by $P = V_{sp}\,C/1000$ (Harris and Kell 1983), we may plot Eq. (3) as a graph of ε'_L versus cell concentration for different chosen values of V_{sp}. Such a graph, together with experimental data obtained by means of plots such as those in Fig. 3, is displayed in Fig. 5, which indicates, inter alia, that electrode polarisation, for instance, does not make a significant contribution to the spectra observed for the β-dispersion. No substantive dispersions at the high frequency end of the range available with the present instrumentation were observed in this organism (Fig. 6). The conductivity increment in this

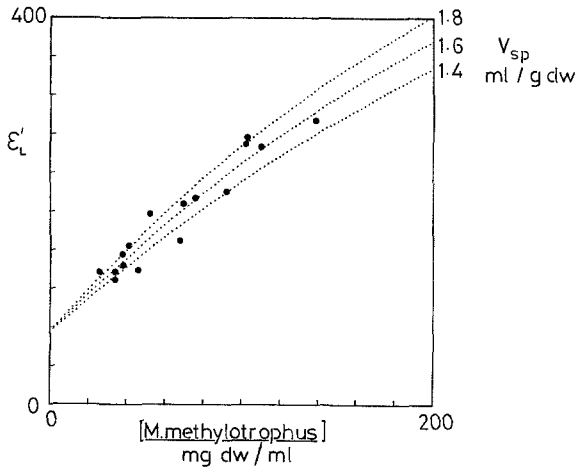

Fig. 5. Effect of cell concentration on the "low-frequency" permittivity of suspensions of *M. methylotrophus*. Data were obtained from plots such as those in Fig. 3. The dotted lines indicate data to be expected from the plots of Eq. (2), for a "classical" Maxwell-Wagner effect if the organisms are treated as spheres with the specific enclosed volumes indicated (see also Harris and Kell 1983)

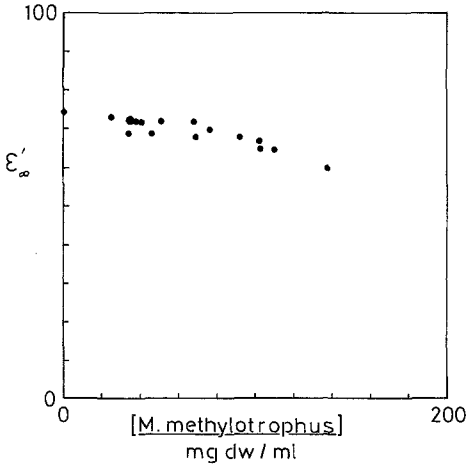

Fig. 6. Effect of cell concentration on the "high-frequency" permittivity of suspensions of *M. methylotrophus*. Data were obtained from plots such as those in Fig. 3

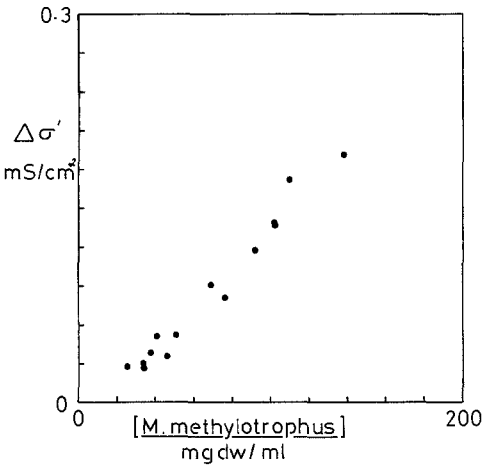

Fig. 7. Effect of the cell concentration on the conductivity increment of the β-dispersion of suspensions of *M. methylotrophus*. Conductivity increments were obtained from complex conductivity diagrams such as those in Fig. 2, and represent the difference in conductivity between the two points where the semi-circular locus intersects the abscissa

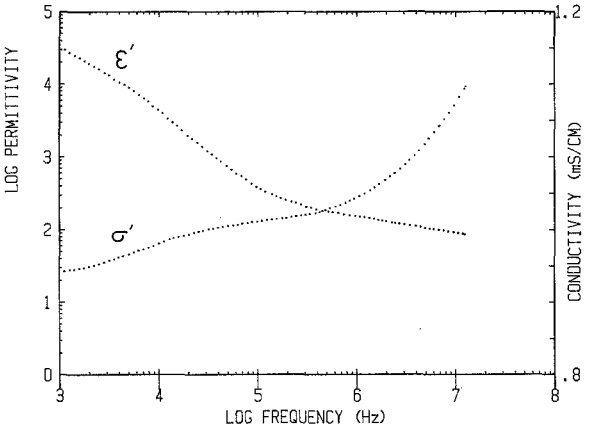

Fig. 8. Dielectric properties of intact cells of *P. denitrificans*. The cell suspension (118 mg dw/ml) was prepared and resuspended, and its dielectric properties assessed, as described in the Experimental section

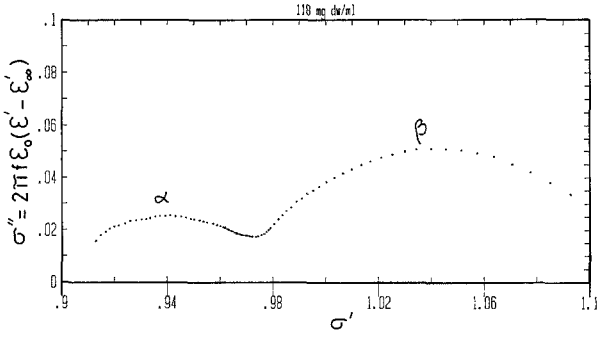

Fig. 9. Complex conductivity of intact cells of *P. denitrificans*. The data of Fig. 8 are plotted in the complex conductivity domain, and indicate clearly the extent of both α- and β-dispersions

organism amounts to approximately 0.1 mS/cm per 100 mg dry weight/ml (Fig. 7). Since this organism is Gram-negative, and rather insensitive to lysozyme treatment, it is not easy to dissect out the contributions of any lipid or protein motions to the observable dielectric properties of intact cells by making protoplasts or membrane vesicles. However, the virtual absence of an α-dispersion would seem to suggest that the outer parts of the cell envelope are either only weakly charged or present only a small effective surface area to the bulk outer phase. Similarly, *Saccharomyces cerevisiae* cells were also found to be devoid of an α-dispersion (Harris and Kell 1983). Thus, whilst the Gram-negative *M. methylotrophus* provides a useful "baseline", we decided to turn our attentions to other, lysozyme-sensitive organisms.

Paracoccus denitrificans

Paracoccus denitrificans is a Gram-negative organism which is nevertheless sensitive to lysozyme (Scholes and Smith 1968; Davis et al.1969; Kell et al. 1978), and which, due to its perceived similarity to mammalian mitochondria, has commanded no little attention from bioenergeticists (e.g. John and Whatley 1977; Vignais et al. 1981). A typical dielectric spectrum of a suspension of intact cells of this organism is shown in Fig. 8; as more clearly demonstrated in the complex conductivity plot of these data in Fig. 9, a very marked α-dispersion is present in addition to the β-dispersion, so that it was in fact not at all easy to separate the two dispersions. To obtain the dielectric increment for the β-dispersion alone, we adopted the same strategy as that described above for *M. methylotrophus*, i.e. that minimum value of σ'_L was chosen which still allowed the data to be fitted adequately to a single semi-circle. The values obtained from the complex plane plots, at several different concentrations of the suspended phase, are plotted in Figs. 10–12.

We observed previously that the α-dispersion of this organism is substantially reduced, but by no means completely eliminated, upon conversion of the cells to protoplasts with lysozyme (Harris et al. 1984). Similarly, the β-dispersion is much broader than seems to be explicable, for spherical protoplasts, in terms solely of a Maxwell-Wagner type of mechanism. As discussed before (Kell 1983, 1984b; Harris et al. 1984; Kell and Harris 1985a, b), one possibility is that there may be a contribution from the motions of the membrane components, a possibility which is amenable to experimental testing by the use of cross-linking reagents. We therefore display in Fig. 13 dielectric spectra of *Paracoccus*

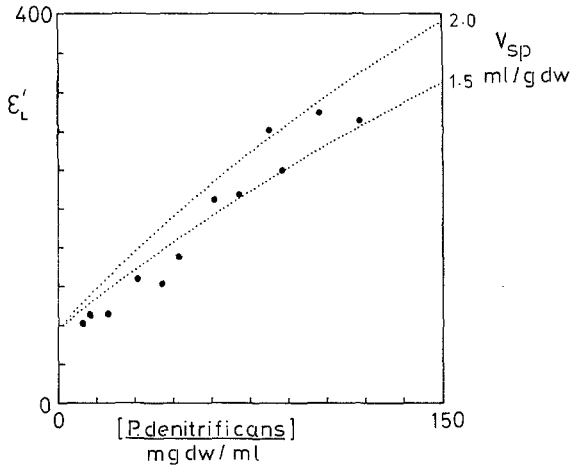

Fig. 10. Effect of cell concentration on the "low-frequency" permittivity of the β-dispersion of *P. denitrificans*. Data were obtained from complex permittivity diagrams such as those in Fig. 3. The relatively greater scatter in this plot compared to that (Fig. 5) for *M. methylotrophus* is considered to be due to the much greater difficulty in the present case (see Fig. 9 and cf. Fig. 2) of choosing an appropriate "low-frequency" conductivity for obtaining the complex permittivity diagrams

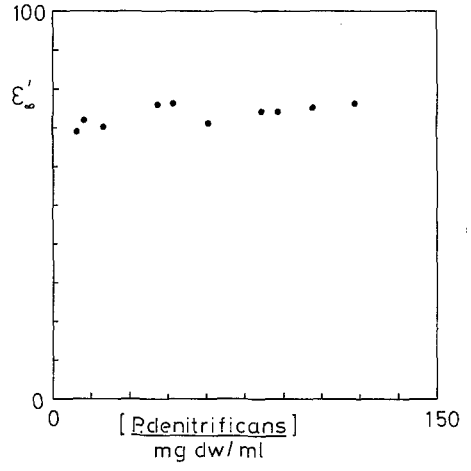

Fig. 11. Effect of cell concentration on the "high-frequency" permittivity of suspensions of *P. denitrificans*. Data were obtained, as in Fig. 10, from complex permittivity plots such as those in Fig. 3

denitrificans protoplasts which either have or have not been treated with 5% glutaraldehyde. It is evident that glutaraldehyde treatment noticeably reduces the β-dispersion and almost eliminates the already small α-dispersion.

It is worth stressing that the available evidence (Peters and Richards 1977) indicates (a) that, at neutral or acid pH, (the polymeric, active form of) glutaraldehyde does not affect the surface charge density of the membrane proteins, and (b) that the transmembrane conductance G_m is not increased, and if anything somewhat decreased, by glutaraldehyde treatment (Zimmermann et al. 1973). This latter point was also checked by looking at the decay of acid pulses in protoplast suspensions, using the method and apparatus described by Hitchens and Kell (1984) (data not shown). Dimethyl suberimidate (Davies and Stark 1970), which also leaves the protein charge intact (Davies and Stark 1970; Means and Feeney 1971; Peters and Richards 1977), was without effect on the dielectric properties of protoplasts (when reacted, as appropriate, at alkaline pH)

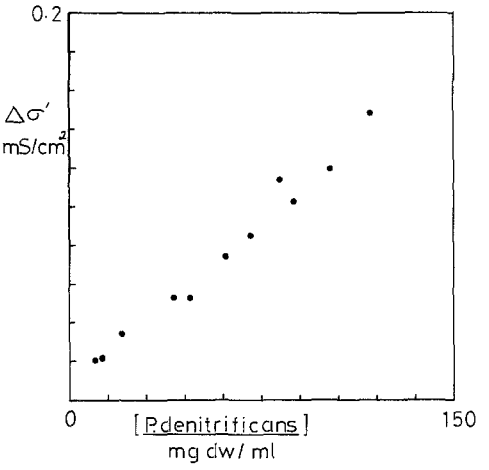

Fig. 12. Effect of cell concentration on the conductivity increment of the β-dispersion of *P. denitrificans*. Conductivity increments were obtained from complex conductivity diagrams such as those in Fig. 9, as described in the legend to Fig. 7

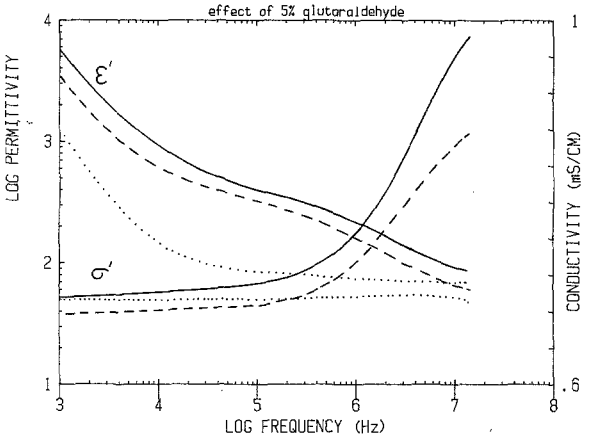

Fig. 13. Effect of glutaraldehyde on the dielectric properties of protoplasts of *P. denitrificans*. A suspension of *P. denitrificans* protoplasts (130 mg dw/ml, assessed turbidimetrically (Hitchens and Kell 1984)) was treated with 5% glutaraldehyde for 1 h. Their dielectric properties (*dashed line*) were assessed as described in the Experimental section, and compared with those (*solid line*) of a suspension of equal concentration which had not been so treated, and whose pH (6.02) and conductivity were adjusted to the same value. The dielectric properties of the suspending medium whose conductivity was adjusted with KCl to a similar "low-frequency" conductivity are also shown (*dotted line*) to indicate the extent of electrode polarisation in the present case

(data not shown), presumably because this reagent is too small to form *inter*-molecular cross-links. Thus, the fact that the cross-linking reagents inhibit dielectric relaxations at frequencies *above* 1 kHz contraindicates the view that the proteins and lipids are *freely* mobile in the plane of the membrane, since their relaxation should be, in particles of this size, approximately 10 Hz (Kell and Harris 1985a). This finding is consistent with several other lines of reasoning in prokaryotes (Kell 1984a).

Bacillus subtilis

In contrast to *P. denitrificans*, which, although lysozyme-sensitive, is reported as Gram-negative, *B. subtilis* is a classically Gram-positive, lysozyme-sensitive organism. The strain used in the present work is a *spoO* mutant which, upon exhaustion of the medium, cannot proceed to sporulation. Upon lysozyme treatment, each mother cell releases two spherical, wall-less protoplasts of equal size (Donnellan et al. 1964; Dunn et al. 1976), so that this preparation is characterised by an especially homogeneous size- and shape-distribution (photomicrographs not shown).

Figure 14 displays the dielectric properties of cells, protoplasts and glutaraldehyde-treated protoplasts of *B. subtilis* strain 34.1 *pheA12 spoOA34*, together with those of a sucrose/KCl solution of equivalent low-frequency conductivity. Several points

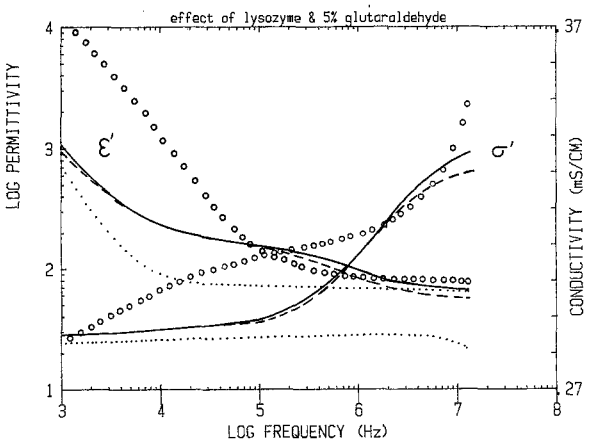

Fig. 14. Dielectric properties of intact cells, protoplasts and glutaraldehyde-treated protoplasts of *B. subtilis*. Preparations were obtained and their dielectric properties assessed, as described in the Experimental section and in the text. *Open circles:* intact cells (25 mg dw/ml); *solid line:* protoplasts derived therefrom, at a similar optical density; *dashed line:* similar protoplasts treated with 5% glutaraldehyde, as described in the legend to Fig. 13. The extent of electrode polarisation is indicated by the dotted line, representing the measured dielectric properties of the suspending medium adjusted with KCl to similar "low-frequency" conductivity

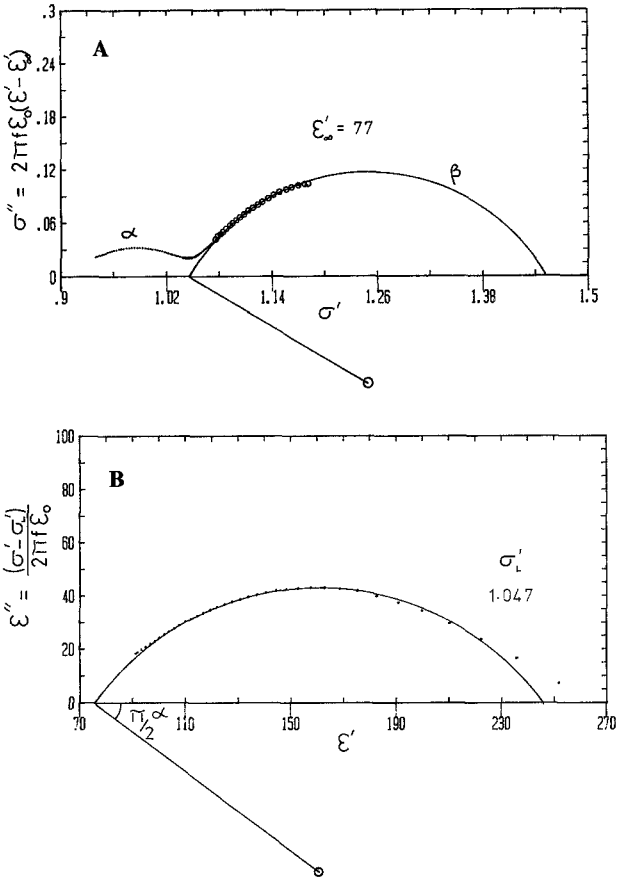

Fig. 15. Dielectric properties of *B. subtilis* cells. The complex conductivity (**A**) and the complex permittivity (**B**) diagrams are plotted using the values of ε'_∞ and σ'_L (in mS/cm) indicated. The cell suspension was the same as that in Fig. 14 except that the cell concentration was 101 mg dw/ml. In (**A**) the open circles serve to indicate the data measured with the highest frequencies available (up to 13 MHz). The Cole-Cole α in (**B**) has the value 0.41. It is evident that the extent to which the entire dispersion is observable is different in the two cases

arise from these data. First, the characteristic frequency of the β-dispersion of intact cells of this organism lies somewhat above those accessible with the present apparatus; this is especially evident in the complex conductivity plot (Fig. 15A) of a more concentrated cell suspension, and this, together with the large conductivity increment derivable from these data (0.408 mS/cm), is consistent with the well-known fact that Gram-positive organisms normally have much higher potassium contents (Tempest 1969), and hence internal conductivities (cf. e.g. Pauly 1962; Marquis and Carstensen 1973; Asami et al. 1980), than do Gram-negative organisms. Secondly, the characteristic frequency as judged from the maximum value of ε'' (Fig. 15B) is significantly lower than that derived from the maximal value of σ'' (Fig. 15B) or from Eq. (7)

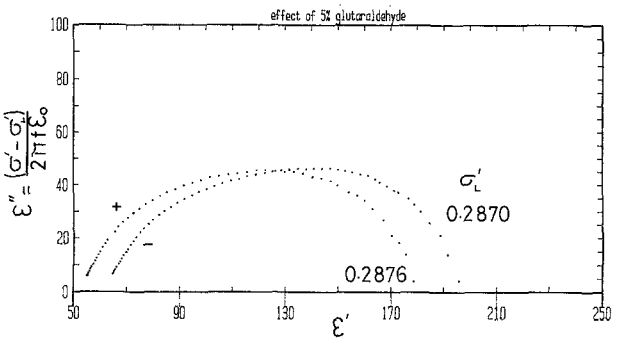

Fig. 16. Effect of glutaraldehyde treatment on the complex permittivity of *B. subtilis* protoplasts. The data are those of Fig. 14, and the presence (+) and the absence (−) of glutaraldehyde are as indicated; the σ_L' values used are in mS/cm. The effect of glutaraldehyde is particularly marked at the high-frequency end of the β-dispersion

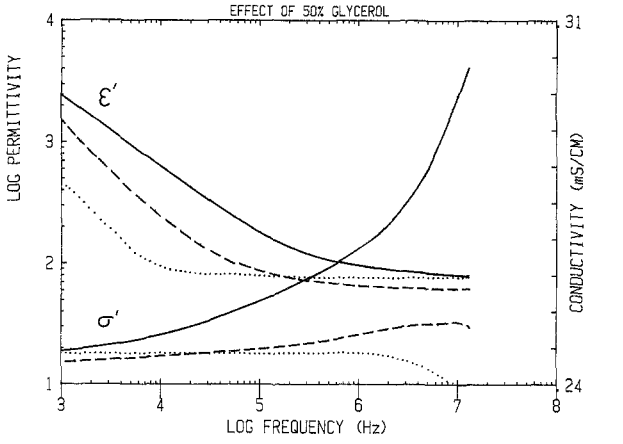

Fig. 17. Effect of glycerol on the dielectric properties of *Rps. capsulata* chromatophores. *Rps. capsulata* chromatophores (1.0 m*M* bacteriochlorophyll) were resuspended in water (*solid line*) or 50% glycerol (*dashed line*) at pH 6.95. The dotted line indicates the (apparent) dielectric properties of a KCl solution of the equivalent conductivity

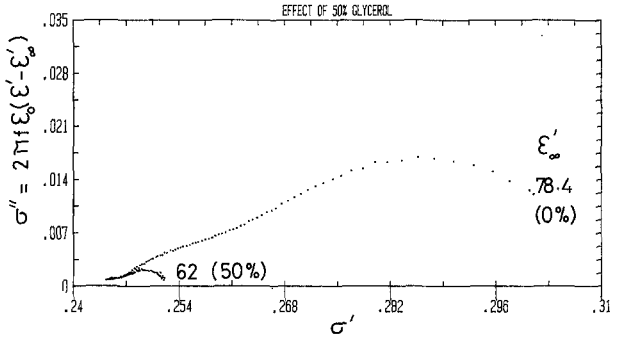

Fig. 18. Effect of glycerol on the complex conductivity of *Rps. capsulata* chromatophores. The data of Fig. 17 are plotted in the complex conductivity domain (in mS/cm) using the "high-frequency" permittivity values indicated for the glycerol concentrations given in parentheses

(ca. 50 MHz). Thirdly, in protoplasts, the α-dispersion is apparently absent, at least from the frequency range above 1 kHz, whilst the β-dispersion is partially sensitive to glutaraldehyde, particularly at the high-frequency end (Fig. 16). Thus, at least part of the β-dispersion cannot be due solely to the simplest type of Maxwell-Wagner mechanism. Nevertheless, the *breadth* (Cole-Cole α) of the β-dispersion is virtually unchanged by glutaraldehyde treatment, indicating that yet other mechanisms of dielectric relaxation, independent of chemical cross-linking reagents and distinct fromt the simplest type of Maxwell-Wagner dispersion, are occurring in this frequency range, and are observable even in the absence of an α-dispersion and despite the excellent sphericity of the protoplasts. Given the foregoing, we are inclined to implicate the relaxation, at higher-than-expected frequencies, of ions in the membrane-solution interphases, which interact with the extramembranal surfaces of the protein complexes at rates, and over distances, conforming to those of the frequency range under consideration. Thus, although the *gross* shape of the protoplasts is spherical, it is their *molecular* roughness (heterogeneity) that leads to a substantial broadening of the β-dispersion.

Membrane vesicles

In earlier work we (Kell 1983; Harris et al. 1984) studied the dielectric properties of bacterial chromatophores in the range 1 kHz to 13 MHz, and observed two dispersions, the more slowly relaxing of which (μ-dispersion) was fully, and the more rapidly relaxing of which (α-dispersion) was partially, destroyed by glutaraldehyde treatment. Neither dispersion was observable at the isoelectric point of chromatophores (Harris et al. 1984). It was concluded (Kell 1983, and see Kell and Westerhoff 1985) that the μ-dispersion was probably due predominantly to the somewhat restricted translational motions of the protein complexes of the chromatophore membrane.

Figure 17 compares the dielectric properties of *Rps. capsulata* chromatophores suspended either in distilled water or in 50% glycerol (pH 6.95 in each case), a treatment which in the latter case raises the bulk aqueous viscosity from ca. 1 cP to 7.5 cP (Rouse 1938). It is evident that the conductivity increment (Fig. 18), and hence the characteristic frequency, of both dispersions is substantially reduced by suspension of the chromatophores in 50% glycerol. The conclusion must be that, *from a dielectric standpoint,* the rotational and translational motions of membrane phospholipids and proteins are

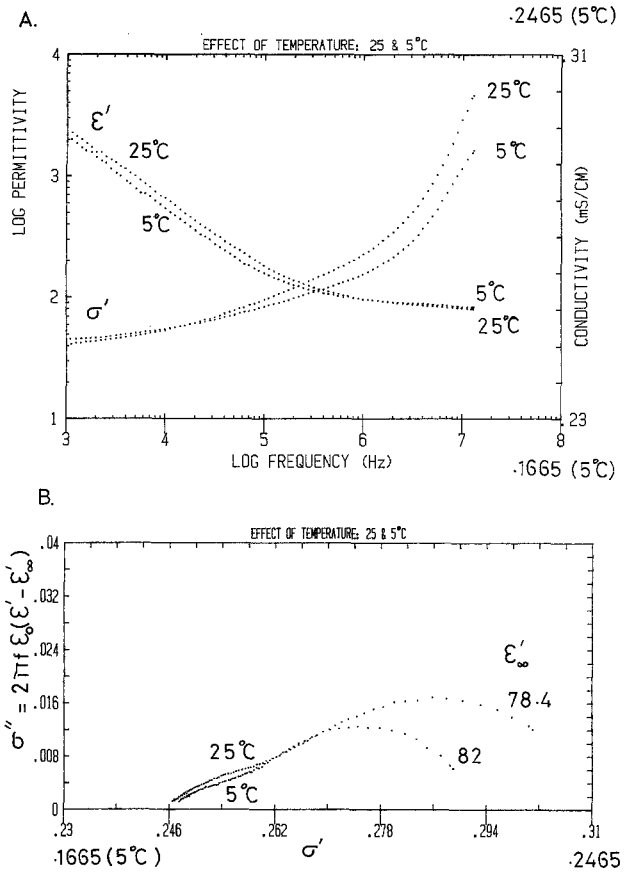

Fig. 19. Effect of temperature on the dielectric properties of *Rps. capsulata* chromatophores. The dielectric properties of a suspension of chromatophores (1.05 m*M* bacteriochlorophyll, pH 6.93) were obtained at 5° and 25 °C as indicated. **A** The data as obtained, **B** Complex conductivity plots of these data using the values of ε'_∞ indicated

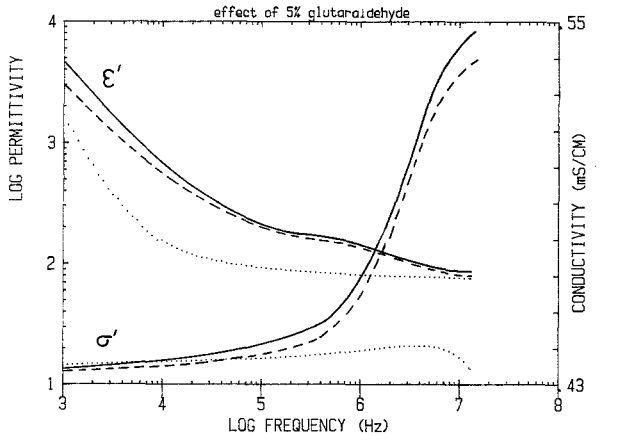

Fig. 20. Effect of glutaraldehyde on the dielectric properties of *P. denitrificans* membrane vesicles. *P. denitrificans* vesicles (12.8 mg protein/ml) were prepared as described in the Experimental section, and treated with glutaraldehyde (*dashed line*) as described in the legend to Fig. 13. A similar aliquot of vesicles was not so treated (*solid line*), and its pH (6.5) and conductivity adjusted to a similar value. The (apparent) dielectric properties of the suspending medium adjusted with KCl solution to a similar low-frequency conductivity, are also shown (*dotted line*)

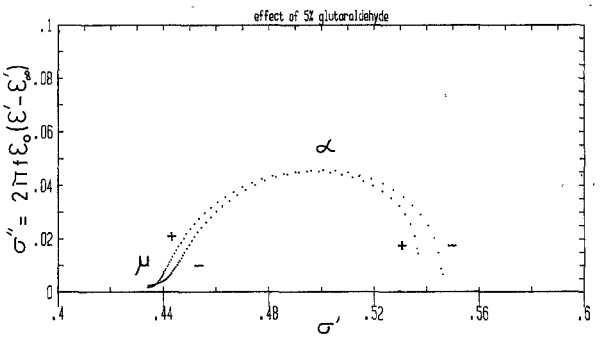

Fig. 21. Effect of glutaraldehyde on the complex conductivity of *P. denitrificans* membrane vesicles. The data of Fig. 20 are plotted for vesicles as prepared (−) or as treated (+) with 5% glutaraldehyde

tightly coupled to those of ions in the adjacent interface, since, in the Saffman-Delbrück treatment (Saffmann and Delbrück 1975, and see Kell and Harris 1985a), the translational diffusion coefficient should be barely affected by changes in the aqueous viscosity of this magnitude. Consistent with this view, lowering the temperature to 5 °C has a much smaller effect upon the dispersions (Fig. 19A and B) than does increasing the viscosity with glycerol, since the relaxation time, and hence the conductivity increment, should be affected by only 1%−2% per °C (Schwan and Foster 1980), if due to the double-layer ionic motions tangential to the charged membrane surfaces.

In contrast to bacterial chromatophores, which possess a fairly substantial μ-dispersion, that exhibited by *P. denitrificans* membrane vesicles is almost unobservable in the frequency range above 1 kHz, and is only partially affected by treatment of the vesicles with 5% glutaraldehyde (Figs. 20 and 21). Similar data (not shown) were obtained when the vesicles were further sonicated, using the method described by Hitchens and Kell (1982), consistent with the view that osmotically prepared vesicles from this organism are already extremely small (approx. 50 nm diameter). Part of the difference between the μ-dispersion of chromatophores and of *P. denitrificans* membrane vesicles may be due to the fact that the latter typically possess an orientation that is only 40% "right-side-out" (Kell et al. 1978b), whilst bacterial chromatophores are believed to have an orientation that is essentially 100% "inside out" relative to that of the intact cell (Kell et al. 1978a). Again, therefore, although part of the dispersions in the present frequency range are abolished by treatment with a cross-linking reagent, part is not, and we are again thus inclined to implicate *molecular*, rather than *vesicular*, heterogeneity as the cause of the broader-than-expected dispersions.

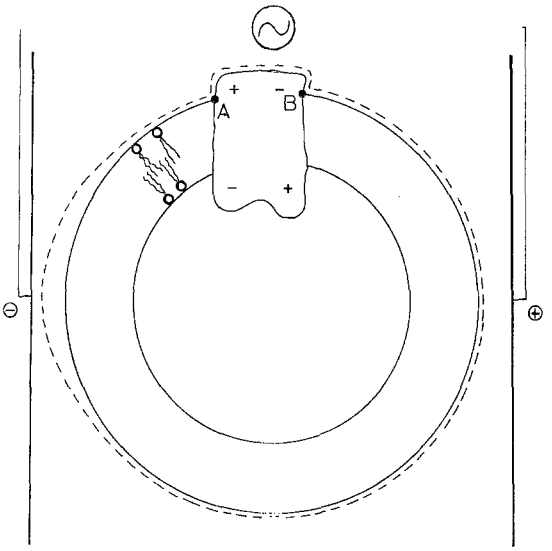

Fig. 22. A diagrammatic representation of the impediment to counterion relaxation caused by the protrusion of proteins above the surface of a typical energy coupling membrane. A single membrane vesicle, taken to be representative of a suspension held between two electrodes, is shown. A single protein complex is displayed, and for the present purposes it is assumed to be immobile. Polarisation of the electrical double layer by the field is impeded at points A and B by the presence of the protein. With the protein and vesicle in the orientation shown, no "vertical" field-induced polarisation of the double layer at the protein/solution interface is possible. Thus there is an impediment to the otherwise "free" relaxation of the double layer ions in a plane tangential to the membrane surface, and the relaxation time(s) of such an ion-cloud polarisation depend(s) not only on the *vesicle* radius, as in the classical model, but also on the molecular roughness of the vesicle surface(s). This phenomenon will have the effect of broadening a relaxation so as to favour the higher-frequency end, as observed

Discussion

Lysozyme sensitivity and the α-dispersion in intact microbial cells

One immediate and striking feature of the present observations is that the presence of a substantial α-dispersion in intact microbial cells is correlated with the lysozyme-sensitivity of the microorganisms. However, even when, as in *M. methylotrophus*, no α-dispersion is present, the β-dispersion is still rather broad to be accounted for solely in terms of a Maxwell-Wagner mechanism, given the reasonably narrow size-distribution to be expected for a typical microbial cell suspension (e.g. Asami et al. 1980). In this regard, one might imagine that if the β-dispersion is caused by *both* the "classical" Maxwell-Wagner mechanisms *and* by molecular motions in and around the cell envelope, then moving the "classical" mechanism to higher frequencies by increasing the internal and/or external conductivity (e.g. see Eq. (6)) would decrease the breadth of the β-dispersion so assessed. Whilst we could not perform this experiment explicitly with the present apparatus, the data of Figs. 2, 9 and 15, obtained with different organisms, are at least consistent with such a view (and see also e.g. Pauly 1962).

As discussed previously (Einolf and Carstensen 1969; Harris et al. 1984), the α-dispersion of intact microbial cells, where existing, is greatly decreased by lysozyme treatment (Figs. 8, 13 and 14). Nevertheless, both the remaining α- and β-dispersions are somewhat broader than one might expect if the mechanisms underlying these dispersions are simply the classical "counterion polarisation" and "Maxwell-Wagner" mechanisms. Could other molecular motions therefore be involved?

Effects of cross-linking reagents on the dielectric properties of bacterial protoplasts

Glutaraldehyde modifies both the α- and β-dispersions in protoplasts of both *P. denitrificans* (Fig. 13) and *B. subtilis* (Fig. 14). In the former case, the effect on the β-dispersion is particularly noticeable at the high-frequency end, whilst the α-dispersion appears to be completely obviated in the frequency range above 1 kHz. That glutaraldehyde significantly decreases dielectric relaxations in this frequency range would seem to argue against the possibility that there are negligible barriers to free, long-range, random lateral motions of lipids and proteins in these membranes since, if this were the case, the characteristic frequency of such a relaxation should be much lower, approximately 10 Hz, in vesicles (protoplasts) of the present size (Kell and Harris 1985a). In the case of *B. subtilis*, the α-dispersion in the absence of the cross-linking reagent is already immeasurably small, so that only the β-dispersion is affected. Nevertheless, although the size heterogeneity of this preparation should be negligible, the β-dispersion remains much broader than that of a pure Debye dispersion. Since, in the presence of the glutaraldehyde, both intra- and inter-molecular motions should be inhibited *within* the membrane, it is necessary to invoke the following concept (Fig. 22) to explain the data.

The classical explanations of the α- and β-dispersions in spherical shell membrane vesicle systems treat the particles as molecularly smooth, so that the relaxation times depend, all other things being equal, on the geometric radius of the spheres alone. However, the typical protein complexes of the energy coupling membrane extend significantly beyond

the "surfaces" delineated by the phospholipid head groups (see e.g. Hackenbrock 1981; Capaldi 1982), so that the relative surface area that they occupy is lower than that of the protein:lipid weight ratio. Thus, even in the absence of molecular motions of the lipids and proteins themselves, a broadening of relaxation times due to the "tangential relaxation" mechanism is to be expected. It is evident that the relaxation time(s) for such "tangential" relaxation will depend rather finely on the height, shape and disposition of the proteins in such a system, and since all of the polypeptides existing in energy coupling membranes have not yet even been *identified* (Kell and Westerhoff 1985), further discussion of this lies outside our present scope. The effects of glutaraldehyde on membrane vesicles from *P. denitrificans* (Figs. 20 and 21) are quite consistent with the above analysis.

The μ-dispersion in bacterial chromatophores

This dispersion was first identified in bacterial chromatophores, since they are sufficiently small that their "classical" α- and β-dispersions are shifted to much higher frequencies than is usual (Kell 1983). It was sensitive to glutaraldehyde (Kell 1983) and was not observed at the chromatophores' isoelectric point (Harris et al. 1984), and was ascribed predominantly to some motional characteristic of the protein complexes of bacterial chromatophores. The present findings (Figs. 17 and 19), that it is much more sensitive to changes in the viscosity of the aqueous phase than to temperature changes, indicate that the molecular motions in question are much more strongly coupled to the ionic and dipolar motions in the double layer than was previously supposed, and are consistent with the suggestions in the previous paragraph that the molecular granularity in this type of system leads to a much broader spectrum of relaxation times than would be expected from a simple macroscopic treatment alone. Studies of reconstituted proteoliposomes containing components of known hydrodynamic properties and gross molecular structure could evidently be of value in furthering our understanding of this type of behaviour. If glutaraldehyde were to cause inter-chromatophore cross links, it is not absolutely impossible that rotation of the entire chromatophore vesicle might contribute to the μ-dispersion (Kell and Harris 1985a); this explanation is not feasible for significantly larger systems. The relative absence of a μ-dispersion in *P. denitrificans* membrane vesicles may be ascribed to one or more of the following: (a) the relatively lower proportion of "inside-out" vesicles in the *Paracoccus* preparation, (b) the even greater restriction on long-range lateral motions of membrane lipids and proteins in the respiratory system relative to the photosynthetic one, or (c) a different isoelectric point. In terms of the second possibility, one should expect a characteristic frequency of approximately 1 kHz (Kell and Harris 1985) if the long-range lateral diffusion were free and random in vesicles of the present size (diameter approximately 50 nm). That no such relaxation is observed adds further weight to the evidence that such "long-range" lateral motions in biomembranes are far more restricted than is implicit in the standard "fluid mosaic" membrane model (Kell 1984a).

Concluding remarks

The studies reported herein allow us to make the following general remarks about the dielectric properties of microbial systems in the frequency range 1 kHz–13 MHz:

(1) The magnitude of the α-dispersion of intact cells is correlated to the sensitivity of the cells to lysozyme treatment;

(2) The dielectric spectra of protoplasts of *P. denitrificans* and *B. subtilis* are affected over the entire frequency range when they are treated with the cross-linking reagent glutaraldehyde, indicating that rotational and translational motions, and perhaps intra-complex motions, contribute to the dielectric relaxations in this case;

(3) Nevertheless, the remaining dispersions are still broader than should be expected in terms of a classical view of the α- and β-dispersions, and suggest that the molecular roughness of the membrane surface(s) contribute(s) to the apparent spread of relaxation times;

(4) The effects of glycerol and of temperature on the dielectric properties of bacterial chromatophores are consistent with this view.

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References

Adey WR (1981) Tissue interactions with nonionising electromagnetic fields. Physiol Rev 61:435–514

Asami K, Irimajiri A (1984) Dielectric dispersion of a single spherical bilayer membrane in suspension. Biochim Biophys Acta 769:370–376

Asami K, Hanai T, Koizumi N (1980) Dielectric analysis of *Escherichia coli* in the light of the theory of interfacial polarization. Biophys J 31:215–228

Capaldi RA (1982) Arrangement of proteins in the mitochondrial inner membrane. Biochim Biophys Acta 694:291–306

Carstensen EL, Marquis RE (1975) Dielectric and electrochemical properties of bacterial cells. In: Gerhardt P, Costilow RN, Sadoff HL (eds) Spore VI. American Society for Microbiology, Washington, pp 563–571

Cole KS (1972) Membranes, ions and impulses. University of California Press, Berkeley

Cole KS, Cole RH (1941) Dispersion and absorption in dielectrics. I. Alternating current chracteristics. J Chem Phys 9:341–351

Curds CR, Roberts DMcL, Wu C-H (1978) The use of continuous cultures and electronic sizing devices to study the growth rate of two species of ciliated protozoa. Soc Bacteriol Weybridge, vol 11. Academic Press, London, pp 165–177

Davies GE, Stark GR (1970) Use of dimethyl suberimidate, a cross-linking reagent, in studying the subunit structure of oligomeric proteins. Proc Natl Acad Sci USA 66:651–656

Davis DH, Doudoroff M, Stanier RY, Mandel M (1969) Proposal to reject the genus *Hydrogenomonas:* Taxonomic implications. Int J Syst Bacteriol 19:375–390

Donnellan EJ Jr, Nags EA, Levinson HS (1964) Chemically defined, synthetic media for sporulation and for germination and growth of *Bacillus subtilis.* J Bacteriol 87:332–336

Dunn G, Torgerson DM, Mandelstam J (1976) Order of expression of genes affecting septum location during sporulation of *Bacillus subtilis.* J Bacteriol 125:776–779

Einolf CW, Carstensen EL (1969) Passive electrical properties of microorganisms. IV. Studies of the protoplasts of *Micrococcus lysodeikticus.* Biophys J 9:634–643

Einolf CW, Carstensen EL (1973) Passive electrical properties of microorganisms. V. Low fequency dielectric dispersions of bacteria. Biophys J 13:8–13

Fricke H, Schwan HP, Li K, Bryson V (1956) A dielectric study of the low-conductance surface membrane in *E. coli.* Nature 177:134–135

Grant EH, Sheppard RJ, South GP (1978) Dielectric behaviour of biological molecules in solution. Oxford University Press, London

Grant FA (1958) Use of complex conductivity in the representation of dielectric phenomena. J Appl Phys 29:76–80

Hackenbrock CR (1981) Lateral diffusion and electron transfer in the mitochondrial inner membrane. Trends Biochem Sci 6:151–154

Harold FM (1977) Ion currents and physiological functions in microorganisms. Ann Rev Microbiol 31:181–203

Harris CM, Kell DB (1983) The radio-frequency dielectric properties of yeast cells measured with a rapid, frequency-domain dielectric spectrometer. Bioelectrochem Bioenerg 11:15–28

Harris CM, Hitchens GD, Kell DB (1984) Dielectric spectroscopy of microbial membrane systems. In: Allen MJ, Usherwood PNR (eds) Charge and field effects in biosystems. Abacus Press, Tunbridge Wells, pp 179–185

Hitchens GD, Kell DB (1982) On the extent of localization of the energised membrane state in chromatophores from *Rhodopseudomonas capsulata* N22. Biochem J 206:351–357

Hitchens GD, Kell DB (1984) On the effects of thiocyanate and venturicidin on respiration-driven proton translocation in *Paracoccus denitrificans.* Biochim Biophys Acta 766:222–232

Houslay MD, Stanley KK (1982) Dynamics of biological membranes. Wiley, Chichester

John P, Whatley FR (1977) The bioenergetics of *Paracoccus denitrificans.* Biochim Biophys Acta 463:129–153

Junge W (1982) Electrogenic reactions and proton pumping in green plant photosynthesis. Curr Top Membr Trans 16:431–465

Kell DB (1983) Dielectric properties of bacterial chromatophores. Biolectrochem Bioenerg 11:405–415

Kell DB (1984a) Diffusion of protein complexes in prokaryotic membranes: Fast, free random or directed? Trends Biochem Sci 9:86–88

Kell DB (1984b) Dielectric spectroscopy of the rotational and translational motions of membrane proteins: Theory and experiment. EBEC Rep 3:645–646

Kell DB, Harris CM (1985a) On the dielectrically observable consequences of the diffusional motions of lipids and proteins in membranes. I. Theory and overview. Eur Biophys J 12:181–197

Kell DB, Harris CM (1985b) Dielectric spectroscopy and membrane organization. J Bioelectricity (in press)

Kell DB, Westerhoff HV (1985) Catalytic facilitation and membrane bioenergetics. In: Welch GR (ed) Organised multienzyme systems: catalytic properties. Academic Press, New York, pp 63–139

Kell DB, Ferguson SJ, John P (1978a) Determination by a flow dialysis technique of the protonmotive force in chromatophores from *Rhodospirillium rubrum.* Comparison with phosphorylation potential. Biochim Biophys Acta 502:111–126

Kell DB, John P, Ferguson SJ (1978b) The protonmotive force in phosphorylating membrane vesicles from *Paracoccus denitrificans.* Magnitude, sites of generation and comparison with the phosphorylation potential. Biochem J 174:257–266

Kosterich JB, Foster KR, Pollack SR (1983) Dielectric permittivity and electrical conductivity of fluid-saturated bone. IEEE Trans Biomed Eng BME-30:81–86

Kubitschek HE (1969) Counting and sizing microorganisms with the Coulter counter. In: Norris JR, Ribbons DW (eds) Methods in microbiology, vol 1. Academic Press, London, pp 593–610

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Marquis RE, Carstensen EL (1973) Electric conductivity and internal osmolarity of intact bacterial cells. J Bacterial 113:1198–1206

McCarthy JEG, Ferguson SJ, Kell DB (1981) Estimation with an ion-selective electrode of the membrane potential in cells of *Paracoccus denitrificans* from the uptake of the butyl triphenyl phosphonium cation during aerobic and anaerobic respiration. Biochem J 196:311–321

McLaughlin SGA, Dilger JP (1980) Transport of protons across membranes by weak acids. Physiol Rev 60:825–863

Means GE, Feeney RE (1971) Chemical modification of proteins. Holden-Day, San Francisco, pp 89–93

Pauly H (1962) Electrical properties of the cytoplasmic membrane and the cytoplasm of bacteria and of protoplasts. IRE Trans Biomed Electron 9:93–95

Pauly H, Packer L (1960) The relationship of internal conductance and membrane capacity to mitochondrial volume. J Biophys Biochem Cytol 7:603–612

Pauly H, Schwan HP (1959) Über die Impedanz einer Suspension von kugelförmigen Teilchen mit einer Schale. Ein Modell für das dielektrische Verhalten von Zellsuspension und von Protein Lösungen. Z Naturforsch 14 B:125–131

Pauly H, Packer L, Schwan HP (1960) Electrical properties of mitochondrial membranes. J Biophys Biochem Cytol 7:589–601

Peters K, Richards FM (1977) Chemical cross-linking reagents and problems in studies of membrane structure. Annu Rev Biochem 46:523–551

Pethig R (1979) Dielectric and electronic properties of biological materials. Wiley, Chichester

Pethig R (1984) Dielectric properties of biological materials: Biophysical and medical applications. IEEE Trans Electr Insulat EI-19:453–474

Pilla AA (1980) Electrochemical information transfer at cell surfaces and junctions. Application to the study and manipulation of cell regulation. In: Keyzer H, Gutmann F (eds) Bioelectrochemistry. Plenum Press, New York, pp 353–396

Ramaley RF, Burden L (1970) Replacement sporulation of *Bacillus subtilis* 168 in a chemically defined medium. J Bacteriol 101:1–8

Rouse H (1938) Fluid mechanics for hydraulic engineers. McGraw-Hill, New York, p 407

Saffman PG, Delbrück M (1975) Brownian motion in biological membranes. Proc Natl Acad Sci USA 72:3111–3113

Schanne OF, Ceretti ERP (1978) Impedance measurements in biological cells. Wiley, Chichester

Scholes P, Smith L (1968) The isolation and properties of the cytoplasmic membrane of *Micrococcus denitrificans*. Biochim Biophys Acta 153:350–362

Schwan HP (1957) Electrical properties of tissue and cell suspensions. In: Lawrence JH, Tobias CA (eds) Advances in biological and medical physics, vol 5. Academic Press, New York, pp 147–209

Schwan HP (1959) Alternating current spectroscopy of biological substances. Proc IRE 47:1841–1855

Schwan HP (1977) Field interaction with biological matter. Ann NY Acad Sci 303:198–213

Schwan HP (1981a) Dielectric properties of biological tissue and biophysical mechanisms of electromagnetic field interactions. ACS Symp Ser 157:109–131

Schwan HP (1981b) Electrical properties of cells: Principles, some recent results and some unresolved problems. In: Adelmann WJ jr, Goldman DE (eds) The biological approach to excitable systems. Plenum Press, New York, pp 3–24

Schwan HP (1983a) Dielectric properties of biological tissues and cells at RF- and MW-frequencies. In: Grandolfo M, Michaelson SM, Rindi A (eds) Biological effects and dosimetry of nonionizing radiation. Plenum Press, New York, pp 195–211

Schwan HP (1983b) Dielectric properties of biological tissues and cells at ELF-frequencies. In: Grandolfo M, Michaelson SM, Rindi A (eds) Biological effects and dosimetry of nonionizing radiation. Plenum Press, New York, pp 549–559

Schwan HP, Foster KR (1980) RF-field interactions with biological system: Electrical properties and biophysical mechanisms. Proc IEEE 68:104–113

Schwan HP, Takashima S, Miyamoto VK, Stoeckenius W (1970) Electrical properties of phospholipid vesicles. Biophys J 10:1102–1119

Smith SRL (1980) Single-cell protein. Philos Trans R Soc B 290:341–354

Stoy RD, Foster KR, Schwan HP (1982) Dielectric properties of mammalian tissue from 0.1 to 100 MHz: a summary of recent data. Phys Med Biol 27:501–513

Tempest DW (1969) Quantiative relationships between inorganic cations and anionic polymers in growing bacteria. Symp Soc Gen Microbiol 19:87–111

Vasey RB, Powell KA (1984) Single-cell protein. Biotechnol Genet Eng Rev 2:285–311

Vignais PM, Henry M-F, Sim E, Kell DB (1981) The electron transport system and hydrogenase of *Paracoccus denitrificans*. Curr Top Bioenerg 12:115–196

Windass JD, Worsey MJ, Pioli EM, Pioli D, Barth PT, Atherton KT, Dart EC, Bryrom D, Powell K, Senior PJ (1980) Improved conversion of methanol to single-cell protein by *Methylophilus methylotrophus*. Nature 287:396–401

Zimmermann U (1982) Electric field-mediated fusion and related electrical phenomena. Biochim Biophys Acta 694:227–277

Zimmermann U, Schulz J, Pilwat G (1973) Transcellular ion flow in *E. coli* B and electrical sizing of bacteria. Biophys J 13:1005–1012