624—DIELECTRIC PROPERTIES OF BACTERIAL CHROMATOPHORES *

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SYMBOLS USED IN THIS PAPER

ϵ'	real part of the complex permittivity
$\epsilon'' = \dfrac{\sigma' - \sigma_l'}{2\pi f \epsilon_r}$	imaginary part of the complex permittivity; dielectric loss
ϵ_r	permittivity of free space $= 8.854 \times 10^{-14}$ F/cm
σ'	real part of the complex conductivity
$\sigma'' = 2\pi f \epsilon_r (\epsilon' - \epsilon_h')$	imaginary part of the complex conductivity
$\Delta\epsilon' = \epsilon_l' - \epsilon_h'$	dielectric increment; magnitude of dispersion
$\Delta\sigma' = \sigma_h' - \sigma_l'$	conductivity increment
P	volume fraction of membrane-bounded space
r	radius of spherical shell membrane
C_m	membrane capacitance per unit area
D	2-dimensional diffusion coefficient
f_c	critical (characteristic) frequency of a dispersion
$\tau = 1/(2\pi f_c)$	relaxation time
f	frequency (Hz)

Subscripts

l	limiting value at low frequencies
h	limiting value at high frequencies

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SUMMARY

(1) The dielectric properties of suspensions of chromatophores from *Rhodopseudomonas capsulata* have been measured in the range 1 kHz to 13 MHz. Neither of the two dielectric dispersions observed is greatly affected by changes in the number or valency of added ions.

(2) The more slowly relaxing (μ-) dispersion is eliminated by treatment of the chromatophores with glutaraldehyde, which has a much smaller effect upon the more rapidly-relaxing (α-) dispersion.

(3) The α-dispersion is ascribed predominantly to the relaxation of ions tangential to the charged chromatophore surface, whilst the novel μ-dispersion is largely ascribed to the mobility of charged protein complexes in the plane of the membrane. Such measurements offer, in principle, a convenient means of estimating the rotational and lateral diffusion coefficients of membrane protein complexes.

INTRODUCTION

Present-day bacterial bioenergetics is dominated by the realisation that the catalytic activity of many membrane-located enzymes is more or less tightly coupled to the transfer of electrical charge, especially protons, across the bacterial cytoplasmic membrane (e.g. Ref. 1). Yet, many experimental approaches (see e.g. Refs. 2–6) have indicated that the charge-separating reactions implicated in processes such as electron transport phosphorylation are more microscopic than is implicit in the widely discussed, and more easily tested, *delocalised* chemiosmotic coupling model.

Since small ions (and other charged groups) exhibit dielectric relaxation at much lower frequencies when they are membrane-associated than when they are freely diffusible in a bulk aqueous phase, we have initiated a study of the dielectric properties of a variety of proton-motive systems in the audio- and radio-frequency ranges, the better to understand the electrical properties of such systems. Such measurements can, in principle [5,7], distinguish energy-dependent changes in bulk- and surface-charge distribution.

Since bacterial chromatophores provide an excellent system for the study of electron transport phosphorylation [2,8], we have carried out measurements of the dielectric properties of chromatophores from *Rhodopseudomonas capsulata* N22. The present article describes the results of such measurements in unilluminated chromatophores.

EXPERIMENTAL

Chromatophores from *Rps. capsulata* N22 were prepared as described [9], except that they were washed twice, and resuspended, in 0.1 M sorbitol/0.5 mM MgSO$_4$, adjusted to pH 7.3 (at room temperature) with Tris base (0.5–1 mM). Photophosphorylation rates, measured as described previously [9], of chromatophores washed in this way were 1–2 μmol/min per μmol bacteriochlorophyll. Where noted, chromatophores were washed in the above medium lacking MgSO$_4$ and containing 1 mM sodium EDTA. Bacteriochlorophyll was measured as described [9].

Dielectric measurements were performed as described [10] using a Hewlett-Packard 4192A Impedance Analyser controlled by an HP 85 microcomputer. Programs and

data were stored on HP 9121D micro floppy discs, and data plotted on an HP 7225A digital plotter. The cell used was of the pin type [11], and electrodes were of Pt, plated with Pt black as described [10]. The temperature was controlled at 25 °C using a water jacket. The cell constant was obtained using solutions of known conductivity and permittivity [10], such measurements also providing controls which ensured that electrode polarisation made a negligible contribution to the dielectric properties under the conditions chosen. Zero open calibrations (see Ref. 10) were carried out at 10 MHz. All measurements were carried out with a black cloth over the sample cell.

RESULTS AND DISCUSSION

Dielectric properties of bacterial chromatophores

A typical plot of the dielectric properties between 1 kHz and 13 MHz of a suspension of *Rps. capsulata* chromatophores is displayed in Fig. 1. From the complex plane plots of these data displayed in Fig. 2, it is evident that in this frequency range chromatophores are possessed of two sizeable dielectric dispersions, dispersions which are most clearly separated in the complex conductivity plot. The magnitude of both dispersions was strictly linear with the concentration of chromatophores up to a bacteriochlorophyll concentration of at least 1.2 mM.
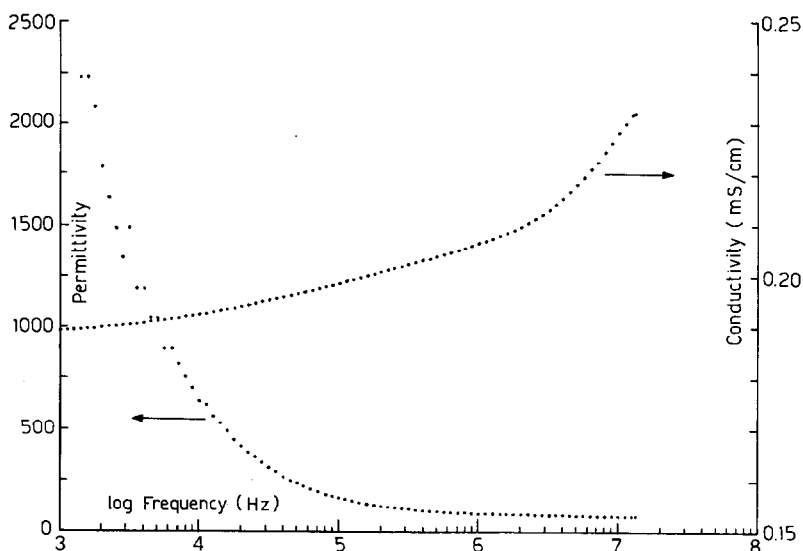

Fig. 1. Dielectric properties of *Rps. capsulata* chromatophores. Measurements were performed as described in the Experimental section on a suspension of chromatophores (0.72 mM bacteriochlorophyll) in 0.1 M sorbitol, 0.5 mM MgSO$_4$, 0.5 mM Tris pH 7.3. Data are displayed exactly as plotted by the microcomputer system.
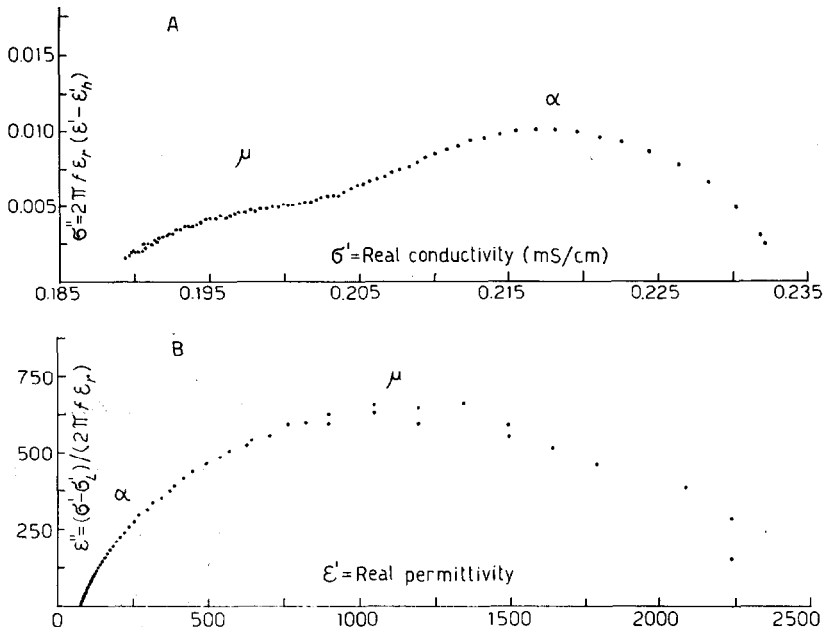
Fig. 2. Complex plane plots of the dielectric properties of *Rps. capsulata* chromatophores. The data from Fig. 1 are plotted as (A) the complex conductivity and (B) the complex permittivity. Symbols as in Ref. 10.

Consideration of the extensive literature covering the dielectric properties of membrane vesicles in this frequency range (see e.g. Refs. 10, 12–17) might lead one to suspect that the two dielectric dispersions could be ascribed to the so-called α- and β-dispersions. The latter is caused by the well-known Maxwell–Wagner mechanism [10,12–17], but several lines of evidence indicate that neither dispersion (Figs. 1 and 2) is in fact due to this latter mechanism.

If we assume that bacterial chromatophores enclose a volume of approx. 50 mm^3/μmol bacteriochlorophyll, and have a radius typically of 18 nm [18,19], the volume fraction P of the chromatophores in Figs. 1 and 2 is approximately 0.036. The dielectric increment $\Delta\epsilon'$ of the Maxwell–Wagner dispersion of such vesicles is given [10,12–17] by $\Delta\epsilon' = 9PrC_m/4\epsilon_r$, where ϵ_r is the permittivity of free space (8.854×10^{-14} F/cm) and C_m is the membrane capacitance, assumed to be approx 1 μF/cm^2 [19]. Substituting appropriate values, we obtain $\Delta\epsilon < 5$ relative permittivity units (compare Refs. 20, 21), a value far lower than those observed (Fig. 2B).

Further, neither dispersion was significantly affected (data not shown) by the presence of 0.5 % w/V Triton X-100, a detergent concentration more than sufficient fully to permeabilise to low molecular weight ions biomembranes such as those of chromatophores [18] and those of a number of intact bacteria (C.M. Harris and D.B. Kell, unpublished). In addition, the dielectric relaxation time of the β-dispersion in these very small vesicles, as calculated in the usual way [10,12–17], as well, of course,

as that of the γ-dispersion, lies somewhat above the range of frequencies presently investigated [20,21]. Thus, in superficial terms, the dielectric dispersions observed in the present work might be regarded as equivalent to that usually referred to as *the* α-dispersion [12]. We do not, however, think that this is an appropriate view, since the quality and quantity of the present data do demonstrate quite unequivocally the presence of *two* separate and clearly, even if imperfectly, resolvable dispersions.

The α-dispersion

The α-dispersion, already discussed in some detail in the 1930s for suspensions of a variety of spherical charged particles [22], is usually broadly and semi-quantitatively ascribed to the relaxation, in a direction parallel (tangential) to the particle surface, of the ion cloud constituting the diffuse double layer surrounding the particles, i.e. to a *surface admittance*.

To summarise a large, detailed and still uncertain theoretical literature, we may make the following remarks. Schwarz's insightful and now classical treatment [23] was criticised because it lacked a *d.c.* conductance contribution [24]. Nevertheless, it was confirmed that the magnitude of the (very large) low frequency permittivity due to the presence of the surface charges was roughly proportional to the size and surface charge density of the particles, whilst the relaxation time was proportional to the square of the particle radius [25,26].

However, in none of these studies were the effects of surface and/or zeta potential and bulk phase ionic composition (valency and concentration) systematically considered. In a suspension such as that of chromatophores, the situation becomes extremely complex if one seeks a rigorous, quantitative treatment. Parameters that must be considered if one wishes adequately to model the interfacial electrical properties of such a system include the surface charge density, bulk pH, ionic strength, degree of particle heterogeneity, and extent and nature of specific and non-specific ion binding (i.e. binding *versus* screening [27,28]).

Certainly the general conception of a surface admittance adequately explains the general properties (relaxation time and dielectric increment) of the α-dispersion. However, given that:

(i) there is already an imperfect separation of the presently observed dispersions,

(ii) there is no universally accepted theory either of the electrical double layer or its effect upon the dielectric properties of suspensions, and

(iii) our interests are predominantly biological, we refer readers to a number of articles which discuss in detail a variety of recent theoretical models [29–35].

In practice, it is not our view that the present experimental studies allow a distinction between the various available treatments. Rather, such urgently needed studies should be performed on homogeneous and durable particles of known surface charge density immersed in suitable concentrations of ions of different valency. However, we note that *charged* phospholipid vesicles of approximately the same radius as chromatophores have been shown to possess a typical α-dispersion corresponding both in relaxation time and magnitude to the *faster-relaxing* process
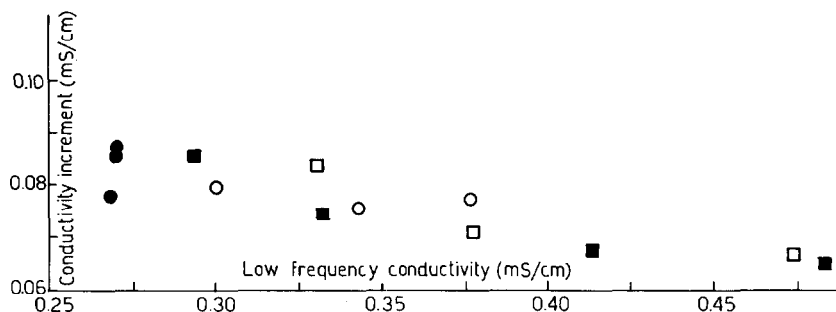
Fig. 3. Effect of small ions on the total conductivity increment of chromatophores of *Rps. capsulata*. Measurements were made as described in the legend to Fig. 1, except that the bulk ionic conductivity was varied by the addition of KCl (○), MgSO$_4$ (■) or tris(ethylene diamine) cobaltic(III) chloride (□). Basal medium (●) was as in Fig. 1. Total conductivity increments were obtained from complex conductivity diagrams (see Fig. 2A) and represent the total contributions of both observed dispersions.

of the two distinguishable in the present work [20,21]. We thus refer to *this* dispersion as the α-dispersion.

Since we have used a 2-electrode technique in the present work, the range of bulk conductivity values testable was restricted by the incidence of significant electrode polarisation (see Refs. 11, 36) at the higher conductivity values. The lower values attainable were limited by our desire to maintain the pH at 7.3 for the present purposes, necessitating the inclusion of Tris base, since the isoelectric point of chromatophores is certainly below pH 5.5 [37,38]. Over the rather limited range of bulk conductivities tested, we could discern only a very weak dependence of the conductivity increment on the magnitude of the bulk conductivity, but none on the valency of the ions contributing to it (Fig. 3); for reasons discussed above, a more systematic study of this point was not undertaken, although the lack of effect of ion valency on the α-dispersion is not easily understood in terms of classical double layer theory.

The μ-dispersion

Our next desire was to understand the type of mechanism underlying the second, more slowly relaxing, dielectrically observable process. It did not seem likely that this might be due to a radial ion current, since this was to be expected (if present) at much higher frequencies (e.g. Ref. 39). One possibility we entertained was that whilst the α-dispersion would be due to the relaxation of ions in the diffuse part of the double layer, the lower-frequency dispersion might be due to a relaxation of more tightly bound (and hence more slowly relaxing) ions in the Stern layer [40]. The strong interaction (and potentially rapid exchange) between ions in the two layers would indeed account for the incomplete separability of the two dispersions (and thus, incidentally, the breakdown of the superposition theorem [41]).

However, neither the magnitude (Fig. 3) nor the form (relative contribution of each relaxation to the total conductivity increment) (data not shown) changed in the
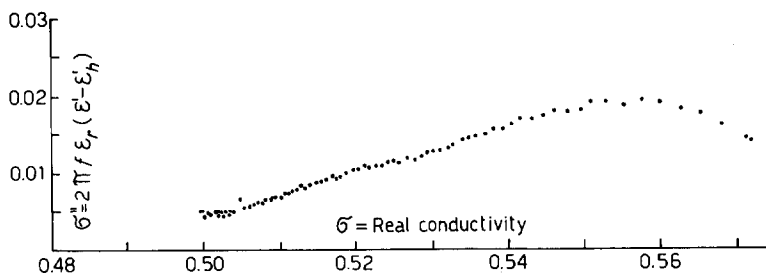
Fig. 4. Lack of effect of EDTA upon the dielectric properties of chromatophores. Measurements were made as described in the legend to Figs. 1 and 2, except that the chromatophores were washed twice, and resuspended in 0.1 M sorbitol, 1 mM EDTA, 1 mM Tris pH 7.3. Bacteriochlorophyll concentration $= 1.05$ mM.

presence or absence of millimolar concentrations of divalent and trivalent ions which might have been expected [27,28,37,42,43] strongly to bind in the Stern layer. Further, when chromatophores were washed twice, and their dielectric properties assessed, in a medium lacking divalent ions and containing 1 mM sodium EDTA/0.1 M sorbitol and 0.5 mM Tris base, both dispersions were still visible (Fig. 4). Thus the more slowly-relaxing dispersion did not seem realistically ascribable to the relaxation of relatively tightly bound ions in the Stern layer.

Field-mediated mobility of protein complexes in the plane of the membrane as the cause of the μ-dispersion

It has been known for some time, and was explicitly mentioned in Singer and Nicolson's celebrated article on the fluid mosaic model [44], that the application of an electrical field to a biomembrane vesicle suspension can alter the disposition of charged protein complexes in the fluid phospholipid membrane phase, and a number of more recent, detailed and elegant studies have beautifully demonstrated the reality of such phenomena (e.g. Refs. 16, 45–49).

Now, such an effect should *necessarily* be accompanied by (and visible as) a frequency dependence of the passive electrical properties of such membrane vesicles (i.e. as a dielectric dispersion), although we are not in fact aware that this type of dispersion has been explicitly discussed or observed in the literature to date. To test whether this mechanism might be the cause of our lower-frequency dispersion, we studied the effect of the cross-linking reagent glutaraldehyde on the dielectric properties of the chromatophores (Fig. 5). It is evident (cf. Figs. 4 and 5) that glutaraldehyde destroyed the more slowly relaxing component whilst having a much smaller effect upon the α-dispersion. The slight shift in the (bulk) low frequency conductivity elicited by glutaraldehyde is due to the release of intra-chromatophore ions. Thus, crosslinking, which will destroy the mobility of protein complexes, removed the more slowly-relaxing dispersion, which we term the μ-dispersion (μ representing mobility). That the α-dispersion remained relatively unaffected by
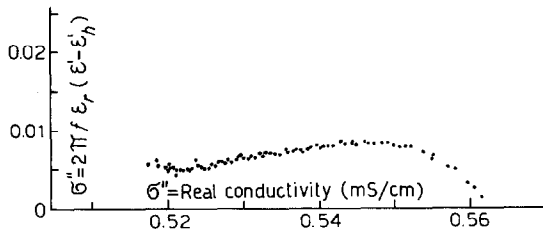
Fig. 5. Effect of glutaraldehyde on the dielectric properties of *Rps. capsulata* chromatophores. Measurements were made on the suspension described in the legend to Fig. 4, except that chromatophores were preincubated with 2.5 % glutaraldehyde for 30 min.

glutaraldehyde confirms our assumption that it reflects, at least partially, the relaxation of the ion cloud surrounding the charged chromatophore membrane, although we recognise that glutaraldehyde is likely to decrease the surface charge density of the chromatophores. Further, the mobility of the charged phospholipid molecules themselves may also contribute to the α-dispersion.

To confirm that the μ-dispersion is indeed due to the movement of the charged protein complexes in the plane of the chromatophore membrane, it would be desirable to study the effect of temperature, since protein mobility should be severely decreased at temperatures below the gel-to-liquid phospholipid phase transition. However, this transition occurs well below 0 °C in chromatophores [50], and the experiment is thus not easily performed. The use of photopolymerisable-phospholipid-containing proteoliposomes (see e.g. Refs. 51 and 52), or vesicles containing
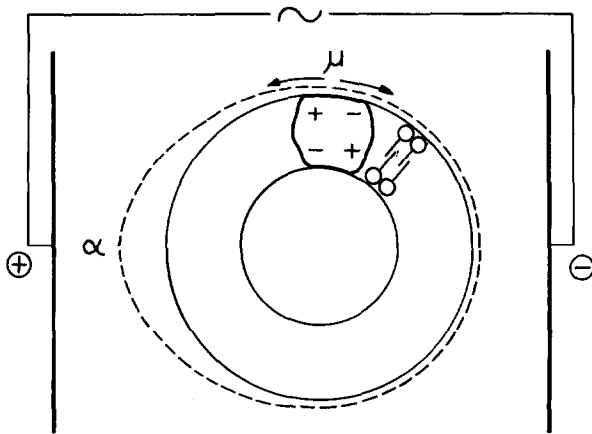

Fig. 6. Diagrammatic representation of proposed mechanisms contributing to the dielectric dispersions observed in bacterial chromatophores. The α-dispersion is caused by polarisation of the ion cloud surrounding the charged chromatophore surface, and possibly partly by the lateral mobility of charged phospholipid molecules, whilst the μ-dispersion is caused, predominantly, by the diffusional and rotational mobility of charged protein complexes in the plane of the fluid chromatophore membrane.

lipids with a more convenient melting temperature than those in chromatophores, should allow a definitive confirmation of our proposed mechanisms (Fig. 6). Other possible mechanisms conceivably contributing to the μ-dispersion, such as trans-membrane ion transfer [12] or vesicle rotation [16], would not seem to explain its sensitivity to glutaraldehyde. Finally, we note that the μ-dispersion is *not* seen in pure liposomes lacking protein [20]. We have not so far extended our studies with the present system to lower frequencies.

Calculation of diffusion coefficients of membrane protein complexes by dielectric measurements

Can we obtain quantitative information from the parameters of the μ-dispersion? At present the exact mechanism(s) of field-induced *lateral electrophoresis* [45] is (are) not yet established [49,53,54]. However, we would concur with the view [54] that the faster-relaxing ion cloud polarisation mechanism will be expected to interact with that due to mobility of the protein complexes, consistent with the lack of separability of the two dispersion (Figs. 1–4).

The relaxation time for the lateral electrophoresis of proteins in a spherical shell membrane is given by

$$\tau = \frac{r^2}{2D} \tag{1}$$

[16,43,55], where r is the vesicle radius, D the diffusion coefficient and τ the relaxation time ($= 1/(2\pi f_c)$, where f_c is the critical frequency). Thus the *average* diffusion coefficient may crudely be calculated from measurement of the relaxation time and particle radius alone.

The relaxation time of a dispersion of interest is given, for systems in which the distribution of relaxation times is not too large, by equation (2)

$$\tau = \frac{\Delta\epsilon'\epsilon_r}{\Delta\sigma'} \tag{2}$$

where $\Delta\epsilon'$ and $\Delta\sigma'$ are respectively the magnitudes of the changes in the real parts of the permittivity and conductivity (in S/cm) through the dispersion. Although it is not easily to obtain exact values for these in the present case, due to the overlap between the μ- and α-dispersions, we may take approximate values of $\Delta\epsilon' = 2100$ and $\Delta\sigma' = 1.5 \times 10^{-5}$ S/cm (Fig. 2). Substituting, we obtain from equation (2) $\tau = 1.25 \times 10^{-6}$ s. From equation (1) we thus obtain $D = 1.35 \times 10^{-7}$ cm^2/s, a value much larger than those generally obtained for such systems (e.g. Refs. 43, 47 and 56). It is thus possible that the μ-dispersion is dominated by *rotational* motion of the protein complexes. Actually, equation (1) permits the physically unreasonable assumption that the protein complexes take up a negligible fraction of the vesicle area, which is certainly not the case for typical energy-coupling membranes, in which they take up at least 30 % of the vesicle area [56]. Thus equation (1) will tend to overestimate the *effective* radius, and hence diffusion coefficient, by an uncertain amount. An

additional possibility, of course, is that the protein complexes are not in fact *randomly* mobile in the plane of the chromatophore membrane. Unfortunately, no clear evidence is as yet available concerning this very important point.

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