THE CHARGING CAPACITANCE OF THE CHROMATOPHORE MEMBRANE

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1. Introduction

The electrical capacitance of artificial lipid bilayers has been determined in a number of laboratories. Although somewhat dependent on lipid composition, capacitance values are generally close to 0.4 $\mu F.cm^{-2}$ [1,2]. The capacitance of natural membranes [3,4], usually measured by less direct methods is higher, in the range of 1.0 $\mu F.cm^{-2}$. The capacitance of the photosynthetic membranes of chloroplasts and chromatophores have not been measured but in view of the probable involvement of membrane potentials in the photosynthetic generation of ATP it becomes a matter of some importance to determine the value of this parameter. In some instances it has been necessary to assume capacitance values choosing either artificial bilayers or 'typical' natural membrane as a model [5–7].

We are, however, now in a position to determine the value for chromatophore membranes with some precision. Three parameters are required:

1. The number of electronic charges, on a bulk bacteriochlorophyll basis, translocated across the membrane during pulsed photo-activation [8–10].
2. The average number of bacteriochlorophyll molecules per chromatophore vesicle (see [11]).
3. The voltage produced across the chromatophore membrane during pulsed activation [6,7,12,13].

The dimensions of the chromatophore membrane are such that its electrical properties are probably best described by a spherical shell dielectric [13]. The capacitance values of single chromatophores of *Rhodopseudomonas sphaeroides* and *Rhodopseudomonas capsulata* are found to be 3.8×10^{-17} F and 4.4×10^{-17} F, respectively. Conversion to parallel plate capacitance gives 1.1 $\mu F.cm^{-2}$.

2. Materials and methods

Cells of *Rps. capsulata* and *Rhs. rubrum* were grown with a malate carbon source and both strains of *Rps. sphaeroides* were grown with a succinate carbon source under anaerobic conditions in constant illumination at 30°C, and harvested after 48 h growth. Harvested cells were washed in 50 mM tricine, 50 mM NaCl, 8 mM $MgCl_2$, pH 7.4 and used immediately. Chromatophores were prepared from the cells by sonication (6 × 30 s at 180 W) followed by the usual differential centrifugation procedures [11]. In some experiments chromatophores were purified by sedimentation through a discontinuous sucrose gradient as in [14] followed by dialysis against 50 mM tricine for 20 h and at 5°C in the dark.

To estimate the bacteriochlorophyll content of the vesicles, chromatophores were mixed with a known concentration of polystyrene latex beads, mean diameter 0.109 μm (Polaron Ltd., Watford) and 2% phosphotungstic acid, pH 7.4, and sprayed on to a formvar and carbon coated, copper electron microscope grid with a Nebulizer gun (E. F. Fullam, Schenectady, USA). The grids were examined in a Phillips model EM301 electron microscope. In later experiments it was found that similar results were obtained by simply washing the grids with the mixture and allowing to dry out. In some cases a chromatophore/latex mixture, sprayed on to a grid was dried in vacuo and then shadowed with carbon and platinum at an angle of 30°.

The bacteriochlorophyll concentrations were determined using the extinction coefficients given in [15].

Spectroscopic measurements were carried out using a two photomultiplier double beam spectrophoto-

meter of conventional design with exciting light provided by a 20 μs Xenon flash tube. Repetitive flashes were analysed using a Datalab DL4000 signal averager coupled to a Datalab microprocessor DL450U.

Alamethicin was a generous gift from Dr Whitfield, Upjohn Ltd, USA.

3. Results and discussion

3.1. *Bacteriochlorophyll content of chromatophore vesicles*

The average bacteriochlorophyll content of a single chromatophore was estimated in [11] by centrifuging a sample of chromatophores, containing a known amount of bacteriochlorophyll, directly on to an electron microscope grid. We experienced some difficulty with this method in ensuring that the chromatophores remained on the grid when it was removed from the centrifuge tube. A spray technique, described in [16] for counting virus particles, appears to give more reproducible results but higher chromatophore counts. The main reason for the higher counts of chromatophores is probably due to the smaller particle diameter (by a factor of 0.62) of current preparations. This may be due to a difference in the breakage procedure (sonication rather than French Press treatment). Disruption by shaking with glass beads gave similar results to sonication. The data are shown in tables 1 and 2 for chromatophores from three species of photosynthetic bacteria. Using dry weight determinations a bacteriochlorophyll/*Rhs. rubrum* chromatophore ratio of 790 was estimated [17], approx. 50% of our value.

The electron micrographs showed a range of particle diameters for each of the 3 species. The average particle diameter was about 30% greater for the negative stained samples than for the carbon replicas but the particle count was similar. We have based our measurements on the former since this technique should be less likely to modify particle diameter. All preparations showed numerous small particles of diameter less than 250 Å which have not been included in the estimations. In this particulate fraction there was no discernible membrane structure and it is most likely that this fraction contains non-chromatophore material including ribosomes and dislodged enzyme complexes such as succinate dehydrogenase and F_1 ATPase. Large particles, greater than 750 Å diameter were also discounted but their low population density, 13% total, would not significantly affect the calculation of the bacteriochlorophyll/chromatophore ratio. Purification of the chromatophores by centrifugation on a discontinuous sucrose gradient [14] did not significantly change the value of bacteriochlorophyll/vesicle for the 250–750 Å particles.

The particle distribution of *Rps. sphaeroides* and *Rps. capsulata* were quite similar but the chromatophore diameter of *Rhs. rubrum* was significantly larger and yielded a higher bacteriochlorophyll/

Table 1
Diameter distribution of *Rps. sphaeroides* chromatophores

Particle diameter (Å)	250–500	500–750	750–1000	1000
% total population	67	19	6.3	6.9
	'chromatophore' fraction mean diam. 360 Å			

Table 2
Particle count of chromatophore preparations

Organism	bacteriochlorophyll/vesicle
Rhodopseudomonas sphaeroides	976 ± 280
Rhodopseudomonas capsulata	approx. 1000
Rhodospirillum rubrum	approx. 1400

chromatophore ratio. The data, summarised in tables 1 and 2 represent average values for at least 2 chromatophore preparations and at least 2 grids/preparation. By this sampling procedure the variation in bacteriochlorophyll/chromatophore was quite wide for each species of bacteria and averaged values are shown. The value for *Rps. sphaeroides* was estimated from 11 fields from 3 chromatophore preparations.

3.2. *Charge transfer across the chromatophore membrane during flash activation*

The cytochrome c_2 content of our chromatophores was largely reduced prior to flash activation and partly re-reduces the $[BChl_2]^+$ within a few μs after the flash. To measure the true extent of $[BChl_2]$ oxidation, a train of saturating flashes was fired at the chromatophore suspension which had been treated with antimycin A (fig.1). Under these conditions the reduced cytochrome c_2 is exhausted after 4–5 flashes [13] and the bleaching at 605 nm is a measure of $[BChl_2]$ oxidation by a single flash. Using the extinction coefficient determined in [8] we obtained an average value of 1 $[BChl_2]$ oxidised/flash/89 bulk bacteriochlorophyll in the preparation used to determine the chromatophore membrane potential. Other preparations showed some variation (± 30%) in this ratio. This represents a mean value of 11 reaction centre bacteriochlorophylls oxidised by a saturating, single turnover flash/chromatophore vesicle.

The extinction coefficients of the 600 nm bands of the reaction centre chlorophyll of *Rps. capsulata*

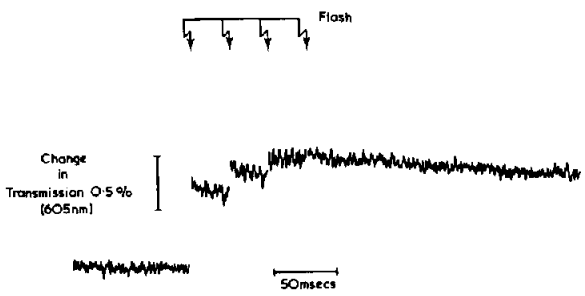

Fig.1. Multipulsed single turnover of $[BChl]_2$ at 605 nm. Chromatophores (bacteriochlorophyll concentration 1.4×10^{-5} M) were suspended in 50 mM tricine, 50 mM NaCl, 8 mM $MgCl_2$, pH 7.4 and 2×10^{-6} M antimycin A. The trace obtained was an average of 4 recordings spaced 40 s apart.

and *Rhs. rubrum* chromatophores have not been precisely determined. Using the coefficient derived for *Rps. sphaeroides* we obtain flash-oxidised values of 13 $[BChl_2]$/*Rps. capsulata* chromatophore and 24 $[BChl_2]$/*Rhs. rubrum* chromatophore.

3.3. *Voltage generated across the chromatophore membrane during flash activation*

The method [12] which has been used earlier to calibrate the chromatophore carotenoid shift presupposes a negligible membrane capacitance. It is assumed that the amount of potassium influx on the valinomycin carrier during the charging process is small and does not immediately result in an increased internal concentration of K^+ (see Discussion in [7]). Deviation from linearity particulary at low added KCl concentrations has been observed [7,18,19], presumably where the endogenous K^+ becomes significant and yet in other studies linearity is maintained [12,20]. In any case, the degree of the carotenoid shift (as indicated by ΔA between the peak and trough of the difference spectrum) shows a linear dependence on the logarithm of the added potassium concentration across the major part of the experimental range ([7,12,18–20], fig.2).

After short flash activation of *Rps. sphaeroides* chromatophores in the presence of antimycin A the carotenoid absorption bands shift in response to charge separation between:

$$\text{cytochrome } c_2 \rightarrow [BChl_2] \rightarrow \text{Q.Fe} \qquad [13].$$

After a few ms the shift begins to decay. The decay processes depend upon the permeability characteristics of the membrane but not upon further electron transport events [6]. Taken with other evidence [9,10] this suggests that the primary events of bacterial photosynthesis are orientated across the membrane and that the carotenoid shift rapidly takes upon a delocalised character. We assume therefore, that the diffusion potential experiments reliably calibrate the flash-induced carotenoid shifts. The data are shown in fig.2 for *Rps. sphaeroides*. Data for *Rps. capsulata* were very similar and gave a value of 48 mV for the single flash potential (in the presence of antimycin A).

The ionophore alamethicin displays voltage-dependent conductance properties in artificial membrane systems [21]. A voltage-dependent effect of ala-

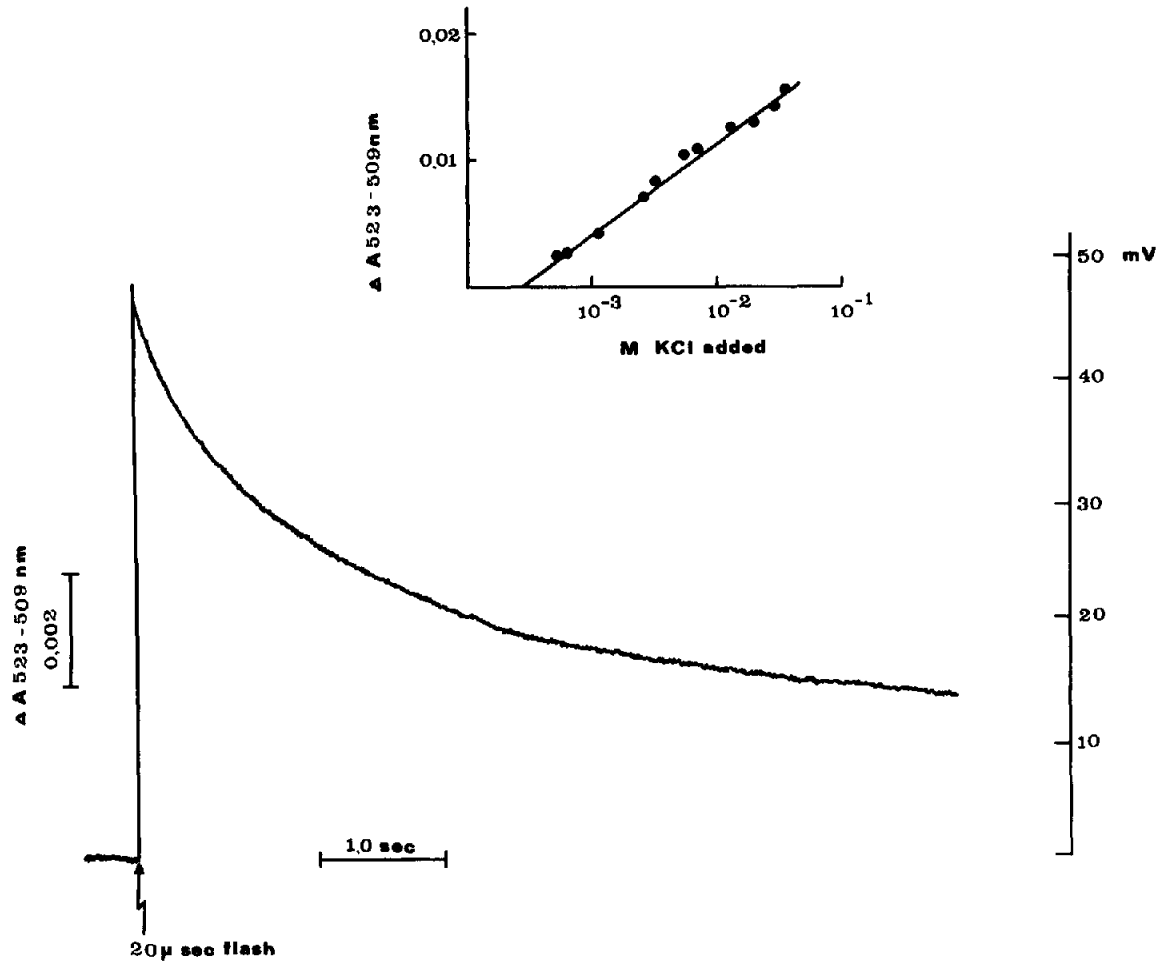

Fig.2. Calibration of the flash-induced carotenoid shift. Cuvette conditions as for fig.1 except for a final bacteriochlorophyll concentration of 2.0×10^{-5} M. Carotenoid-shift kinetics after single-flash activation. The trace was an average of 8 recordings spaced 40 s apart. Inset: Calibration of the carotenoid shift using KCl diffusion potentials. 0.5 μg valinomycin was present.

methicin on the 515 nm shift of chloroplasts was described [22]. Applying their data to the empirical finding [23,24] that the 'characteristic voltage' in phospholipid bilayers shows a logarithmic dependence on alamethicin concentration, they calculated a value of 105–135 mV for the flash-generated membrane potential. Qualitatively similar data to [22] may be observed with chromatophore membranes (N.K. P., unpublished observations). The relaxation of the carotenoid shift of *Rps. sphaeroides* and *Rps. capsulata* after a single or multiple flashes is polyphasic even in the absence of ionophore. An additional break in the semi-log plot of the decay, which we identify as the characteristic voltage in absorbance units, ΔA_c is observed in the presence of alamethicin (fig.3a). The plot of:

$$\frac{\Delta A_{c1} - \Delta A_{c2}}{\Delta A_o} \text{ versus } \log \frac{C_2}{C_1}$$

is shown in fig.3b. ΔA_o is the maximum amplitude of the carotenoid shift after a train of 5 saturating flashes, ΔA_{c1} is the break point at one concentration of alamethicin, c_1 and ΔA_{c2} is the break point at a

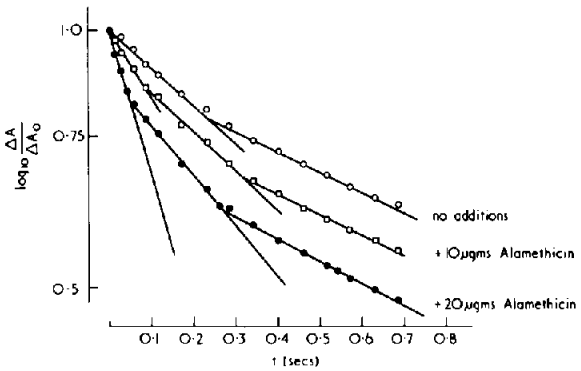

Fig.3a

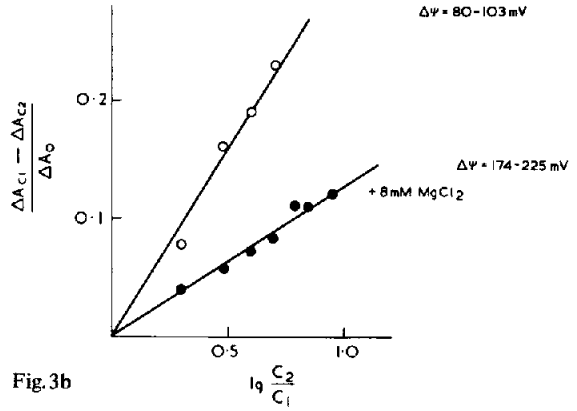

Fig.3b

Fig.3. Acceleration of the carotenoid band-shift decay by alamethicin. Conditions as for fig.1 except for a final bacteriochlorophyll concentration of 1.8×10^{-5} M and antimycin was not present. Addition of alamethicin as shown. (3a) Semi-logarithmic plot of carotenoid shift decay. (3b) Calibration of carotenoid shift using alamethicin (see text for details).

second concentration of alamethicin, c_2 (see [22]). Using the constant α, which relates the characteristic voltage to the alamethicin concentration, at a value of 0.72–0.93 [24] we can calculate that the membrane potential produced by a single flash in the presence of antimycin A ($\times$ 0.29 ΔA_0) lies between 58 mV and 86 mV.

This is in remarkably good agreement with the value determined by diffusion potential experiments but some caution must be exercised in interpreting the alamethicin data:

(i) In the presence of 8 mM $MgCl_2$ the slope of the data shown in fig.3b differs by a factor of two. This gives a corresponding doubling in the value of the membrane potential. The data in [22] also shows a variation in slope with the cation present but in the phospholipid bilayer experiments the slope is constant for different cations [23,24].

(ii) The value of α falls within the range 0.72–0.93 for phosphatidylethanolamine, phosphatidylcholine and phosphatidylserine [23,24]. It may be appreciably different in biological membranes of high protein content.

(iii) Further uncertainty is introduced by the fact that alamethicin is adsorbed on the outside of the chromatophore vesicles and yet the light-driven membrane potential is positive inside, a situation in bilayers which does not favour ion conduction (see Discussion in [22]).

3.4. *Chromatophore charging capacitance*

During saturating, single turnover flash activation, in the presence of antimycin, an average of 11 e^- are translocated across the membrane of a single vesicle of *Rps. sphaeroides* chromatophores, generating a membrane potential of about 47 mV. The charging capacitance of an 'average' chromatophore is therefore approx. 3.8×10^{-17} F. For *Rps. capsulata*, acknowledging the uncertainty in the 605 nm extinction, the value is 4.4×10^{-17} F.

Owing to the small size of chromatophore vesicles, the radius of curvature of the internal interface is significantly smaller than that of the external interface. The chromatophore capacitance is therefore probably best described by the equation for a spherical shell dielectric [13]:

$$C = \frac{4\,\Pi\epsilon}{\frac{1}{a} - \frac{1}{b}}$$

where a and b are the radii of curvature of the internal and external membrane interfaces and ϵ is the permittivity of the membrane.

Using values of a = 150 Å, b = 180 Å, the dielectric constant (ϵ_r) of the chromatophore membrane would be approx. 3.8. In terms of a parallel plate capacitor for comparison with other membranes, this would give us a value of 1.1 $\mu F.cm^{-2}$ for a membrane with an effective thickness of 30 Å.

3.5. *Chromatophore membrane composition*

The value of the dielectric constant derived from the chromatophore membrane charging capacitance falls within the range found for other biological membranes [2] and is about 2-fold higher than that of artificial lipid bilayers [1].

Our chromatophore preparations of *Rps. sphaeroides* and *Rps. capsulata* possess approx. 10 μmol lipid phosphorus/μmol bacteriochlorophyll (S. D. Shukla, unpublished results), i.e., about 10^4 molecules phospholipid/vesicle. Taking the cross-sectional surface area of a phospholipid molecule in a bilayer to be 50 $Å^2$ [25] then bilayer can roughly account for all of the chromatophore surface. The protein content of chromatophore membrane is in fact expected to be rather high since each reaction centre complex is associated with light-harvesting pigment–protein complexes and numerous electron-transport carriers. Adsorption of protein to bilayer surface is unlikely to significantly affect the membrane capacitance directly [1] but may structurally support an expanded, thinner bilayer structure with a consequently higher capacitance than that of an artificial black membrane [25]. Alternatively, since there is good reason to believe that many of the chromatophore proteins actually span the membrane, it may be that their presence raises the mean dielectric constant above that of the pure lipid hydrocarbons. The presence of even 1% or 2% hydrophylic pores ($\epsilon_0 = 80$) within the membrane would raise the capacitance of a lipid bilayer to about 1 $\mu F.cm^{-2}$ [1].

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References

[1] Hanai, T., Haydon, D. A. and Taylor, J. (1965) J. Theoret. Biol. 9, 422–432.
[2] Jain, M. K. (1972) in: The Bimolecular Lipid Membrane, p. 91, Van Nostrand Reinhold Company, New York.
[3] Fricke, H. (1953) Nature 172, 731–732.
[4] Pauly, H., Packer, L. and Schwan, H. P. (1960) J. Biophys. Biochem. Cytol. 7, 589–601.
[5] Schliephake, W., Junge, W. and Witt, H. T. (1968) Z. Naturforsch. 23b, 1616–1617.
[6] Jackson, J. B. and Crofts, A. R. (1971) Eur. J. Biochem. 18, 120–130.
[7] Baccarini-Melandri, A., Casadio, R. and Melandri, B. A. (1977) Eur. J. Biochem. 78, 389–402.
[8] Dutton, P. L., Petty, K. M., Bonner, H. S. and Morse, S. D. (1975) Biochim. Biophys. Acta 387, 536–556.
[9] Prince, R. C., Baccarini-Melandri, A., Hauska, G. A., Melandri, B. A. and Crofts, A. R. (1975) Biochim. Biophys. Acta 387, 212–227.
[10] Petty, K. M. and Dutton, P. L. (1976) Arch. Biochem. Biophys. 172, 335–345.
[11] Saphon, S., Jackson, J. B., Lerbs, V. and Witt, H. T. (1975) Biochim. Biophys. Acta 408, 58–66.
[12] Jackson, J. B. and Crofts, A. R. (1969) FEBS Lett. 4, 185–189.
[13] Jackson, J. B. and Dutton, P. L. (1973) Biochim. Biophys. Acta 325, 102–113.
[14] Gorchein, A., Neuberger, A. and Tait, G. H. (1968) Proc. Roy. Soc. London B 170, 229–246.
[15] Clayton, R. K. (1963) in: Bacterial Photosynthesis (Gest, H., San Pietro, A. and Vernon, L. P. eds) p. 397, Antioch Press, Yellow Springs, OH.
[16] Kellenberger, E. and Arber, W. (1957) Virology 3, 245–255.
[17] Kakuno, T., Bartsch, R. G., Nishikawa, K. and Horio, T. (1971) J. Biochem. Tokyo 70, 79–94.
[18] Symonds, M., Swysen, C. and Sybesma, C. (1977) Biochim. Biophys. Acta 462, 706–718.
[19] Matsuura, K. and Nishimura, M. (1977) Biochim. Biophys. Acta 459, 483–491.
[20] Evans, E. H. and Crofts, A. R. (1974) Biochim. Biophys. Acta 333, 44–51.
[21] Mueller, P. and Rudin, D. O. (1968) Nature 217, 713–719.
[22] Zickler, A., Witt, H. T. and Boheim, G. (1976) FEBS Lett. 66, 142–148.
[23] Eisenberg, M., Hall, J. E. and Mead, C. A. (1973) J. Membrane Biol. 14, 143–176.
[24] Boheim, G. (1974) J. Membrane Biol. 19, 277–303.
[25] Fettiplace, R., Andrews, D. M. and Haydon, D. A. (1971) J. Membrane Biol. 5, 277–296.