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Comparison of a Lipophilic Cation and Microelectrodes to Measure Membrane Potentials of the Giant-Celled Algae, *Chara australis* (Charophyta) and *Griffithsia monilis* (Rhodophyta)

Raymond J. Ritchie

The School of Biological Sciences, The University of Sydney, Sydney, New South Wales, Australia

Summary. The vacuolar equilibrium potential of the lipophilic cation $TPMP^+$ (triphenyl methyl phosphonium) in the giant algae *Chara australis* and *Griffithsia monilis* was directly measured. The $TPMP^+$ equilibrium potential was approximately 100 mV less negative than the measured vacuolar electrical potential. Thus $TPMP^+$ does not act as a probe of the vacuolar electrical potential and appears to be extruded against an electrochemical gradient. Measurement of the plasmalemma equilibrium potential of $TPMP^+$ showed that extrusion of $TPMP^+$ apparently occurred at both the tonoplast and plasmalemma in *Chara* and at the plasmalemma in *Griffithsia*. It is concluded that $TPMP^+$ cannot be used as a membrane potential probe in *Chara* or *Griffithsia*.

Key words giant algal cells · membrane potentials · microelectrodes · lipophilic cations

Introduction

Lipophilic cations such as $TPMP^+$ (triphenyl methyl phosphonium) have been used to measure the membrane potentials of isolated mitochondria (Liberman, Topaly, Tsofina, Jasaitis & Skulachev, 1969; Kamo, Muratsugu, Hongoh & Kobatake, 1979; Mewes & Rafael, 1981) and a variety of bacteria (*Halobacterium halobium*: Michel & Oesterhelt, 1976; *Escherichia coli*: Ghazi, Schechter, Letellier & Labedan, 1981; and *E. coli* and *Streptococcus faecalis*: Bakker, 1978). Some attempts have been made to use lipophilic cations to measure plasma membrane potentials of eukaryote cells (*animal cells*: Lichtshtein, Kaback & Blume, 1979; Cheng, Haspel, Vallano, Osotomehin & Sonenberg, 1980; *fungal cells*: Barts, Hoeberichts, Klaassen & Borst-Pauwells, 1980; Hauer & Hofer, 1978; *plant cells*: Komor & Tanner, 1976; Rubenstein, 1978; Beardall & Raven, 1981; Reed & Collins, 1981).

The use of lipophilic cations to calculate membrane potentials is based on the observation that they can cross biological membranes passively without any carrier system (Grinius, Jasaitis, Kadziauskas, Liberman, Skulachev, Topali, Tsofina & Vladimorova, 1970). Thus if the equilibrium accumulation ratio can be measured, the membrane potential should equal the equilibrium potential of the lipophilic cation. The lipophilic cation must be used at very low concentrations because lipophilic cations depolarize (short circuit) membranes (*see* Michel & Oesterhelt, 1976). Unfortunately, there is some evidence that lipophilic cations can be transported by some cells against their electrochemical gradient. Harold and Papineau (1972) found that *S. faecalis* cells loaded with K^+ did not accumulate DDA^+ (dibenzyl dimethyl ammonium) or $TPMP^+$. Barts et al. (1980) found that yeast cells (*Saccharomyces cerevisiae*) partially excluded a wide variety of lipophilic cations including DDA^+ and $TPMP^+$ and transported them as thiamine analogs. Passive distribution of lipophilic cations is therefore not a valid assumption but a hypothesis which has to be tested before uptake of a lipophilic cation can be used to calculate membrane potentials.

The total cellular uptake of a lipophilic cation consists of the sum of the lipophilic cation uptake by the mitochondrial matrix, cytoplasm, vacuoles, lipid bodies and perhaps other cell incursions. Thus the mean accumulation ratio, based on whole cell uptake of a lipophilic cation, is not related to the cytoplasmic accumulation ratio. Beardall and Raven (1981) and Reed and Collins (1981) incorrectly supposed that the mean accumulation ratio would give some indication of the cytoplasmic accumulation ratio. It is clearly impractical to attempt to estimate the cytoplasmic accumulation ratio by subtracting the contents of the various intracellular compartments from the total cellular uptake.

The accumulation ratio of an ideal lipophilic cation in any intracellular compartment, relative to the loading medium, would reflect the electrical potential between this phase and the loading medium. For example, the vacuole to loading medium accumu-

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lation ratio ($[C_v]/[C_o]$) would reflect the vacuolar potential ($\psi_{v,o}$). The vacuoles of the giant-celled freshwater green alga *Chara australis* and the giant-celled marine red alga *Griffithsia monilis* are large enough to sample the vacuoles directly by microsurgery and so the uptake of a lipophilic cation into a cell organelle can be followed directly. The vacuole does not contain any inclusion bodies capable of accumulating a lipophilic cation.

The electrophysiology of *Chara* and *Griffithsia* is well known and the vacuolar potential can be readily measured using microelectrodes (*Chara*: Hope & Walker, 1975; Smith & Walker, 1981; Walker, 1982; *Griffithsia* species: Findlay, Hope & Williams, 1969, 1970). The null hypothesis that the equilibrium potential of a lipophilic cation in the vacuole of a cell relative to the external loading medium was equal to the vacuolar electrical potential can be directly tested. Such a direct test of the validity of membrane potentials of eukaryote cells calculated from uptake of lipophilic cations is not possible on smaller cells.

Theory

An ideal lipophilic cation can be defined as a lipophilic cation which distributes itself between two phases separated by a membrane according to the electrical potential difference between the two phases, i.e. the equilibrium potential of the lipophilic cation equals the electrical potential. The equilibrium potential (E) is calculated using the Nernst equation,

$$E_{i,o} = \frac{-RT}{zF} \operatorname{Log} \frac{[C_i]}{[C_o]} \tag{1}$$

where $[C_i]$ is the concentration of a lipophilic cation (C), at equilibrium in phase (i), e.g. a bacterial cell or the vacuole of a plant, $[C_o]$ is the concentration of a lipophilic cation in the loading medium, R, T, z and F are the universal gas constant, absolute temperature, valency ($+1$) and the Faraday, respectively.

The vacuole of a plant cell is in series with the cytoplasm and thus any exclusion of a lipophilic cation from the vacuole could be occurring at either the plasmalemma and/or the tonoplast. To determine which of these membranes excluded a lipophilic cation (C) it is necessary to determine the apparent equilibrium potential across the plasmalemma. The cytoplasmic accumulation ratio ($[C_c]/[C_o]$) of a lipophilic cation can be estimated using a simultaneous equation approach and is the only valid method for determining the equilibrium potential of a lipophilic cation across the plasma membrane of a eukaryotic cell with mitochondria. The lipophilic cation uptake of cells is measured under control conditions and under conditions where the plasmalemma potential is zero (but all potentials of the intracellular compartments relative to the cytoplasm are unchanged). A plasmalemma potential of zero can be achieved by placing cells in an artificial cytoplasm containing similar concentrations of K^+, Na^+ and Cl^- as the cytoplasm. It can be shown that the equilibrium potential of an ideal lipophilic cation (E) would be;

$$E_{c,o} = \frac{-RT}{zF} \operatorname{Log} \frac{c_i v_i' [C_o']}{c_i' v_i [C_o]} \quad \text{or} \quad \frac{-RT}{zF} \operatorname{Log} \frac{[\bar{C}_i]}{[\bar{C}_i']} \tag{2}$$

where, c_i and c_i' are the total cellular uptake of the lipophilic cation (C) of control cells and cells equilibrated to artificial cytoplasm, respectively, v_i and v_i' are the volumes of the cells used in the two experiments, $[C_o]$ and $[C_o']$ are the concentrations of lipophilic cations in the respective loading media, $[\bar{C}_i]$ and $[\bar{C}_i']$ are the mean accumulation ratios of the control cells and cells in artificial cytoplasm, respectively. (For the derivation of Eq. (2) *see* the Appendix).

In most plant cells there is the additional problem that the fixed negative charges of the cell wall take up lipophilic cations (Briggs, Hope & Robertson, 1961; Ritchie & Larkum, 1982). Thus the total intracellular uptake is the total tissue uptake minus the cell wall 'free space' component. The cell wall uptake must be measured under both experimental conditions because the cell wall uptake of a cation is a complex function of ionic strength and the species of the cations in the loading medium (Ritchie & Larkum, 1982).

Provided the free space component is taken into account, Eq. (2) can be validly used on both prokaryotic and eukaryotic cells. Most previous studies which have used lipophilic cations to calculate plasma membrane potentials of eukaryotic cells have not used correct forms of the Nernst equation. Most studies have incorrectly ignored the problem of intracellular compartmentation of lipophilic cations into the organelles of eukaryotic cells and used the simple form of the Nernst equation (Eq. 1). This leads to an overestimate of the cytoplasmic accumulation ratio ($[C_c]/[C_o]$). Other studies have used algebraically incorrect modifications of the Nernst equation to correct for mitochondrial accumulation of lipophilic cations (e.g. Lichtshtein et al., 1979). It seems that the only previous study where the appropriate form of the Nernst equation has been used on eukaryotic cells is that of Cheng et al. (1980).

Materials and Methods

Chara australis R.Br. plants were a gift from N.A. Walker. Results obtained on these cells can be directly compared to the extensive physiological knowledge of this particular cell line of the plant (Hope, 1971; Hope & Walker, 1975; Raven, 1976; Smith & Walker, 1981; Walker, 1982). Morphologically the giant internodal cells are cylinders about 1 mm in diameter and up to 15 cm long. The vacuole occupies about $96.1 \pm 0.5\%$ of the cell volume (Bostrom, 1976, pp. 67-69). All experiments were conducted on trimmed internodal *Chara* cells equilibrated to artificial pond water (APW): KCl 0.1 mM, NaCl 1.0 mM, $CaCl_2$ 0.1 mM; buffered to pH 6.5 using 1.0 mM MES (2' |N-morpholino| ethane sulphonic acid), titrated with NaOH.

The giant-celled marine alga *Griffithsia monilis* Harvey, was collected from the low tidemark in the Athol Wharf area, Sydney Harbour (*cf.* Larkum & Weyrauch, 1977; Bisson & Kirst, 1979*a*, *b*). Cells had been kept in the laboratory for one to three weeks when used. They survived and grew well in enriched seawater medium *f*/2 (Stein, 1973) at 20 °C and in dim light (< 20 μE m^{-2} sec^{-1} (400–700 nm)[1]). The giant cells of *Griffithsia monilis* are up to 2 mm in diameter; cells larger than 1 mm were used. The cytoplasm occupies about 1.8% of the protoplasm of *G. monilis* (Bisson & Kirst, 1979*a*).

|^{3}H|TPMP$^+$ iodide (designated TPMP* in this paper) was purchased from the Radiochemical Centre-Amersham (Batch No. H/858). To avoid possible toxic effects of TPMP$^+$ and to use the maximum specific activity, TPMP* was not diluted with non-radioactive TPMP$^+$ in labeling experiments. The concentration of TPMP* stock was 92 μM (extinction coefficient $\varepsilon = 29.1 \times 10^3$ M cm^{-1} at 223 nm). TPMP* was used at concentrations of 10 to 50 nM in experiments and the tritium beta radiation was counted in a standard Packard 3375 scintillation counter (1 c/min in the red channel $\equiv 224 \times 10^{-18}$ mole of TPMP*). Unlabeled TPMP$^+$ was a gift from N.A. Walker. TPMP* in labeled tissue could not be counted directly because of inadequate extraction into scintillant and subsequent quenching and self absorption problems. 200 μM of the cationic detergent, S-decyl isothiuronium (DIU$^+$) was found to be an efficient extractant for TPMP* and caused negligible quenching. Treatment of TPMP* standards, efflux aliquots and tissue extracts were rigorously standardized so that quench corrections were unnecessary. S-decyl isothiuronium chloride and bromide were gifts from C.S.I.R.O. Plant Industry, Canberra.

To measure the TPMP* content of vacuoles of *Chara* cells, the cells were first rinsed in unlabeled APW for 3 min to reduce contamination due to the cell wall TPMP*. Each cell was blotted dry, weighed, and placed on a folded waxed filter paper. The cell ends were cut off and the vacuolar sap collected in a 5 μl capillary (Microcap) (*cf.* Hope & Walker, 1975) and the TPMP* counted under standard conditions. The cell ends and remainder of the cells were extracted and counted separately; added to the vacuolar sap, this gave the total cellular TPMP* uptake. Cell wall contamination was very small.

Vacuolar contents of *Griffithsia* were measured using a similar method. The cells were first rinsed in seawater for 3 min and blotted dry. Several cells were placed on a waxed filter paper, then each cell was stabbed with a sharp needle and the vacuolar sap collected. Normally 3 cells were necessary to fill a 5 μl Microcap. Little cytoplasmic contamination (red color) was observed. provided the cells were stabbed with a sharp needle and the sap collected quickly. Total tissue TPMP* uptake was measured separately on a parallel group of cells. The 'free space' component was estimated by relabeling the same cells in TPMP* after the total TPMP* uptake of live cells had been extracted using DIU$^+$. Rinsing methods which were satisfactory for *Chara* were unsatisfactory for *Griffithsia* because the cell wall took up large amounts of TPMP* and exchanged very slowly.

Artificial cytoplasm solutions of *Chara* and *Griffithsia* were used to measure the TPMP* uptake of cells in which the plasmalemma potential was zero and hence, using Eq. (2), to calculate the plasmalemma equilibrium potential for TPMP*. The artificial *Chara* cytoplasm (ACC) was based on Smith and Walker (1981) and had the following composition (in mM): NaOH 1; KOH 80; $MgSO_4$ 2; KCl 10; EGTA (ethyleneglycol-bis-(β amino-ethyl ether) N,N'-tetra-acetic acid) 5; MES 93; pH 7. Plasmalemma membrane potential *vs.* K^+ external concentration curves for *Griffithsia* showed that the plasmalemma potential ($\psi_{c,o}$) is zero at about 300 mM K^+ and the K^+/Na^+ selectivity is about 0.0002 (Findlay et al., 1969). The artificial *Griffithsia* cytoplasm devised had the following composition (in mM): KCl 220; sucrose 300; NaOH, KOH, $MgSO_4$; EGTA, MES and pH as for ACC. Both *Chara* and *Griffithsia* cells could survive for at least 24 hr in their respective artificial cytoplasms provided they were slowly equilibrated to these media over about 2 hr. Slow cytoplasmic streaming could be seen in *Chara* cells equilibrated to ACC. *Chara* cells could survive being first equilibrated to ACC then being returned slowly to APW 24 hr later (6 cells).

Vacuolar potentials were measured using glass microelectrodes with a central glass core filament and a standard microelectrode apparatus. The microelectrodes had tip diameters of less than 0.5 μm, tip potentials of less than 10 mV and a resistance of about 1 MΩ. Bathing medium was passed continuously past the cells.

For the compartmental analysis of a TPMP* efflux curve on *Griffithsia*, cells were labeled for 3 days, then effluxed in a continuous flow apparatus and the effluent aliquots collected and counted. The efflux seawater medium contained unlabeled TPMP$^+$ at the same concentration as the TPMP$^+$ in the loading medium. At the end of the experiment, the TPMP$^+$ remaining in the cells was extracted and counted. A plot was made of Log_e (counts remaining in the tissue) *vs.* time, and analyzed as described by Walker and Pitman (1976) and Atkins (1969).

As a check that TPMP* was not metabolized by *Chara* cells, cells were labeled for 7 days and the TPMP* taken up extracted from the cells and vacuum dried. Samples of the extract were passed through cation and anion exchange columns (Zeo-Karb 2.25 and Dowex-1 strongly basic anion exchange resin, respectively). They showed that the ^{3}H present was still predominantly present as an organic cation ($94 \pm 3\%$, $n = 4$, $\pm 95\%$ confidence limit). Thin-layer chromatography, using *n*-butanol |H_2O| acetic acid (12:5:3), as recommended in the Amersham data sheet, showed no significant decomposition into nonvolatile products. Any decomposition was therefore into ^3H_2O.

All experiments were conducted in continuous light (20 to 40 μE m^{-2} sec^{-1} (400 to 700 nm).

Results

Time course studies in *Chara* and *Griffithsia* showed that the total cellular and vacuolar uptake of TPMP* did not significantly increase after labeling cells for 24 hr. All TPMP* uptake experiments were routinely labeled for three days except the uptake experiments in artificial cytoplasm which were labeled for 24 hr. Both *Chara* and *Griffithsia* showed no ill effects and actively grew in TPMP$^+$ solutions from 3 to 300 nM, left for up to 14 days. The average TPMP* concentration used in tracer studies was 10 nM.

[1] E = one micromole of photons.

Table 1. Comparison of vacuolar electrical potentials and TPMP* equilibrium potentials

Alga	Vacuolar electrical potential $\psi_{v,o}$ (mV)	Vacuolar accumulation ratio $[\bar{C}_v]$ of TPMP*	Vacuolar TPMP* equilibrium potential $E_{v,o}$ (mV)
Chara australis	-183 ± 10 ($n=16$)	0.550 ± 0.15 ($n=20$)	$+18\pm7$ ($n=20$)
Griffithsia monilis	-55 ± 4 ($n=18$)	0.110 ± 0.018 ($n=12$)	$+56\pm4$ ($n=12$)

Error bars about means are $\pm95\%$ confidence limits.

Table 2. Equilibrium potential of TPMP* across the plasmalemma

Alga	Mean accumulation ratio of control cells $[\bar{C}_i]$	Mean accumulation ratio of cells in artificial cytoplasm $[C_i']$	Cytoplasmic accumulation ratio $[\bar{C}_i]/[\bar{C}_i']$	Plasmalemma TPMP* equilibrium potential $E_{c,o}$ (mV)
Chara australis	0.967 ± 0.184 ($n=16$)	0.172 ± 0.023 ($n=16$)	5.62 ± 1.31	-42^{+7}_{-5}
Griffithsia monilis	0.241 ± 0.036 ($n=14$)	1.11 ± 0.185 ($n=16$)	0.218 ± 0.049	$+38^{+7}_{-5}$

Error bars about means are $\pm95\%$ confidence limits.

Table 1 is a comparison of vacuolar potentials measured using microelectrodes to the TPMP* equilibrium potentials calculated from the accumulation of TPMP* into the vacuole. There is a very large discrepancy between the microelectrode measurements and those which would be inferred from the TPMP* equilibrium potential. Less than 1% of the TPMP* expected to be present in the vacuole was measured. This suggests that $TPMP^+$ does not behave as an ideal lipophilic cation and is extruded by the cells against its electrochemical gradient. The vacuolar potentials measured in this study were made in the presence of 50 nM $TPMP^+$ and compare well to previous measurements of the vacuolar potential of *Chara* and *Griffithsia* (Hope & Walker, 1975; Raven, 1976; Walker, 1982). Thus the TPMP* concentrations used in labeling experiments were not sufficient to depolarize the vacuolar potential.

The discrepancy between the vacuolar potentials and the $TPMP^+$ equilibrium potentials gives no information on whether the apparent exclusion of TPMP* was occurring at the plasmalemma and the tonoplast or only at the plasmalemma (it would be very unlikely that TPMP* was excluded only at the tonoplast). To determine the site of exclusion of TPMP*, the cytoplasmic equilibrium potentials were measured from the ratio of TPMP* uptake of control cells and cells equilibrated to artificial cytoplasm, using Eq. (2). The mean accumulation ratios are set out in Table 2, as well as the calculated cytoplasmic accumulation ratios and the TPMP* equilibrium potentials across the plasmalemma.

The vacuolar potential of *Chara* cells in artificial cytoplasm was measured to check if the depolarization of the plasmalemma electrical potential had effected the tonoplast potential. The vacuolar potential was found to be $+21\pm3$ mV ($n=12$, $\pm95\%$ confidence limits); i.e. if the plasmalemma potential was zero then the tonoplast potential was not different from values quoted by Hope and Walker (1975) and Raven (1976). The vacuolar TPMP* equilibrium potential of *Chara* cells was also measured. The vacuolar equilibrium of depolarized *Chara* cells was $+61\pm8$ mV ($n=8$, $\pm95\%$ confidence limits), i.e. significantly more positive than the measured vacuolar electrical potential. This suggests that some exclusion of TPMP* from the vacuole of *Chara* occurred in depolarized cells.

Figure 1 shows an example of a TPMP* efflux curve for *Griffithsia* cells labeled in seawater for 3 days. This curve shows that the slow phase was equivalent to the vacuolar compartment and had a 50% exchange time of about 67 min. The normal labeling time of three days thus allowed for complete equilibration of the vacuole. The very low accumulation ratios of the vacuoles of *Chara* and *Griffithsia* thus cannot be dismissed by postulating that the tonoplast had a very low permeability to TPMP* which did not allow complete equilibration of the vacuole to occur. The rapid exchange phase consisted of the cytoplasm (including organelles) and the cell wall. It was not possible to separate these phases from each other by compartmental analysis. Relabeling of the dead cells after DIU^+ extraction of remaining TPMP* at the end of the efflux experi-

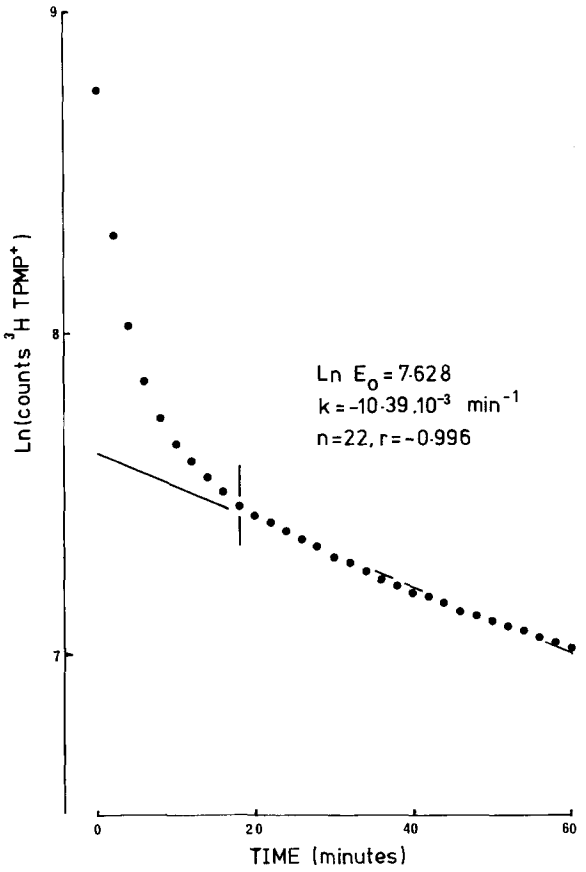

Fig. 1. Example of an efflux curve of TPMP* from *Griffithsia* cells labeled for three days. The efflux curve is plotted as Log_e counts remaining in the tissue *vs.* time in minutes. The line of best fit for the slow phase was fitted by regression analysis. The loading medium specific activity was 92,000 c/ml. The calculated vacuolar volume was 160 µl, giving a vacuolar accumulation ratio of 0.14. The average accumulation ratio of five efflux experiments was 0.124 ± 0.015 ($\pm 95\%$ confidence limits)

ment showed that the cell wall compartment made up about 77% of the fast exchange phase.

Discussion

This is the first study in plants where microelectrode potentials and the TPMP* equilibrium potentials have been made on the same cells. Vacuolar potentials and vacuolar TPMP* equilibrium potentials do not correspond. There is a discrepancy in excess of -100 mV between the TPMP* vacuolar equilibrium potentials and the measured vacuolar electrical potentials of *Chara* and *Griffithsia*. This discrepancy cannot be explained in terms of dissolution of TPMP* into organelles, lipid bodies, etc., because vacuoles do not contain any kind of inclusion body capable of taking up TPMP*. The conclusion must be drawn that TPMP* does not behave as an ideal lipophilic cation; i.e. the vacuolar electrical potential cannot be estimated from the vacuolar TPMP* equilibrium potential. The rapid turnover of the vacuolar phase of *Griffithsia* shows that the low accumulation ratios were not due to inadequate equilibration and so they are due to extrusion of TPMP* from the vacuoles against an electrochemical gradient. The vacuolar accumulation ratios in both species were less than 1% of those which would be predicted from the vacuolar electrical potentials. The vacuolar potentials were not significantly depolarized by the concentrations of TPMP* used in this study and compare well to previously reported values.

Plasmalemma membrane potentials were not measured in this study; however, they are well documented. The plasmalemma potential of *Chara* is about 20 mV more negative than the vacuolar potential; i.e. $\psi_{c,o} \simeq -200$ mV and the tonoplast potential ($\psi_{v,c}$) $\simeq +20$ mV (Hope & Walker, 1975; Raven, 1976; Smith & Walker, 1981; Walker, 1982). Findlay et al. (1969) have shown that the plasmalemma potential of *Griffithsia* species is about -82 mV and the tonoplast potential is about $+30$ mV. The cytoplasmic TPMP* equilibrium potentials shown in Table 2 are very different from the published plasmalemma electrical potentials and so TPMP* is extruded at the plasmalemma in *Chara* and *Griffithsia*, against an electrochemical gradient.

If there was no TPMP* extrusion mechanism at the tonoplast, TPMP* would distribute passively between the cytoplasm and the vacuole governed by the tonoplast electrical potential ($\psi_{v,c}$). Thus $\psi_{v,c}$ would equal the TPMP* tonoplast equilibrium potential ($E_{v,c} = E_{v,o} - E_{c,o}$) calculated from Tables 1 and 2. The TPMP* equilibrium potential across the tonoplast of *Griffithsia* is $+18 \pm 9$ mV; there is no need to invoke a TPMP* extrusion mechanism at the tonoplast of *Griffithsia*. In *Chara* the tonoplast equilibrium potential for TPMP* calculated from the difference in the TPMP* equilibrium potentials of the vacuole and the cytoplasm is $+60 \pm 14$ mV, which is not significantly different from the vacuolar TPMP* equilibrium potential of depolarized cells ($+61 \pm 8$ mV). Thus depolarization of the plasmalemma did not alter the TPMP* vacuole/cytoplasm accumulation ratio; hence the use of Eq. (2) to calculate the cytoplasmic accumulation ratio of TPMP* is valid. The vacuolar electrical potential of *Chara* cells in artificial cytoplasm was $+21 \pm 3$ mV, i.e. similar to published tonoplast potential values. It can be concluded that the extrusion of TPMP* from the vacuole of *Chara* was the product of extrusion mechanisms at both the tonoplast and plasmalemma.

The use of lipophilic cations to calculate plasma membrane potentials is a deceptively simple technique which has encouraged its use without consideration of the underlying assumption that lipophilic cations are not actively transported or cotransported by a H^+ or Na^+ cotransport system (Tanner, 1980). This assumption is based on the premise that an "unphysiological" lipophilic cation would distribute passively because no enzymes already present in the cell would bind and transport them against an electrochemical gradient (Grinius et al., 1970; Komor & Tanner, 1976). Lipophilic cations do occur in nature, e.g. thiamine and a variety of quaternary ammonium compounds. Barts et al. (1980) found that $TPMP^+$ and a wide variety of other lipophilic cations were transported as thiamine analogs by yeast even though these cations do not resemble thiamine in structure. Although not intuitively obvious, the chemiosmotic hypothesis (Mitchell, 1966) implicitly requires that eukaryotic cells would have an extrusion mechanism for lipophilic cations at the plasma membrane and be able to maintain very low concentrations of lipophilic cations in the cytoplasm, otherwise mitochondria would not be capable of generating a negative membrane potential of about -200 mV across the cristae. Mitochondria *in vitro* (Liberman et al., 1969; Mitchell & Moyle, 1969; Kamo et al., 1979; Mewes & Rafael, 1981) and *in vivo* (Hoek, Nicholls & Williamson, 1980) seem to lack an extrusion mechanism for lipophilic cations because the equilibrium potentials of lipophilic cations seem to correspond well to the electrical potential values obtained using other methods to measure the cristae membrane potential. Thus any lipophilic cation present in the cytoplasm will distribute passively between the cytoplasmic and mitochondrial matrix phases governed by the cristae membrane potential ($\psi_{m,c}$). The cristae membrane potential of about -200 mV is equivalent to a concentration ratio of about 10^4 (Mitchell & Moyle, 1969; Kamo et al., 1979; Hoek et al., 1980; Mewes & Rafael, 1981). The plasmalemma and mitochondrial cristae are in series with each other and so the electrical potential between the mitochondrial matrix and the external medium is the sum of the electrical potential differences across the plasmalemma and mitochondrial cristae, respectively. The plasmalemma potential in *Chara* is about -200 mV and so the accumulation ratio of an ideal lipophilic cation between the mitochondrial matrix and the external medium would be about 10^7. Mitochondria *in vitro* are depolarized by lipophilic cations when they reach concentrations in the mitochondrial matrix of about 10 to 50 mM (Kamo et al., 1979; Mewes & Rafael, 1981; and for a bacterial example *see* Michel & Oesterhelt, 1976). Thus an ideal lipophilic cation would be expected to uncouple *Chara* cells when in nanomolar concentrations in the loading medium. Table 1 clearly shows that there was no effect of 50 nM $TPMP^+$ on the vacuolar potential of *Chara* or *Griffithsia* even though the concentration of $TPMP^+$ in the mitochondrial matrix of the *Chara* cells should have been 5 M if $TPMP^+$ was an ideal lipophilic cation. Furthermore Beardall and Raven (1981) noted no ill effects of 0.3 mM TPP^+ on *Chlorella* even though they state that impossibly high accumulation of TPP^+ into the mitochondria should have occurred. A eukaryote cell which did not have an exclusion mechanism for lipophilic cations would be vulnerable to any prokaryotic competitor capable of producing a lipophilic cation as an antibiotic.

In summary the use of lipophilic cation to calculate plasmalemma potentials of *Chara* and *Griffithsia* is not valid. The validity of previous claims that lipophilic cations can be successfully used on normal eukaryotic cells (i.e. containing functional mitochondria) need to be reassessed.

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Appendix

To show that for an ideal lipophilic cation (i.e. one for which the equilibrium potential across a membrane (m) equals the electrical potential across the membrane),

$$\frac{[C_c]}{[C_o]}=\frac{c_i v_i'[C_o']}{c_i' v_i[C_o]} \quad \text{or} \quad \frac{[\bar{C}_i]}{[\bar{C}_i']} \tag{A.1}$$

where, $[C_c]$ and $[C_o]$ are the lipophilic cation concentrations in the cytoplasm and external loading medium of control cells, respectively, $[\bar{C}_i]$ and $[\bar{C}_i']$ are the mean accumulation ratios (based on whole cell uptake c_i, cell volume v_i and loading medium concentration $[C_o]$) of lipophilic cations in control cells and cells equilibrated to artificial cytoplasm, respectively.

$$c_i = c_c(1+\bar{M}v_m/v_c+\bar{V}v_v/v_c+\bar{P}v_p/v_c+\bar{F}v_f/v_c)$$

and therefore,

$$\frac{c_i}{v_c[C_o]}=\frac{[C_c]}{[C_o]}(1+\bar{M}v_m/v_c+\bar{V}v_v/v_c+\bar{P}v_p/v_c+\bar{F}v_f/v_c) \tag{A.2}$$

where, $\bar{M}$, $\bar{V}$, $\bar{P}$ and $\bar{F}$ are the distribution coefficients of the mitochondrial matrix, vacuole (s), chloroplast (s) and lipid bodies, respectively, v_m, v_v, v_p and v_f are the volumes of the mitochondrial matrix, vacuole (s), chloroplast (s) and lipid bodies, respectively, and v_c is the cytoplasmic volume. When the plasmalemma electrical potential is zero, $[C_c]=[C_o]$, therefore,

$$\frac{c_i'}{v_c'[C_o']}=(1+\bar{M}v_m/v_c+\bar{V}v_v/v_c+\bar{P}v_p/v_c+\bar{F}v_f/v_c). \tag{A.3}$$

Combining the two equations above by substituting the left-hand side of Eq. (A.3) into Eq. (A.2),

$$\frac{[C_c]}{[C_o]}=\frac{c_i v_c' [C_o']}{c_i' v_c [C_o]}$$

since $v_c'/v_c = v_i'/v_i$ (a reasonable assumption),

$$\frac{[C_c]}{[C_o]}=\frac{c_i v_i' [C_o']}{c_i' v_i [C_o]} \quad \text{Q.E.D.}$$

Note that provided the distribution coefficients of the intracellular compartments, relative to the cytoplasm, are independent of the plasmalemma membrane potential, then it is irrelevant how many intracellular compartments there are or the values of their distribution coefficients.

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