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EFFECTS OF CATIONS ON THE PLASMA MEMBRANE OF *BLASTOCLADIELLA EMERSONII* ZOOSPORES

AN ESR STUDY

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Summary

The physical properties of the plasma membrane of the aquatic phycomycete *Blastocladiella emersonii* were investigated, in particular the effects of cations on membrane structure. Intact zoospores and lipid extracts were labelled with the spin-labels 5-nitroxystearate (5-NS), 12-nitroxystearate (12-NS), and 2,2,6,6-tetramethylpiperidine-1-oxyl (Tempo). Electron spin resonance spectroscopy indicated a total of three breaks in plots of the hyperfine splitting parameter, $2T_{\parallel}$, order parameter, S, and the partition coefficient, f, vs. temperature. The first and third break points (T_L and T_H) were found to be independent of the external K^+, Ca^{2+}, or Mg^{2+} concentrations. They were similar to the break points found in aqueous dispersions of lipid extracts and correlate well with the temperature limits for zoospore viability. In contrast, the middle break point (T_M) was markedly influenced by the external Ca^{2+} concentration. Ca^{2+} increased T_M from 12°C (no Ca^{2+} added) to 22°C (10 mM Ca^{2+}), i.e., growth temperature. K^+ reversed this Ca^{2+} effect, downshifting T_M from 22°C to 10°C. A comparison of the physico-chemical effects of these ions on the membrane, as revealed by the cation-induced shift in T_M, is closely correlated with the temperature dependence and physiological effects of cations on zoospore differentiation. This suggests that cations may modify the physical state of the plasma membrane and be involved in regulating the initial changes during zoospore encystment.

Abbreviations: Mops, 3-(*N*-morpholino)propanesulfonic acid; Tempo, 2,2,6,6-tetramethylpiperidine-1-oxyl; 5-NS, 5-nitroxystearate; 12-NS, 12-nitroxystearate.

Introduction

Zoospores of the aquatic phycomycete *Blastocladiella emersonii* are highly differentiated cells which can undergo rapid morphological changes in response to their environment [1—5]. Prior to encystment and germination the motile zoospore is characterized by the absence of a cell wall and an extensive spatial segregation of its internal components [6,7]. When zoospores are induced to encyst they proceed irreversibly through a sequence of development changes which results in the formation of a chitinous cell wall and the breakdown of their internal organization [5,7,8]. The encystment process occurs rapidly and seems to require neither protein nor RNA synthesis [9—14]. However, the first detectable changes during encystment do involve alterations of the plasma membrane, specifically changes in cell surface fluorescence properties [15], the induction of cell adhesiveness [6], and the fusion of vesicles derived from the γ-particles with the plasma membrane [16,17].

Ions and temperature play an important role(s) in the regulation of the encystment process. Physiological studies on *B. emersonii* have demonstrated that K^+ can trigger encystment, and that this induction is temperature dependent [2]. In contrast, Ca^{2+} (but not Mg^{2+}) can prevent encystment [2,3,6]. This Ca^{2+} effect can be reversed by increasing the temperature, or including K^+ buffer [3]. Cations (especially Ca^{2+}) and temperature are known to affect the physical properties of model and biomembranes, including many membrane associated functions [18—24]. Since the initial changes during encystment involve the plasma membrane, it is reasonable to hypothesize that at least part of the effects of ions on zoospore differentiation involve changes in the physical properties of this membrane. To test this hypothesis we monitored the state of the zoospore plasma membrane, in vivo, with ESR spectroscopy, as a function of temperature and cation concentration using the spin-labels 12-nitroxystearate (12-NS), 5-nitroxystearate (5-NS), and 2,2,6,6-tetramethylpiperidine-1-oxyl (Tempo). The information presented in this paper indicates that cations do affect the dynamic state of the plasma membrane, in vivo, in a way which correlates very well with the previously reported effects of ions on zoospore differentiation.

Materials and Methods

Organism, medium and growth

Blastocladiella emersonii was kindly supplied by Dr. E.C. Cantino (Department of Botany and Plant Pathology, Michigan State University). The organism was routinely grown on PYG agar at 22°C as previously described [25]. Zoospores were harvested from first generation plants by flooding each plate with a buffered (5 mM Mops, final pH 6.6—6.8) solution containing the particular Ca^{2+} or Mg^{2+} concentration being tested (K^+, final concentration 50 mM, was added after resuspension of the pellet to avoid encystment during harvesting). The zoospores suspension was then collected, filtered to remove germlings and plants, and gently pelleted by centrifugation (1000 × *g* for 5 min at room temperature).

Spin-labelling procedure

Pellets were resuspended, 0.5 ml samples removed ($4.0 \cdot 10^9$ to $5.0 \cdot 10^9$ cells) and labelled with an ethanolic solution of 12-NS, 5-NS, or Tempo (concentration approx. 1 mol spin-label/6000 mol lipid). Just prior to transfer of the sample into the ESR cuvette, $K_3Fe(CN)_6$ was added (final concn. 1 mM) to prevent disappearance of the spin-label. Control experiments (not shown) indicated that neither the concentration of ethanol (approx. 1%) nor $K_3Fe(CN)_6$ used altered the ESR spectra recorded. The ethanol concentration used was also shown not to affect cell viability. In some experiments ethylene glycol (final concn. 33%) was added to the sample just before transfer into the ESR cuvette to depress the freezing point. Control experiments demonstrated that though there was an increase in the hyperfine splitting parameter ($2T_{\parallel}$) values obtained, there was less than a one degree shift in the temperature at which break points were found in plots of $2T_{\parallel}$ vs. temperature.

Lipid extraction and vesicle preparation

Lipids were isolated from zoospores as previously reported [26]. Aqueous dispersions were prepared from the total lipid extract by drying an aliquot of the mixture (30—40 mg dry wt.) first under N_2, and then under vacuum. The sample was resuspended on a vortex stirrer into 0.5 ml buffer (pH 6.7) with a glass bead. The suspension was then sonicated (125 watt sonic water bath), spin-label was added, and the suspension was again sonicated. The concentration of spin-label was less than 0.2% (w/w).

Spin-label measurements and analysis

ESR measurements were made with a Varian X-band spectrometer (Model E-112). The temperature was regulated with a Varian variable temperature controller and monitored inside the cuvette with a calibrated thermocouple which was connected to a digital readout meter (Omega model 250). All spectra were recorded at power and modulation amplitude settings which were previously determined to be below those causing saturation or line width broadening. The preferential incorporation of the spin-label into the plasma membrane was ascertained by ascorbate reduction of the spin-probe [27], and protection of the spin-label from reduction using $K_3Fe(CN)_6$ or $Na_3Fe(CN)_6$ [28].

The spectra were analyzed with a Varian 620/L-100 computer. The order parameter (S) was calculated according to method number two of Jost and Griffith [29]. The partition parameter (f) was determined according to the method of Shimshick and McConnell [30]. The experimental plots (hyperfine splitting parameter $2T_{\parallel}$, S, or f vs. temperature) were analyzed using two methods. The first method was in terms of linear components by fitting regression lines to appropriate sections using the method of least squares. This method of analysis has been frequently used in the interpretation of results obtained from ESR experiments, and the temperature at which break points are observed (indicated by the intersections of straight lines) are in good agreement with those obtained by other physico-chemical techniques [22,30, 31]. This methods also included the determination of the correlation coefficient, r, a measure of the goodness of fit, which indicated whether the initial

assumption of a straight line was valid. An r value of 1.0 indicates a perfect fit between the data points and the calculated line. In all cases $r > 0.96$ with most lines having $r > 0.98$. The second method of analysis was in terms of an iterative least squares program (Brunder, Coughlin, and McGroarty, unpublished) using normalized β-splines developed by Dierckx [32]. This method involved the simultaneous analysis of $2T_{\parallel}$, S, or f over the entire temperature range, and determined the temperatures at which break points occurred. Both methods of analysis gave the same results. The spectra obtained with 12-NS, however, could not be analyzed in this fashion due to absence of a high field trough even at 0°C, which made measurements of $2T_{\parallel}$ impossible. Use of this spin-label was therefore discontinued.

Results

Representative spectra of 5-NS labeled zoospores recorded at different temperatures are shown in Fig. 1. The spectra obtained, especially at higher temperatures, are similar to those observed for spin-labeled sarcoplasmic reticulum, spinach chloroplasts, yeast cells [18],erythrocytes [33], and *Escherichia coli* membranes [34]. These previously published spectra were interpreted as indicating the presence of at least two superimposed spectral components, one corresponding to a relatively immobilized spin-label population, and another to a relatively mobile population, here associated with peaks A and B. To gain further insight into the spectral shape changes observed, the heights of the two low field peaks, A and B, were measured at different temperatures. The ratio of the two peak heights (H_A/H_B) was found to change as a function of temperature (Table I). This change is similar to that recently observed for spin-labeled *E. coli* membranes, where changes in the (H_A/H_B) ratio were measured as a function of divalent cation concentration [34]. In *B. emersonii* zoospores the spectral contribution of peak B increased with

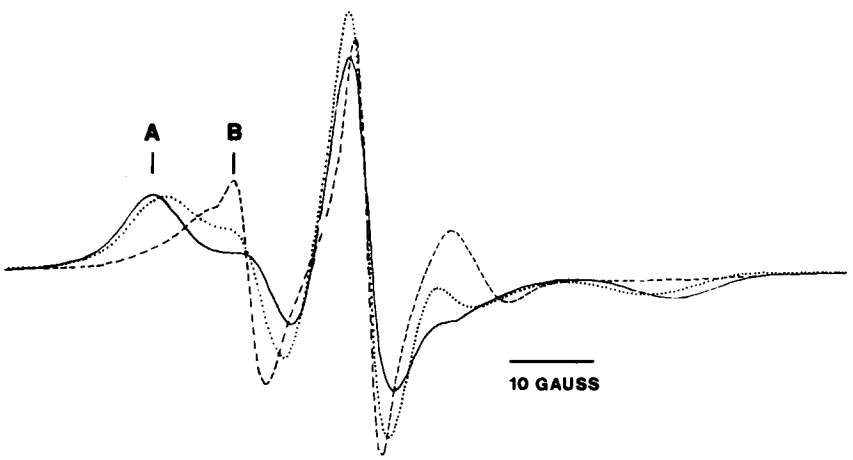

Fig. 1. Representative ESR spectra of 5-nitroxystearate labelled *B. emersonii* zoospores recorded at different temperatures. The ratio of the two low field peak heights (H_A/H_B), changed reversibly as a function of temperature. ———, 2°C; · · · · · ·, 9°C; — — —, 36°C.

TABLE I

RATIO OF THE HEIGHTS OF THE LOW FIELD PEAKS (H_A/H_B) OBSERVED IN ESR SPECTRA AND MEASURED AT DIFFERENT TEMPERATURES

B. emersonii zoospores spin-labelled with 5-nitroxystearate (5-NS). Values are averages of 4 experiments.

	Temperature (°C)					
	0	5	10	15	20	25
Ratio H_A/H_B	3.54	2.38	1.62	1.24	0.97	0.84
S.D.	±0.22	±0.18	±0.13	±0.07	±0.048	±0.059

increasing temperature. However, there was little, if any, change in its position. In contrast, the spectral contribution of peak A changed both in relative peak height and position as a function of temperature (Fig. 1). Addition of ascorbate to reduce the spin-label resulted in the simultaneous and total disappearance of both low field peaks (data not shown).

The effects of ion addition on the plasma membrane of intact zoospores labelled with 5-NS were evaluated by measuring the hyperfine splitting parameter, $2T_{\parallel}$, and where possible, the order parameter, S. The hyperfine splitting value, $2T_{\parallel}$, is highly sensitive to changes in the molecular environment of the spin-label [35], and is related to the freedom of motion of the spin-label in the membrane, lower $2T_{\parallel}$ values being associated with a greater freedom of motion of the probe [36,37]. The order parameter, S, is dependent on $2T_{\perp}$ as well as $2T_{\parallel}$. Various models have been proposed for the interpretation of the order parameter at the molecular level, including models based upon molecular motion [29], and others based upon molecular orientation [38]. In either case a large value for S (up to 1.0) indicates a high degree of order and a small value for S (down to 0) indicates a low degree of order. Plots of these two parameters vs. temperature have been used extensively to determine changes in the physical properties of both model and natural biomembranes [22,24,31]. To eliminate possible artifacts due to overlap of the two low field peaks, $2T_{\parallel}$ and S were only measured at temperatures where the two low field peaks of the spectrum were clearly resolvable (up to 26—28°C). By restricting the measurement of $2T_{\parallel}$ to this temperature range and observing the spectra obtained at low temperatures, where the spectral contribution of peak B was minimized, it was possible to attribute changes in $2T_{\parallel}$ to changes in peak A. Fig. 2 illustrates the effects of various ions and ion concentrations on $2T_{\parallel}$ and S as a function of temperature. In all cases two break points were observed. Plots of $2T_{\parallel}$ and S exhibited the same break point temperatures. The results obtained in the presence of buffer only, buffer + 10 mM $MgCl_2$, or buffer + 50 mM KCl were very similar (Fig. 2a). The first break point occurred at 3—4°C in buffer + 50 mM KCl, or at 4—6°C in buffer only or buffer + 10 mM $MgCl_2$. The second break point varied only slightly, occurring at 8—9°C in buffer + 50 mM KCl, at 10—12°C in buffer only, or at 11—13°C in buffer + 10 nM $MgCl_2$. Ca^{2+} did not significantly alter the temperature at which the first break occurred (5—7°C), but had a marked affect on the temperature at which the second break point, T_M, was observed (Figs. 2b

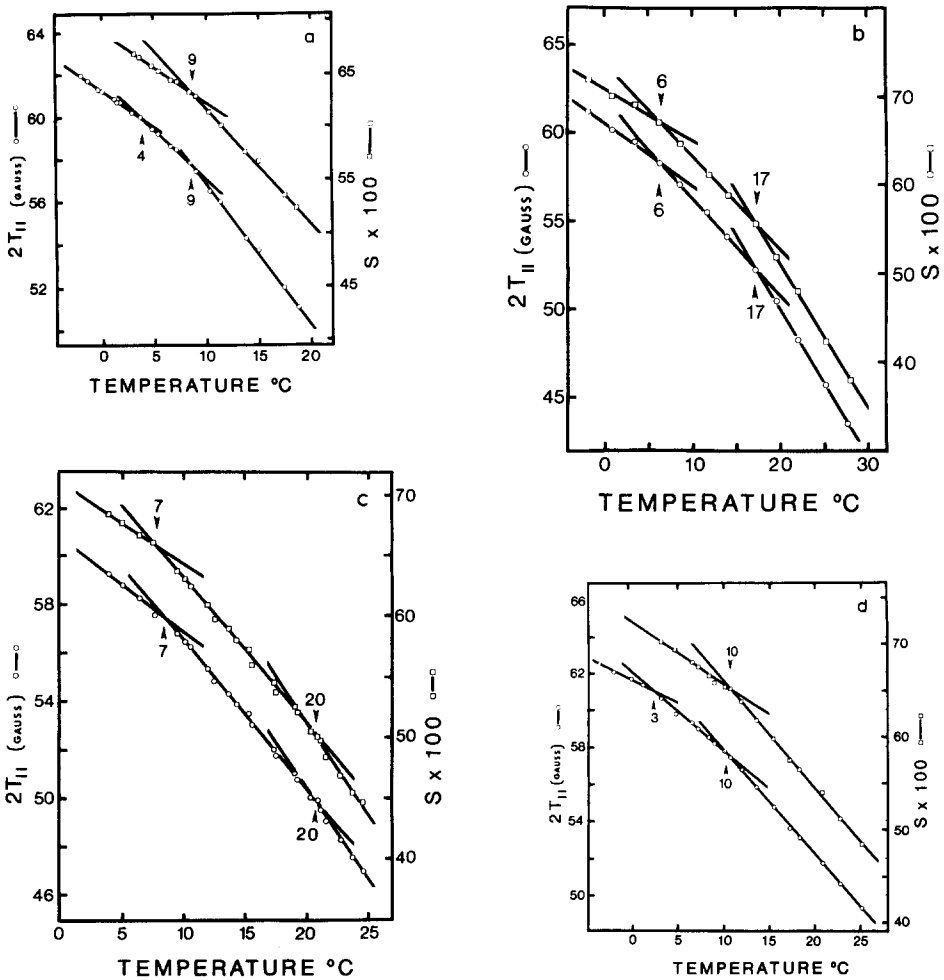

Fig. 2. Plots of $2T_{\parallel}$ and S vs. temperature for *B. emersonii* zoospores labelled with 5-nitroxystearate showing the effects of ions on the position of the break points. Two breaks for $2T_{\parallel}$ were found in all cases. S was calculated where possible. Arrows indicate break points. Note the agreement between the break points determined with $2T_{\parallel}$ and those obtained with S (pH 6.6 to 6.7). (a) Zoospores in the presence of 50 mM KCl. Break points were very similar to those determined in the presence of buffer only, or 10 mM $MgCl_2$. ○——○, $2T_{\parallel}$; □——□, S. (b) Zoospores harvested in the presence of 1 mM $CaCl_2$. ○——○, $2T_{\parallel}$; □——□, S. Note increase in the temperature at which the second break occurred. (c) Zoospores harvested in the presence of 10 mM $CaCl_2$. Break points were the same as those obtained in the presence of 20 mM $CaCl_2$. ○——○, $2T_{\parallel}$; □——□, S. (d) Reversibility of the Ca^{2+} effect by K^+. Zoospores were harvested as in experiments represented in (c). After spin-labelling, but before transfer to the ESR cuvette, KCl was added (final concn. 50 mM KCl). ○——○, $2T_{\parallel}$; □——□, S.

and 2c). In the presence of 1 mM Ca^{2+} this break point was shifted from 10—12°C to 17—18°C. Increasing the Ca^{2+} concentration to 5 mM Ca^{2+} shifted T_M to 19—20°C, and 10 mM Ca^{2+} further shifted the break point 20—22°C. 20 mM Ca^{2+} did not further change the temperature at which the second break point occurred, suggesting a possible saturation effect. The Ca^{2+} effect observed could be reversed by K^+ (Fig. 2d). When KCl was added (50 mM final concn.) to samples already in the presence of 10 mM Ca^{2+}, the second break

point T_M was shifted back to 10—11°C. To ascertain whether any break points occurred below 0°C, 5-NS labelled zoospores were studied in the presence of ethylene glycol. No additional break points were detected between −12°C and 0°C, nor were the positions of the break points above 0°C significantly altered (data not shown).

To determine whether there were any break points at higher temperatures (>25°C) which could not be observed with 5-NS, we used the spin-label Tempo. Tempo is a molecule which has been shown to be soluble in water and in fluid, liquid-crystalline membranes, but not in bilayers in the gel state [29,30,39]. The spectral parameter, f, is related to the fraction of the membrane which is accessible to the spin probe. Since f is determined by the partitioning of the spin-label between the aqueous phase and the fluid membrane, Tempo not only allowed us to measure the physical behavior of the zoospore membrane at higher temperatures, but also provided a different method of verifying the results obtained with 5-NS.

Fig. 3 illustrates the data obtained for zoospores labelled with Tempo in the presence of different Ca^{2+} concentrations as a function of temperature. In these experiments it was not possible to determine the absolute amount of membrane lipid in the liquid-crystalline state, due to the variable number of cells present. However, changes in the partitioning of the spin-label measured in the same sample as a function of temperature do reflect changes in the relative fraction of the zoospore plasma membrane which was accessible to the spin-label. In all cases, an upper break point, T_H, was observed between 32 and 34°C which was independent of Ca^{2+} concentrations. In contrast to this upper break point, another break occurred at lower temperatures which was influenced by the Ca^{2+} concentration. For samples in the presence of 10 mM $CaCl_2$ this break occurred at 22—23°C. Reducing the Ca^{2+} concentration

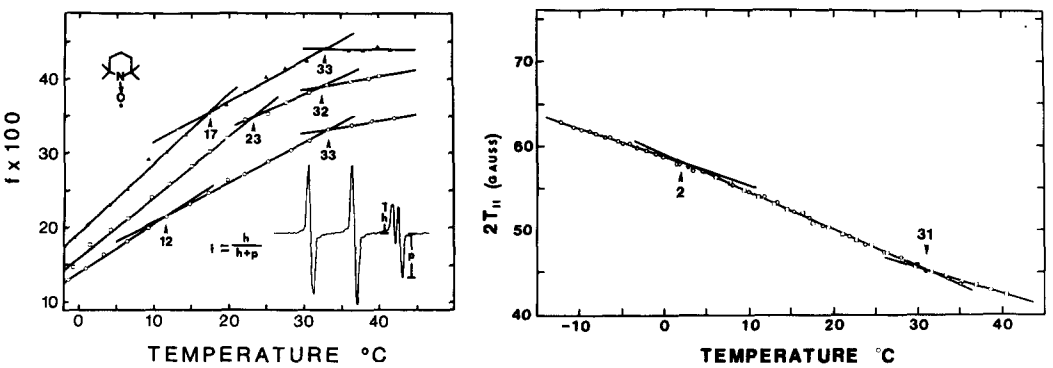

Fig. 3. Plot of $f \times 100$ vs. temperature for *B. emersonii* zoospores labelled with Tempo (pH 6.6—6.7). f was calculated as indicated since the line widths for both H and P were found to be equal. Zoospores harvested: in buffer only (○———○); 1 mM $CaCl_2$ (△———△); 10 mM $CaCl_2$ (□———□). Plots of 10 mM $MgCl_2$ were the same as buffer only.

Fig. 4. Plot of $2T_{\parallel}$ vs. temperature for aqueous dispersions of *B. emersonii* zoospore lipid extract labelled with 5-nitroxystearate. ○, sample run in the presence of buffered ethylene glycol (final concn. 33%). □, sample run in the presence of buffer only. Note: the ethylene glycol did affect the absolute value of $2T_{\parallel}$ observed and the data presented were normalized to those obtained with buffer only. Break points were shifted less than one degree in the presence of ethylene glycol.

TABLE II

THERMAL TRANSFORMATION POINTS OF MEMBRANES OF *B. EMERSONII* AS A FUNCTION OF ION CONCENTRATION

Spin-label	Ion concn. (mM)	Break point		
		Lower T_L (°C)	Middle T_M (°C)	Higher T_H (°C)
5-NS	Buffer only *	4—6	10—12	
	+ 10 mM $MgCl_2$	4—6	11—13	
	+ 50 mM KCl	3—4	8— 9	
	+ 1 mM $CaCl_2$	5—7	17—18	
	+ 10 mM $CaCl_2$	5—7	19—20	
	+ 20 mM $CaCl_2$	5—7	20—22	
	+ 10 mM $CaCl_2$ + 50 mM KCl	2—3	10—11	
Tempo	Buffer only		10—12	32—34
	+ 1 mM $CaCl_2$		17—18	33—34
	+ 10 mM $CaCl_2$		22—23	33—34
	+ 10 mM $MgCl_2$		10—12	33—34
5-NS labeled lipid extract				
	Buffer only	2—4		30—32

* Buffer: 5 mM Mops, pH 6.7.

to 1 mM $CaCl_2$ shifted break point down to 17—18° C. Zoospores in the presence of buffer only, or buffer + mM $MgCl_2$ were characterized by a shallow break which occurred at 10—12° C. Thus, the results derived from the Tempo experiments confirm those obtained with 5-NS for the Ca^{2+}-dependent break point, T_M. These results are summarized in Table II. Breaks were not detected at 4—6° C using the spin-label Tempo. However, no firm conclusions can be drawn since it was not possible to determine f accurately below 0° C.

To examine the role(s) of lipids in these observed phenomena, zoospore lipids were isolated, aqueous dispersions were labelled with 5-NS, and the temperature dependence of the hyperfine splitting parameter, $2T_{\parallel}$, was determined over the temperature range −12° C to 42° C. A plot of $2T_{\parallel}$ as a function of temperature is shown in Fig. 4. Two break points were observed, one at 2—4° C and the other at 30—32° C. These values are very similar to the lower and upper break points, T_L and T_H, observed in whole cells with 5-NS and Tempo respectively (Table II). Ca^{2+} addition did not affect the position of these break points. However, it did increase the $2T_{\parallel}$ values obtained (by 5—7 gauss) over the entire temperature range. In addition, no breaks were detected between these two, in the presence or absence of Ca^{2+}. Since the H_A/H_B ratios were significantly different from those obtained with zoospores labelled with 5-NS, there are probably differences in the relative amounts and organization of the extracted lipids as compared to the plasma membrane of the zoospore. Such differences could explain the absence of the middle, ion-dependent break point, T_M.

Discussion

The data presented in this paper support two conclusions. (1) The temperature limits of zoospore viability are related to the phase transformation

temperatures of the lipid matrix. If T_L and T_H represent the onset and completion of a gel-to-liquid crystalline phase transition, respectively, zoospore viability is related to a mixed lipid state. (2) Specific ions markedly affect the physical properties of the plasma membrane, which in turn are related to the physical effects of these ions on zoospore differentiation.

The hyperfine splitting parameter ($2T_{\parallel}$) values obtained for 5-NS labelled zoospores indicate that the plasma membrane is much more fluid at its growth temperature than the plasma membrane of organisms such as *T. acidophila* [22], or *E. coli* [31]. Initial experiments utilizing 12-NS also suggest a very fluid microenvironment. This observation is consistent with preliminary findings in our laboratory showing that the isolated plasma membrane contains a large percentage of unsaturated fatty acyl chains, including arachidonic acid (20 : 4). Similar levels of unsaturated lipids have also been reported in *B. emersonii* zoospore lipid extracts [26].

Analysis of the data obtained from spin-labelled zoospores indicated three break points in plots of spectral parameters ($2T_{\parallel}$, S, f) vs. temperature (Table II). Of these three, the lower and upper transition points, T_L and T_H, were found to be independent of ion concentration. They were also very similar to the two break points found in 5-NS labelled aqueous dispersions of whole cell lipid extracts. Both intact cells and lipid extracts were characterized by the same transformation temperature range ΔT of about 28°C, where $\Delta T = T_H - T_L$. In lipid extracts T_L and T_H were shifted to lower temperatures by approximately three degrees as compared to the break points determined in spin-labelled zoospores. Such a downshift of T_L and T_H probably reflects the absence of proteins in the lipid extract dispersions, or organizational differences in the two membranes.

Break points observed with ESR spectroscopy in multicomponent systems have been correlated with lipid phase transitions, lateral phase separations, lipid clusters or lipid-protein interactions [30,39,40—43]. In our experiments both protein-free lipid extracts and intact zoospores had similar break points (Table II). In addition, the partition parameter, f, only increased slightly above T_H (Fig. 3). This has previously been interpreted to indicate the presence of a fluid phase membrane [29,30,39]. The two temperatures, T_L and T_H, may therefore represent the onset and end of a gel-to-liquid-crystalline phase transformation of the lipid environment associated with spectral component A.

The lower and upper phase transformation temperatures, T_L and T_H, correlate well with the temperature range of zoospore viability. Previously studies indicated a lower temperature viability limit of 1—4°C [5], and upper growth temperature limit of 37—39°C [2]. We confirmed these results finding temperature limits of 1—3°C and 34—36°C (data not shown). These observations suggest that the temperature range over which the zoospore remains viable is correlated with that where plasma membrane lipids exist in a mixed state. The necessity of a mixed lipid state for cell viability is in accordance with findings for *E. coli* outer membranes [31].

In contrast to the lower and upper break points, T_L and T_H, the temperature at which the middle break point occurred (T_M) was markedly influenced by the ionic environment (Fig. 2 and 3, Table II). The reference value determined

for T_M in buffer alone was about 12°C. Addition of either K^+ or Mg^{2+} changed this value only slightly. However, increasing the external Ca^{2+} concentration progressively shifted the T_M value in both 5-NS and Tempo labelled cells up to about 22°C, i.e., around the growth temperature, where this effect saturated. These results suggest that the shift of T_M upwards was Ca^{2+} selective rather than an electrostatic divalent cation effect. Furthermore, ionic strength could not account for this shift since the concentrations of K^+ employed were much greater than those of Ca^{2+}.

A comparison of these ionic effects on T_M with the temperature dependence and physiological effects of the same ions on zoospore differentiation indicated a high degree of similarity. A dramatic change in zoospore encystment kinetics was found to occur between 10°C and 15°C in the presence of K^+ [2]. At 15°C or above zoospores encysted within minutes, whereas at 10°C or lower it took hours if it occurred at all.

In the presence of millimolar Ca^{2+} concentrations (K^+ not added), zoospores did not encyst at their growth temperature, i.e., 20°C [3,4]. However, zoospores did encyst if the temperature was raised to 27°C, even in the presence of much higher Ca^{2+} concentrations [3]. These results indicate that there is an upper limit for the Ca^{2+} effect, which lies between 20 and 27°C. This is consistent with our experiments which demonstrated an upper limit for T_M at about 22°C as a function of Ca^{2+} concentration. In addition, Ca^{2+} was found to be more effective than Mg^{2+} in preventing encystment, indicating that the inhibition was relatively Ca^{2+} selective [5]. Similar effects of these ions on T_M are shown in Table II.

Physiological experiments indicate that K^+ can reverse the effect of Ca^{2+} on zoospore encystment [3]. This implies that a similar effect should be observed physico-chemically for T_M, if the two ion effects are indeed related. The results presented in Fig. 2(c,d) demonstrate that K^+ does reverse the effects of Ca^{2+} on the plasma membrane. This hypothesis is in accord with findings that $^{45}Ca^{2+}$ was immediately released from preloaded zoospores upon K^+ addition [3].

The molecular nature of the phenomena responsible for the middle break point and the ion-induced shifts in T_M remains unknown. Since both Tempo and 5-NS were sensitive to these shifts in T_M, the membrane lipids are certainly involved. In addition, ESR experiments with the isolated zoospore plasma membrane, spin-labelled with 5-NS, also show a distinct middle break point (T_M) at 23—25°C in the presence of 10 mM $CaCl_2$ in plots of $2T_\parallel$ vs. temperature, measured over the temperature range 0—40°C (Leonards, K.S. and Haug, A., unpublished). It is noteworthy, however, that irrespective of the position of T_M, the $2T_\parallel$ and S values observed remained relative unchanged as determined by the 5-NS spin probe (Fig. 2). In model systems addition of Ca^{2+} to negatively charged phospholipids resulted in an increase in $2T_\parallel$ and S as well as a shift in the transition point [18,34,40,44]. This suggests that the actual fluidity of the bulk phase lipids in the zoospore membrane may not be affected by Ca^{2+}. Instead, the Ca^{2+} and K^+ may be acting more specifically at the cell surface, perhaps by altering lipid/protein interactions, lipid headgroup orientations, or the clustering of membrane components. These interactions may be similar to the surface antagonism of Na^+ and Mg^{2+} observed in

chloroplast grana thylakoid membranes [45]. Such interactions would be consistent with the observation that addition of K^+ alters the fluorescence properties of the cell surface within 15 s [15]. These possiblilities are now being investigated.

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