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Phase transitions as a function of osmotic pressure in *Saccharomyces cerevisiae* whole cells, membrane extracts and phospholipid mixtures

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Abstract

Fourier Transform Infrared spectroscopy (FTIR) was used to determine the phase transition temperature of whole *Saccharomyces cerevisiae W303-1 A* cells as a function of Aw in binary water–glycerol media. A phase transition occurred at 12 °C in water, at 16.5 °C at Aw=0.75, and at 19.5 °C at Aw=0.65. The temperature ranges over which transition occurred increased with decreasing Aw. A total lipid extract of the plasma membranes isolated from *S. cerevisiae* cells was also studied, with a phase transition temperature determined at 20 °C in pure water and at 27 °C in binary water–glycerol solutions for both Aw levels tested. The pure phospholipids dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE) and three binary mixtures of these phospholipids (percentage molar mixtures of DMPC/DMPE of 90.5/9.5, 74.8/25.2, and 39.7/60.3) were studied. For DMPC, there was no influence of Aw on the phase transition temperature (always 23 °C). On the other hand, the phase transition temperature of DMPE increased with decreasing Aw for the three aqueous solutions tested (glycerol, sorbitol and sucrose), from 48 °C in water, to 64 °C for a solution at Aw=0.67. For the DMPC/DMPE mixtures, transitions were found intermediate between those of the two phospholipids, and a cooperative state was observed between species at the gel and at the fluid phases.

Keywords: Phase transition; Osmotic pressure; *S. cerevisiae*; Phospholipid; Fourier Transform Infrared spectroscopy

1. Introduction

The dehydration and dry storage of *Saccharomyces cerevisiae* have been studied for years, because of its uses in industry. Many have tried to find ways to prevent cell death during dehydration, but the mechanisms involved are not well understood. Cell viability depends strongly on the osmotic pressure of the medium [1,2], and the kinetics of dehydration and rehydration is very important to prevent cell death [3,4]. Indeed, optimized kinetics using hyper-osmotic modifications leading to post-dehydration survival has been determined for both *S. cerevisiae* [5] and lactic bacteria [6]. Some studies have shown that viability is preserved if rehydration is carried out at a temperature higher than the phase transition temperature [7–9]. Previous studies have allowed us to propose a hypothesis explaining yeast mortality during dehydration or rehydration. This relates mortality following rapid dehydration or rehydration to water flow through an unstable membrane undergoing phase transition [10]. The aim of the present study was to better understand membrane behavior, using non-invasive Fourier Transform Infrared spectroscopy on whole cells, membrane extracts, and phospholipid mixtures. Most previous studies using this method have been carried out on model phospholipids to study the behaviors of hydrocarbon chains [11] or polar head groups [12], and interactions between phospholipids and ions [13–15], or saccharides [16]. Some reports concerned whole cells [17,18], but only dried cells or fully hydrated cells, and never at an intermediate hydration level. Lyotropic transitions of some pure phospholipids have been studied [19] by varying the proportions of water to lipids and determining RH. In this paper, a novel approach is used, studying

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phase transitions of whole cells as functions of the water activity, using solutes to depress it.

The main phase transition of biological membranes is the L_β to L_α shift, during which the fatty acid chains pass from a rigid state to a fluid state. This conformational change is characterized by FTIR as a displacement of the wave number of the bands corresponding to the symmetric stretching vibrations of CH_2 groups (νCH_2 bands) from 2 to 3 cm^{-1}. For pure phospholipids in the gel state, these symmetric stretching vibrations give characteristic peaks around 2850 cm^{-1} and 2853 cm^{-1} when they are in a fluid state: These values vary with the nature of the phospholipid being studied. The transition temperature from L_β to L_α phase corresponds to the inflection point of the curves, reflecting the temperature at which there are as many phospholipids in the gel state as in the liquid–crystalline state.

Using this method, we have determined the phase transition temperatures of whole *S. cerevisiae* cells, in water and with various binary water–glycerol solutions. To explain the results, we repeated the experiments using a mixture of phospholipids extracted from *S. cerevisiae* plasma membranes, and with the pure phospholipids, dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), and with three binary mixtures of these phospholipids.

2. Materials and methods

2.1. Yeast strain

S. cerevisiae W303-1 A (*MATa leu2-3,112 his3-11,15 trp1-1 can1-100 ade2-1 ura3-1*) [20] was a gift from Laboratorium voor Moleculaire Celbiologie, Katholieke Universiteit Leuven, The Netherlands.

2.2. Culture conditions

Cells were maintained on Petri dishes with a modified Malt Wickerham medium supplemented with 20 g L^{-1} of agar (VWR International, Fontenay-Sous-Bois, France). *S. cerevisiae W303-1A* was grown aerobically as previously described [21] in 250-mL conical flasks containing 100 mL of modified Malt Wickerham medium, containing 10 g glucose (VWR International), 3 g pancreatic peptone (VWR International), 3 g yeast extract (Institut Pasteur, Paris, France), and 1.5 g NaH_2PO_4 (VWR International), for 1 L of water. The pH was adjusted to 5.35 by the addition of orthophosphoric acid (Sigma, Saint Quentin Fallavier, France) before sterilization by autoclaving at 121 °C for 20 min. The flasks were shaken at 250 rpm on a rotary shaker (New Brunswick Scientific, USA) at 25 °C for 48 h. One milliliter of a subculture was transferred into a conical flask containing the same medium and allowed to grow to early stationary phase (48 h, final population of 1×10^8 cells mL^{-1}).

2.3. Preparation of binary water–glycerol solutions

Three solutes were used in these experiments: glycerol, sucrose, and sorbitol (all from Sigma).

The mass of solute to be added to 1000 g of distilled water to obtain the desired Aw was calculated using the Norrish equation [22]:

$$\mathrm{Aw} = (1 - \mathrm{X_s})e^{-KXs^2}$$

Where Xs is the molar fraction of the solute and K, the norrish coefficient of the solute used to depress Aw.

Values of K and M_w are given in Table 1.

The Aw of all the solutions was verified using a dew point osmometer (Decagon Devices Inc., USA).

2.4. Preparation of samples

2.4.1. Whole cells

Stationary phase *S. cerevisiae W303-1 A* cells were harvested by centrifugation (5 min, 2000×*g*), and washed twice in a binary water–glycerol solution at Aw=0.99. Cells were deposited on the FTIR crystal surface, and placed in a desiccator for 24 h to eliminate most of the water.

2.4.2. Plasma membrane isolation

The isolation of plasma membranes of *S. cerevisiae W303-1 A* was performed essentially as described by Serrano [23]. Stationary phase cells of 2 L culture were harvested (10 min, 2000×*g* at 4 °C) and washed twice with distilled water and buffer A (25 mM Tris, 5 mM EDTA, 0.5 mM Phenylmethylsulfonuyl fluoride (PMSF), pH 7.5). Cells were resuspended in 150 mL of buffer A before being ruptured in a homogenizer Bio101 FP120 (2.2 kbar, three times). The supernatant was then centrifuged for 10 min at 700×*g* to remove cell debris. The pellet was suspended in buffer B (10 mM Tris, 0.2 mM EDTA, 1.5 mM β-mercaptoethanol) and centrifuged as described above to increase the yield of the membrane. The combined supernatants were centrifuged at 20 000×*g* for 20 min. The resulting pellet, which contained crude plasma membrane, was suspended in buffer B containing 20% glycerol (w/w) with 10 strokes in a loose-fitting Dounce homogenizer, and applied to a discontinuous gradient made of 1 volume 53% (w/w) and 2 volumes 43% (w/w) sucrose in buffer B, followed by a 2 h centrifugation at 100 000×*g* in a swing-out rotor. The plasma membrane fraction was then recovered at the 43/53% sucrose interface. After three-fold dilution with ice-cold water, membranes were sedimented at 80 000×*g* for 20 min. The pellet was suspended in 1 mL chilled distilled water.

Table 1
K and M_w values for the three solutes used

Solute	K	M_w (g mol^{-1})
Glycerol	1.16 [41]	92.09
Sorbitol	1.65 [42]	182.2
Sucrose	6.47 [42]	342.3

2.4.3. Total lipid extraction of plasma membrane isolate

Total lipids were extracted by a modified method of Bligh and Dyer [24]. 3.75 mL of chloroform:methanol 1:2 (v/v) was added to the membrane suspension and the mixture was stirred for 15 min. Lipids were extracted during 1 h before 1.25 mL chloroform was added. The mixture was stirred again and centrifuged at 2000×*g* for 15 min. The chloroform layer was recovered and stored under nitrogen at −30 °C.

All steps of isolation and extraction protocols were performed at 4 °C to avoid enzymatic degradation and to limit oxidation.

2.4.4. Phospholipids and binary mixtures

DMPC and DMPE were selected for study as they carry the same fatty acid chain (myristoyl, C14:0), but differing polar head groups. Phospholipids carrying these two head groups can be found in *S. cerevisiae* membranes, with the PE component mainly in the internal layer of the plasma membrane, and the PC component in the external layer. Three binary mixtures were studied: (1) 90.5% DMPC, 9.5% DMPE; (2) 74.8% DMPC, 25.2% DMPE; and (3) 39.7% DMPC, 60.3% DMPE (all molar percentages). For each mixture, 10 mg of the phospholipids was diluted in 5 mL of ethanol.

2.5. FTIR measurements

2.5.1. Synthetic phospholipids

Experiments were performed using a Fourier Transform Infrared spectrophotometer IFS Vector 22 (Bruker, Germany), equipped with a ZnSe ATR crystal and a dialysis cell, and linked to a computer with OPUS software (Bruker), permitting spectra acquisition and data treatment. The dialysis cell (Bio-ATR: Bruker, Germany) allows liquid circulation on the phospholipid film to be studied via a dialysis membrane. This system permits the user to hydrate samples in a non-perturbing manner and to maintain close contact between the crystal and the film being studied. The phospholipids in this study were diluted in ethanol. When deposited on the hot crystal surface, the ethanol evaporates, leaving a dry phospholipid film. The hydration of phospholipids needs to be performed at a temperature higher than the phase transition temperature, to ensure a fluid conformation facilitating the entry of water molecules. Phospholipids were therefore heated to 70 °C by circulation of hot water near the crystal. Temperature was controlled using a thermocouple placed in contact with the film. When the desired temperature was reached, the dialysis cell was placed on the crystal and a hot solution was injected and diffused through the dialysis membrane. The time needed to hydrate samples varies with the solution used and the type of phospholipid or mix studied. Hydration was controlled by following the anti-symmetric stretching vibrations of the PO^{2-} groups ($\nu_a PO^{2-}$ peaks), as a wave number of about 1220–1230 cm^{-1} is characteristic of hydrated head groups [25,26]. When the sample had been hydrated, the crystal was cooled by the circulation of glycol from a cryostat (Huber). When the appropriate temperature had been reached, experiments could begin. The sample temperature was then increased at 0.1 °C/min, and spectrum acquisition was performed at a resolution of 2 cm^{-1} over 20 scans.

2.5.2. Plasma membrane total lipid extract

500 μL of lipid extract in chloroform was deposited on the crystal at 70 °C. Chloroform evaporated under a nitrogen stream leaving a dried lipid film. Solutions of glycerol at the appropriate Aw were used to hydrate the sample. The experiment was then carried out as described above.

3. Results

3.1. Whole S. cerevisiae cells

For the whole cells, the study was performed in water and in two binary water–glycerol solutions (Aw=0.75 and 0.65). Fig. 1 shows CH_2 symmetric stretching vibration wave numbers of whole cells as functions of temperature, for the three media. In water, there was a phase transition at about 12 °C, with a shift in wave number from 2850.6 cm^{-1} at 9 °C to 2852.4 cm^{-1} at 15 °C. Below 9 °C and above 15 °C, the wave number increased slightly with temperature. With a water–glycerol solution at Aw=0.75, transition took place between 12.5 °C and 21 °C (T_m determined at 16.5 °C), and between 14.5 °C and 28 °C (T_m 19.5 °C) at Aw=0.65. Thus, the range of temperature in which transition occurred increased with osmotic pressure, as it was 6 °C in water, 8.5 °C at Aw=0.75, and 13.5 °C at Aw=0.65. Moreover, the maximal wave number, corresponding to the fluid state, increased with the glycerol concentration, with an increase from 1.8 cm^{-1} in water to 2.5 cm^{-1} at Aw=0.65.

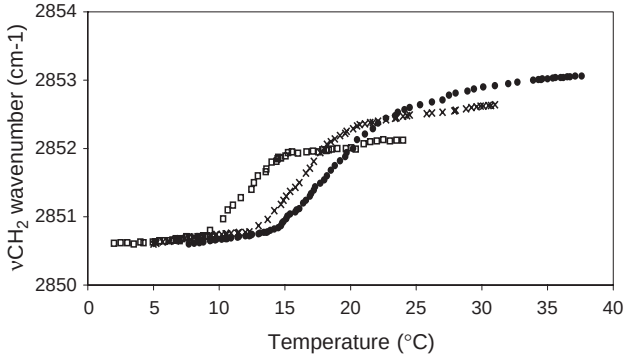

Fig. 1. Symmetric vibrations of the CH_2 groups of *S. cerevisiae W303-1 A* as a function of temperature, in water (□) and binary water–glycerol solution at Aw=0.75 (×) and 0.65 (●).

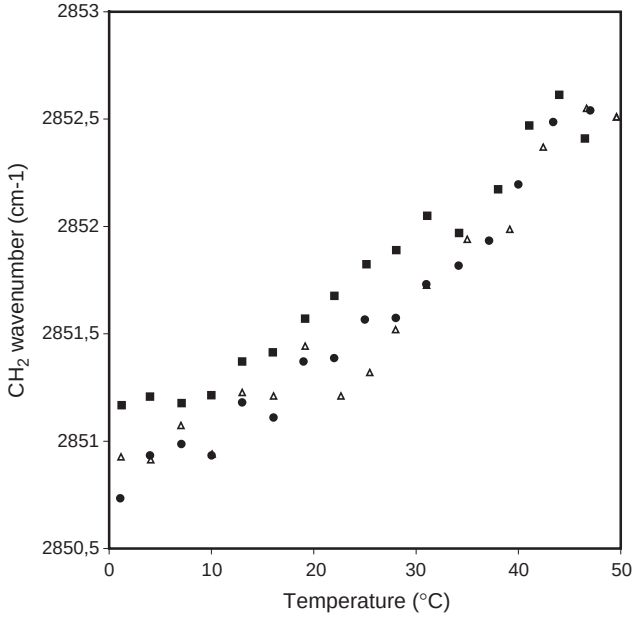

Fig. 2. Symmetric stretching vibrations of the CH_2 groups of *S. cerevisiae W303-1 A* plasma membrane as a function of temperature, in water (■) and binary water–glycerol solution at Aw=0.75 (Δ) and 0.65 (●).

3.2. Total lipid extract from plasma membrane

In a second study, lipids extracted from the *S. cerevisiae* plasma membrane were tested. As with whole cells, these were studied in water and with water–glycerol solutions at Aw=0.75 and 0.65 (Fig. 2). In water, we observed a first phase transition between 10 °C and 30 °C and a second phase transition between 37 °C and 44 °C. Actually, CH_2 symmetric stretching band slightly increased around 2851 cm^{-1} between 0 °C and 10 °C and increased to 2852 between 10 °C and 30 °C. Then, the signal slightly increased until 36 °C and increased again to 2852.5 cm^{-1} at 44 °C.

In glycerol solutions, the signal was a little lower if compared to water and no significant difference was observed between curves obtained at Aw=0.75 and 0.65. In both cases, phase transitions were less marked and a constant increase was observed between 16 °C and 44 °C. The shift in wave number ranged from 2851 to 2852.5 cm^{-1} in this temperature range and a mean temperature of phase transition around 30 °C was estimated in both cases.

Finally, it is important to notice that for all cases, the range of temperature in which transition occurred was wide (20 °C in water and 28 °C in binary water–glycerol solutions) when the wavelength of the CH_2 symmetric stretching band was subjected to a weak increase.

3.3. Pure phospholipids

Pure phospholipids were studied to better understand the behaviors of the membrane extracts and whole cells in relation to osmotic pressure. For DMPC in water, at temperatures lower than 21.5 °C, the wave numbers of νCH_2 bands were stable according to the temperature, around 2850 cm^{-1}. Between 21.5 °C and 24.5 °C, wave numbers increased quickly, until stabilizing around 2852.5 cm^{-1} for temperatures higher than 24.5 °C. Here the transition took place over a temperature range of 3 °C, and the phase transition temperature was 23 °C. The same experiment was carried out using two binary water–glycerol solutions (Aw=0.80 and 0.69), but the curves obtained did not show any difference from those carried out in water.

Experiments were also performed with DMPE (Fig. 3). In water, the DMPE is in a gel state at temperatures lower than 47 °C. It changed state between 47 °C and 49 °C and was in a fluid state at higher temperatures. The phase transition temperature was 48 °C. The same experiment was carried out with various binary water–glycerol solutions (Aw=0.90, 0.80, 0.72, 0.69 and 0.67), and, in contrast with DMPC, for which no shift of the phase transition temperature was observed, the curves (Fig. 3) showed a phase transition temperature increasing with decreasing Aw. There was no linear relation between the decrease of Aw and the increase in the phase transition temperature, as a solution at Aw=0.90 induced a very weak shift (about 2 °C), whereas a solution at Aw=0.80 induced a shift to 60 °C or 12 °C above the temperature recorded in water. At Aw=0.72, the phase transition temperature was 62 °C, whereas it was 63 °C at Aw=0.69 and 64 °C at Aw=0.67. In addition, the wave number corresponding to the fluid phase increased, whereas that corresponding to the rigid phase decreased slightly for Aw lower than 0.80.

As a shift of the phase transition temperature was not observed for DMPC, but only with DMPE, the same experiments were carried out with a binary water–sorbitol solution and another of water–sucrose. The aim of these experiments was to check if the shift previously observed was caused by the decrease of Aw or by a particular interaction of glycerol with phospholipids. As for glycerol, these two aqueous solutions induced a shift in the phase transition temperature of the DMPE. However, the presence of sucrose or sorbitol did not induce any increase in the

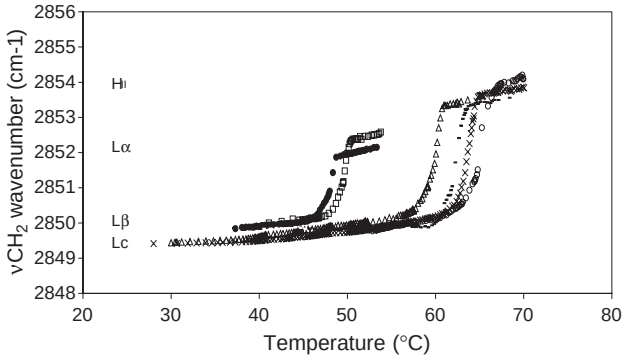

Fig. 3. Symmetric stretching vibrations of the CH_2 groups of the DMPE as a function of temperature, in water (●) and in binary water–glycerol solution at Aw=0.90 (□), 0.80 (Δ), 0.72 (-), 0.69 (×) and 0.67 (○).

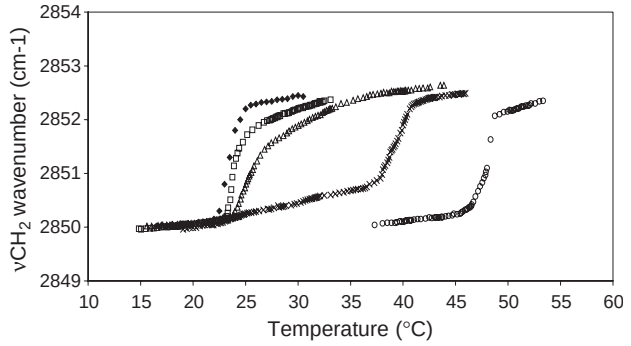

Fig. 4. Symmetric stretching vibrations of the CH_2 groups as a function of temperature for the DMPC (♦) and DMPE (○) mixtures 1 (□), 2 (Δ), and 3 (×).

wave number corresponding to the fluid state, as observed for glycerol. The lowest wave number observed at a low temperature for glycerol was also seen in the sucrose solution, but not in sorbitol.

3.4. DMPC/DMPE mixtures

Finally, three DMPC/DMPE mixtures were studied, in water and in a water–glycerol solution at Aw=0.80. In water, the mixtures' behaviors in relation to Aw were intermediate between those of DMPC and DMPE individually (as shown in Fig. 4). For mixtures 1 and 2 (DMPC/DMPE molar percentages 90.5:9.5 and 74.8:25.2 respectively), the increase in wave number of νCH_2 bands began at a temperature close to that of pure DMPC. On the other hand, the range of temperatures for which the transition occurred was extended, and increased with the quantity of DMPE present in the mixture. For pure DMPC, this was between 21.5 °C and 24.5 °C. For mixture 1, in the rigid state, the wave number was around 2850.2 cm^{-1}. A rapid increase was observed starting from 23 °C. This increase slowed at 25 °C, but the wave number stabilized only around 32 °C, at 2852.3 cm^{-1}. For mixture 2, the range of temperature for which the transition occurred was even wider (about 15 °C). For this mixture, the variation of the wave number was more progressive than for mixture 1. At 23 °C, the wave number was about 2850.2 cm^{-1} and it increased above this temperature to stabilize around 2852.5 cm^{-1} at 38 °C. The behavior of mixture 3 differed from that of the first two (see Fig. 4). Here there was a progressive increase in the wave number, reaching 2850.8 cm^{-1} at 37 °C, followed by a fast rise and a stabilization around 2852.3 cm^{-1} at 41 °C. The phase transition temperature was 39 °C.

The same experiments were carried out on each mixture with a water–glycerol solution at 30 MPa. As previously, the behavior of mixtures 1 and 2 (main phospholipid DMPC) differed from that of mixture 3. For mixture 1, the transition commenced around 24 °C and arrested at 32 °C, which differs only little from the mixture in water. However, the increase in wave number was slower in the first phase than in the case of the mixture in water. The average phase transition temperature was estimated here at 26.5 °C, or a shift of 3 °C compared with mixture 1 in water. For mixture 2, we observed again that the range of temperature over which the transition occurred differed little from that of the same mixture in water (23 °C to 38 °C). In this case, the slope of the curve is steeper, with a more progressive increase in wave number. T_m was determined at 31 °C, an increase of 4 °C compared with the phase transition temperature of the mixture in water. In the case of mixture 3, we also observed a progressive increase in the wave number up to 39 °C (c.f. 37 °C in water). The phase transition of most of the phospholipids occurred between 39 °C and 45 °C, but over a range of 2 °C more, compared with that observed in water. Consequently, compared with the same mixture in water, the most rapid change in the wave number occurred 2 °C higher, and extended over a range of 2 °C greater. Thus there was a shift of 4 °C in the temperature at which all the phospholipids were in a fluid state. The phase transition temperature was estimated at 42.5 °C, against 39 °C in water: a shift of 3.5 °C.

4. Discussion

The whole results are synthesized in Table 2 to facilitate comparisons between samples i.e., pure phospholipids, mixture of phospholipids, plasma membrane extracts, whole cells.

4.1. Pure phospholipids

In the case of DMPC, there was no influence of Aw on the phase transition temperature. On the other hand, the phase shift of DMPE increased with decreasing Aw for the three aqueous solutions tested. In water, the average phase transition temperatures of DMPC and DMPE were estimated at 23 °C and 48 °C, respectively. These temperatures were determined in the past by a variety of methods (DSC, ^{31}P NMR, Raman spectroscopy), giving values for DMPC varying from 22.5 °C to 25 °C, with an average value of 23.5 °C. In the same way, studies carried out on DMPE gave an average value of 49 °C. The values obtained here are a little lower, which may be because of our experimental conditions. Studying DMPE with various water–glycerol solutions, there was an increase in the maximum wave number reached by DMPE in the presence of glycerol. Thus, the phase transition of DMPE in water is reflected in an increase in the wave number between 2850 cm^{-1} and 2852 cm^{-1}, whereas at 55 MPa this variation occurred between 2850 cm^{-1} and 2854 cm^{-1}. The PEs are able to adopt a non-lamellar hexagonal phase H_{II}, which results in a high wave number. This transition from liquid–crystalline state to the H_{II} phase is characterized in FTIR by a wave number variation between 2853 cm^{-1} and 2855 cm^{-1}, but small differences have been observed according to the type of

Table 2
Wave number limits, amplitude of shifts, temperature limits, range of temperature and phase transition temperature for whole cells, total lipid extract of plasma membranes, DMPC, DMPE, and DMPC/DMPE mixtures for the various osmotic pressures tested

		Wave number limits (cm^{-1})		Wave number shift (cm^{-1})	Temperature (°C)		Temperature range (°C)	T_m (°C)
Intact cells	Water	2850.6	2852.4	1.8	9	15	6	12
	0.75	2850.8	2852.8	2	12.5	21	8.5	16.5
	0.65	2850.9	2853.4	2.5	14.5	28	13.5	19.5
Total lipid extract from plasma membranes	Water	2851.2	2852	0.8	10	30	20	20
	0.75	2851.2	2852.5	1.3	16	44	28	30
	0.65	2851.1	2852.5	1.4	16	44	28	30
DMPC	Water	2850	2852.5	2.5	21.5	24.5	3	23
	0.75	2849.9	2852.5	2.6	21.5	24.5	3	23
	0.65	2850	2852.6	2.6	21.5	24.5	3	23
DMPE	Water	2850.1	2852	1.9	47	49	2	48
	0.90a	2850.2	2852.5	2.3	49	51	2	50
	0.82 Sorbitol	2850.1	2852.5	2.4	56	58	2	57
	0.80	2850.1	2853.1	3	59	61	2	60
	0.79 Sucrose	2850.1	2853.2	3.1	60	62	2	61
	0.72	2849.9	2853.2	3.3	61	63	2	62
	0.69	2849.8	2853.7	3.9	62	64	2	63
	0.67	2850	2854.2	4.2	63	65	2	64
Mix A—90.5:9.5 (mol%)	Water	2850.2	2852.3	2.1	23	32	9	23.5
	0.80	2850.3	2852.4	2.1	24	32	8	26.5
Mix B—74.8:25.2 (mol%)	Water	2850.2	2852.5	2.3	23	38	15	27
	0.80	2850.2	2852.5	2.3	23	38	15	31
Mix C—39.7:60.3 (mol%)	Water	2850.8	2852.3	1.5	37	41	4	39
	0.80	2850.9	2852.4	1.5	39	45	6	42.5

studies and the nature of the fatty acid chains of phospholipid. It has been shown that in pure glycerol, DSPE is unable to adopt the lamellar liquid–crystalline phase (L_α) and passes directly from the lamellar gel phase (L_β) to hexagonal phase H_{II} [27]. In the same way, the presence of glycerol or sucrose induces a reduction in the wave number corresponding to the rigid state of phospholipids. This lower wave number could correspond to a lamellar crystalline state (L_c). Indeed, this state is characterized by a wave number slightly lower than that of the lamellar gel state, and the two types of aqueous solutions are known to induce this conformation of phospholipids. Nevertheless, the curves obtained do not make it possible to affirm here that the phase transition observed corresponds to the direct state change from lamellar crystalline state to hexagonal H_{II} ($L_c \rightarrow H_{II}$), and the DMPE could go through various intermediate states (L_c, L_β and H_{II}) successively.

4.2. Mixtures of pure phospholipids

We studied three mixtures of DMPC and DMPE. The phase transition temperature of each mixture was intermediate between those of the pure phospholipids. This was also observed by Crowe et al. [28] for binary mixtures of DOPC and POPC (dioleylphosphatidylcholine/palmitoleylphosphatidylcholine). In water, for mixtures 1 and 2 (with a preponderance of DMPC), transition began at a temperature close to that of pure DMPC. Nevertheless, the range of temperatures for which the phase transition of phospholipids occurred was extended and increased with the quantity of DMPE present in the mixture. We also observed a rapid initial increase in wave number in the first phase (faster, as the quantity of DMPC is significant), but it continued to increase more slowly with the increasing temperature. As the phase transition temperature corresponds to the temperature at which 50% of phospholipids are in the gel state and 50% in a fluid state, as in the case of pure phospholipids, the phase transition temperature of mixture 1 is 23.5 °C. At 25 °C, the wave number was 2851.6 cm^{-1}, implying that about 80% of the phospholipids went through a fluid state over a range of 3 °C, whereas the remaining 20% still required a rise of 7 °C. For mixture 2, about 55% of the phospholipids changed state over a range of 4 °C, whereas the remaining 45% required a further rise of 11 °C. The average phase transition temperature was estimated to be 27 °C. In water–glycerol solutions at 30 MPa, the range of temperatures over which transitions occurred did not change compared with those observed in water alone. However, higher phase transition temperatures were recorded. These two mixtures (1 and 2) were mainly composed of DMPC, showing that the phase transition temperature does not vary with the Aw. The small quantity of DMPE present is then responsible for the deceleration of the increase in wave number observed above a certain temperature. We believe that molecules of DMPC change state starting from 23 °C quickly and are responsible for the steep slopes of the curves between 23.5 °C and 25 °C for mixture 1 and 23.5 °C and 27 °C for mixture 2. At higher temperatures, the DMPE molecules in their turn change state. However, the phase transition temperature of pure DMPE was estimated at 48 °C, but

for mixture 1, all phospholipids were in a fluid state above 32 °C, and for mixture 2 this occurred above 38 °C. This indicates equilibration between the fluid and gel states. Here, the phase transition of DMPE was affected by the presence of DMPC, so it was slower and occurred over a larger range of temperatures. This phenomenon was increased by the presence of more DMPE in the mixture.

The behavior of mixture 3 differed from that of mixtures 1 and 2. Indeed, we observed a progressive increase in the wave number up to 37 °C, followed by a fast rise of this value and a stabilization starting from 41 °C. The progressive increase in the wave number observed below 37 °C represents the transition of a minority of the phospholipids, followed between 37 °C and 41 °C by a rapid transition of the other molecules into the fluid state. The phase transition temperature was estimated at 39 °C. In the water–glycerol solution at Aw=0.80, as for the two preceding mixtures, we observed a shift in T_m with the decreasing Aw. In addition, whereas the range of temperature for which the state change of the first two mixtures was observed did not show evolution with the decrease in Aw, for mixture 3 we noted a widening of this range. We believe that this increase is because the difference between the phase transition temperatures of the two phospholipids increases with decreasing Aw and is associated with a higher proportion of DMPE. In the case of this mixture, all phospholipids were in a fluid state at a temperature lower than for pure DMPE, indicating again an influence of the presence of DMPC. As the T_m of pure DMPC has been found to be around 23 °C, we believe that the first part of the curve corresponds to changes in the state of the DMPC molecules, but the slow increase in wave number over a wide range of temperatures shows an influence of the DMPE in limiting this change in state.

4.3. Lipids extracted from plasma membranes

For membrane extracts in water, the mean phase transition temperature was estimated to be 20 °C, with a change in the state of phospholipids observed from 10 °C to 30 °C. Moreover, another significant increase in wave number was observed between 36 °C and 44 °C. This second increase in wave number reached only 2852.5 cm^{-1}, a level which is normally attributed to the L_α phase. It could be explained by the occurrence of hexagonal H_{II} phases concerning only a minor fraction of the lipids (e.g. PE) in the total lipid membrane extract.

When studied in binary water–glycerol solutions, a progressive phase transition between 16 °C and 44 °C occurred for both Aw levels tested. The wave number varied from 2852 to 2852.5 cm^{-1}. The mean phase transition temperature was thus estimated to be 30 °C.

For all levels of Aw tested, the variation in wave number was weaker than in the case of pure phospholipids, as the amplitude was only around 1 cm^{-1}. The very varied composition in lipids of the membrane extract can explain the wide range of temperature corresponding to the transition between gel and liquid–crystalline phases. Moreover, the weak variation in the wave number can be attributed to the high content of ergosterol in the plasma membrane of *S. cerevisiae* [29]. Actually, this phenomenon has already been observed in model membranes with increased ergosterol concentrations [9]. Sterols are known to interact with phospholipids and to modify their conformation. Indeed, these molecules induce an increase in the fluidity of the hydrocarbon chains at temperatures lower than the phase transition temperature between gel and liquid (T_m), whereas they decrease it above T_m [30–33]. Furthermore, the fact that the wave numbers under and above phase transitions slightly increased with temperature is in agreement with results obtained by Leslie et al. [9] in unilamellar vesicles containing ergosterol.

Comparing this behavior with what was observed for binary mixtures, we note that only in the case of mixture 3 did the initial temperature of transition increase with decreasing Aw. This we attributed to the high proportion of DMPE. It could be concluded that the membrane extract contained a sufficient relative quantity of phospholipids whose conformation is sensitive to Aw to make it sensitive to increasing concentrations of glycerol. In fact, according to Zinser and Daum [34], PE represents 20.3% of total plasma membrane phospholipids when PC is only 16.3%. Nevertheless, the behavior of mixture 3 does not reflect this of membrane extract as decreasing Aw from 0.75 to 0.65 didn't change the membrane extract phase transition.

4.3.1. Lipids of whole cells

For the whole cells in water, the main lipid phase transition temperature was estimated to be 12 °C, with a transition observed between 9 °C and 15 °C. A shift of the phase transition and an increase in the variation of the wave number was noted in the presence of a binary water–glycerol solution. The phase transition was estimated at 16.5 °C for the solution at Aw=0.75, and at 19.5 °C for the solution at Aw=0.65. At 0.75, the transition occurs between 12.5 °C and 21 °C (range of 8.5 °C) and at 0.65 it was between 14.5 °C and 28 °C (range of 13.5 °C).

For the plasma membrane extract, phase transition was estimated at 20 °C in water, with a transition of lipids observed between 10 °C and 30 °C. Thus, for the whole cells the transition occurred over a smaller range of temperature (6 °C, against 20 °C for the extract), and at a lower temperature, 12 °C. Such a shift had already been observed between membrane isolates and whole cells [9] and had been attributed to the selected stretching band to follow the phase transition (phosphate group for whole cells and CH_2 group for membrane isolates).

In the present case, several explanations can elucidate this shift and the different sensitivity to Aw of the samples (whole cells and membrane extracts).

Firstly, for the whole cells, the CH_2 stretching band not only represents the behavior of plasma membrane lipids but

of the whole CH_2 groups in the cells which include all subcellular membranes and probably triglyceride reserves of the cells.

Secondly, distribution of lipids is greatly different for each one of the biological samples. Indeed, the membrane extract is a mixture of lipids distributed in a random manner on the surface of the measuring crystal. In contrast, in whole cells, certain lipids are mainly present in the external layer and others in the internal layer of the cell membrane. In particular, the PEs are mainly found in the internal layer of the *S. cerevisiae* plasma membrane. This distribution could have an influence on the appearance of H_{II} phases and their detection by FTIR.

A third difference between the two samples relates to the absence of proteins in the membrane extract. Indeed, proteins modify the structure of surrounding lipids [35], and can maintain PEs in a lamellar state [36–39]. In whole cells, the phospholipids thus do not seem to have adopted a hexagonal H_{II} conformation whereas H_{II} phase is suspected in the plasma membrane extract.

Numerous studies have related phase transition occurrence of the plasma membrane to mortality of yeasts when submit to dehydration [7–9]. Our objective was to localize temperature and Aw ranges where transitions occurred by the mean of the rapid and non-invasive FTIR method. Thus, it must be considered if the samples tested (whole cells and plasma membrane extracts) could permit the measurement of phase transitions into plasma membrane of *S. cerevisiae*. A positive relationship between Aw and the range of temperature over which the variation of phospholipid fluidity occurs has been observed in a study of membrane fluidity using fluorescence polarization [40]. This was confirmed here. At Aw=0.73, this temperature range was between 13 °C and 21 °C, whereas at Aw=0.64 it was between 14 °C and 28 °C. The average corresponding phase transition temperatures had thus been estimated at 17 °C and 21 °C, respectively. Here, the ranges of temperatures observed were in agreement with the results from fluorescence polarization: 12.5 °C to 21 °C at Aw=0.75, and 14.5 °C to 28 °C at Aw=0.65. However, the estimated phase transition temperatures—although close-differed, as they were 16.5 °C for the solution at Aw=0.75 and 19.5 °C for Aw=0.65, but the methods used do not necessarily reflect the same kind of phenomenon.

In addition, in a preceding study using FTIR, Crowe and Crowe [18] determined the phase transition temperature of whole cells of *S. cerevisiae* in water to be about 10 °C. Here we found it to be 12 °C. This difference could be due to experimental conditions, but also to the strain used, as it was also found to be 12 °C by fluorescence polarization.

According to these works, results obtained when studying CH_2 stretching band of whole cells give a good evaluation of phase transition occurrence in plasma membrane. Nevertheless, it is probable that a great part of the CH_2 groups did not come from phospholipids plasma membranes and made part of the signal measured. For this reason, data should be interpreted with caution. However, it is possible, that in this range of temperature, the triglyceride influence is only minor and that the transition observed mainly resulted from the transition of the membranes phospholipids.

5. Conclusion

This FTIR study has allowed us to better understand modifications of phospholipid molecular behavior at different temperatures according to Aw. It has also highlighted the limitations of using simple membrane extracts to study the complex behavior of living cells. In fact, the presence of proteins in whole cells plus the positioning of phospholipids in the cell membranes would induce very different results. The behavior of pure phospholipids, and mixtures of them, was not relevant to the study of membrane extracts. Indeed, lipids extracted from plasma membrane where much more complex not only by the phospholipid composition but also by the presence of other lipids like ergosterol.

When measuring CH_2 stretching band of whole cells, results obtained for T_m in different Aw conditions were close to data found in literature. However, it seems that this method may reflect the behavior of the total lipid of the cells.

It thus appears that neither the study of membrane extracts nor the study of whole cells, while providing important information, can reflect the state of the membrane of living cells. However, our results showed that the measurement of CH_2 symmetric stretching band of whole cells could permit the localization of phase transitions close to those found using fluorescence polarization [40]. Thus it seems that this technique may reflect membrane conformations. Nevertheless, for a better comprehensive approach, further works will permit to discriminate between the contribution to FTIR signal of plasma membranes lipids with regard to other cells lipids.

Such a rapid and non-invasive method could be advantageously used to predict the Aw and temperature ranges of membrane lipids transitions and the ability of microorganisms to be dried.

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