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OSMOTIC BEHAVIOUR OF *ACHOLEPLASMA LAIDLAWII B* CELLS WITH MEMBRANE LIPIDS IN LIQUID-CRYSTALLINE AND GEL STATE

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SUMMARY

The osmotic behaviour of *Acholeplasma laidlawii B* cells was investigated with combined spectrophotometric and enzymatic measurements. The conclusion could be drawn that this osmotic behaviour depends largely on the physical state of the membrane lipids. When part of the membrane lipids is in the liquid-crystalline phase the cell is able to swell and behaves as a good osmometer. However, when the membrane lipid is in the gel phase, the cell is unable to swell and the change in absorbance of the cell suspension is then completely due to lysis.

INTRODUCTION

Since the lipid composition of the membranes of *Acholeplasma laidlawii B* can be varied considerably by changes in the growth medium [1], this organism was chosen for many studies on the significance of membrane lipid composition for the membrane functions [2–6]. Furthermore, the cells behave as osmometers with selectively permeable membranes and in comparative experiments conclusions on relative permeabilities have been drawn from the initial swelling rates in isotonic solutions which can be measured optically [2–5].

By studying the effect of temperature on the permeability of the cells it has been found that there is a sudden change in the optical behaviour of the cells, corresponding with the lower limit of the gel-to-liquid-crystalline phase transition of the membrane lipids as measured by differential thermal analysis [5] and differential scanning calorimetric studies [7]. In view of the increasing interest in biomembranes with paraffin chains in an ordered state we thought it important to study the osmotic properties of cells with membranes in the gel state in comparison with those with the membrane lipids in the liquid-crystalline state in order to find a mechanism that accounts for the swelling behaviour in both phases.

MATERIALS AND METHODS

Organism and growth conditions. Cells of *A. laidlawii B* were grown on a lipid-poor tryptose medium as described [3, 8]. Elaidic acid was added to the growth

medium to a total concentration of 0.12 mM. Cells were harvested in late log phase, washed with 150 mM NaCl and resuspended in a 300 mM sucrose solution. This suspension was diluted such that 100 μl suspension, added to 10 ml 300 mM sucrose solution came to an absorbance (450 nm) of 0.5–0.7. The cell suspension was equilibrated at room temperature for 1 h before use.

Isolation of membranes. Membranes of *A. laidlawii* were isolated as described by Van Golde et al. [9].

Determination of free and total glucose-6-phosphate dehydrogenase. The amount of free cytoplasmic enzyme glucose-6-phosphate dehydrogenase was measured by continuously recording the rate of formation of NADPH at 340 nm [10]. The absorption was measured using a Perkin-Elmer double beam spectrophotometer Model 356 in split-beam mode and thermostated 1.0 cm cuvettes.

Each cuvette contained 1.2 ml sucrose solution of a certain osmolarity, 10 μl buffer (containing 7.5 μmol Tris · HCl (pH 7.4), 1.5 μmol 2-mercaptoethanol), 10 μl $NADP^+$ solution (20 μg/ml), 10 μl glucose-6-phosphate solution (32 mg/ml) (omitted in reference cuvette) and 50–200 μl cell suspension. Conditions were such that $\partial A_{340\ nm}/\partial t$ was proportional to the concentration of enzyme.

The ratio of enzyme activity before and after addition of 5 μl of a 10 % solution of Triton-X-100 was taken as a measure of the lysed fraction θ. (This amount was enough for complete cell lysis). It appeared that Triton-X-100 had a slightly inhibitory effect on the enzyme activity, the inhibition being proportional to the amount of Triton added. A correction was made for this by adding subsequent amounts of Triton-X-100 (3 × 5 μl of the 10 % solution), noting graphically the recorded enzyme activities and extrapolating to zero Triton concentration.

The lysed fraction was calculated as a function of sucrose concentration. This concentration was corrrected for the amount of buffer and cell pellet added.

Materials. All reagents used were of Analytical Reagent Grade and used without further purification.

RESULTS AND DISCUSSION

It has been found [3, 5] that cells of *A. laidlawii*, resuspended in solutions of different sucrose concentration at room temperature, exhibit a linear relationship between the reciprocal of the absorbance at 450 nm and the reciprocal of the sucrose concentration (for concentrations between 50 and 200 mM). This was taken as a strong indication that cells of *A. laidlawii* behave as ideal osmometers as was also found for liposomes [11].

However, lysis of cells results, just like swelling, in a decrease of the absorbance. As it is likely that, especially at low sucrose concentrations, a high proportion of cells will be lysed, a correction for the amount of lysis should be made in order to obtain a good understanding of the real osmotic behaviour of a cell. To correct for the amount of lysis we carried out the following procedure; it appeared that under measuring conditions in a 300 mM sucrose medium the absorbance as a result of light scattering was approximately proportional to the concentration of cells or membranes as can be seen from Fig. 1. Now we presume that at any concentration below 1.0:

$$A = (1-\theta)A_{cell} + \theta A_{lys} \tag{1}$$

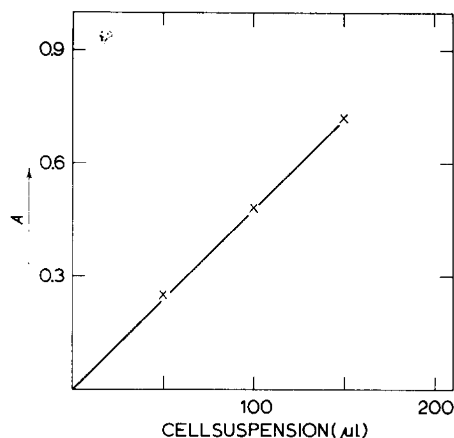


Fig. 1. Relationship between absorbance at 450 nm and concentration of cells. Successive amounts of cells were added to a 300 mM sucrose solution. Temp. 12 °C. At 28 °C the behaviour is identical.

A, measured absorbance; A_{cell}, absorbance if no cells are lysed; A_{lys}, absorbance if all cells are lysed; θ, lysed fraction of cells = number of lysed cells/number of (lysed+intact) cells. From this equation we can calculate the absorbance we expect to measure if all cells were intact:

$$A_{cell} = \frac{A - \theta A_{lys}}{1 - \theta} \tag{2}$$

so we can calculate A_{cell} if we can measure, in addition to the absorbance the lysed fraction θ and the absorbance of cells after complete lysis. The lysed fraction can be determined by comparing the amount of free cytoplasmic [10] enzyme glucose-6-

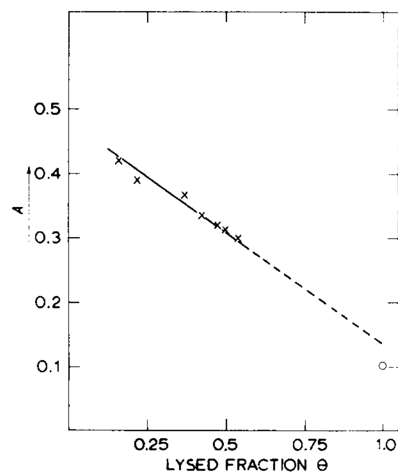


Fig. 2. Relationship between absorbance measured (450 nm) and lysed fraction θ in different sucrose solutions. The extrapolated value for E_{lys} was 0.135. The value obtained with lysed cells was 0.105. Temperature was 12 °C. At 28 °C a similar behaviour was observed.

phosphate dehydrogenase and the enzymatic activity after lysis of the cells with Triton-X-100 as described in Materials and Methods.

The absorbance of completely lysed cells was taken to equal the absorbance of a sample after fracturing of the cells by shaking on a Vortex mixer during 20 min at 0 °C. Cells fractured this way satisfied the following conditions: 1. The absorbance changed no more after prolonged shaking. 2. All glucose-6-phosphate dehydrogenase was available. 3. "Cells" did not swell any more in isotonic glycerol. 4. The value for A_{lys} obtained by extrapolating to $\theta = 1$ in a graph representing the measured absorbance A as a function of θ (Fig. 2), was comparable with the value for A_{lys} obtained from these fractured cells. Combining these criteria the assumption was made that cells, fractured this way, behave optically like cells fractured due to osmotic lysis. From differential thermal analysis and scanning calorimetric measurements [7] it is known that membranes of *A. laidlawii* cells grown on elaidic acid show a broad gel to liquid-crystalline phase transition from 18 up to 42 °C. The osmotic behaviour of these cells was tested at 12 °C, which is below the lower limit of this phase transition, and at 28 °C where a large part of the membrane lipids exists in the liquid crystalline phase.

Figs 3 and 4 represent the lysed fraction θ as a function of the sucrose concentration. For convenience, the concentration axis is represented as a reciprocal. As can be seen, in both cases there is a certain amount of lysis in a 300 mM sucrose medium, due to the resuspending of the cells. Otherwise, the behaviour of the cells above and below the lower limit of the phase transition is quite different. At 28 °C lysis occurs quite gradually when the sucrose concentration is lowered, whereas at 12 °C the lysis is more abrupt. Apart from this in the latter case there was also a relevant increase in the amount of lysis when the sucrose concentration was increased. This behaviour was also observed below the transition temperature of stearic acid grown cells (not shown). From the values of θ found, combined with the absorbance measured, the osmotic swelling behaviour of intact cells can be calculated from Eqn 2 (θ and A were measured within equal time limits, that is within 15 min after resuspending into the medium). The osmotic behaviour of cells above and below the transition temperature is represented in Figs. 5 and 6. In these graphs a second correction

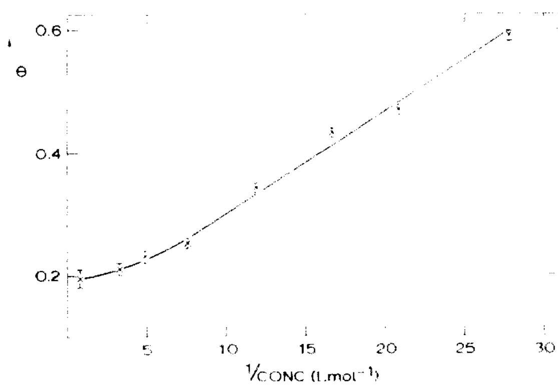


Fig. 3. Lysed fraction θ, measured as described in Material and Methods as a function of sucrose concentration. Temperature 28 °C.

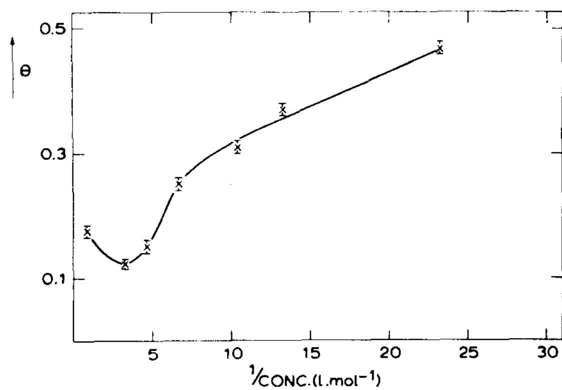
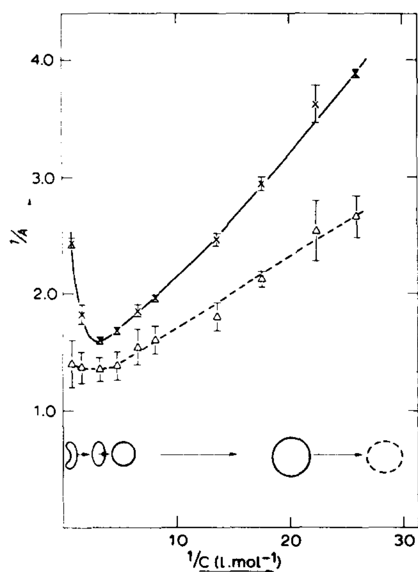


Fig. 4. Lysed fraction θ, measured as described in Material and Methods as a function of sucrose concentration. Temperature 12 °C.

was made, because it appeared that for sucrose concentrations above 300 mM the absorbance of any solution was enlarged due to the increasing refractive index of the sucrose solution. The correction was made, assuming this influence being essentially the same for solutions containing cells, lysed cells of purified membranes.



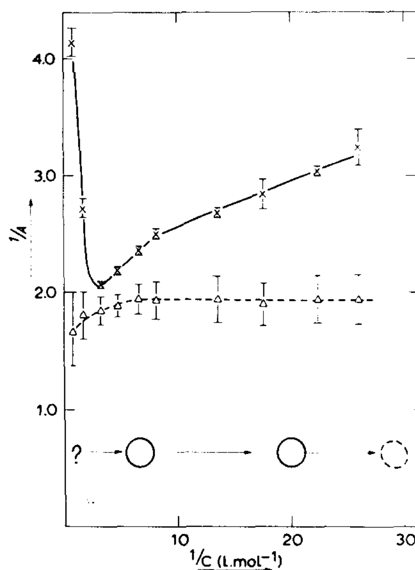


Fig. 5. Double reciprocal plot of absorbance (450 nm) versus sucrose concentration. 100 μl cell suspension was added to 10 ml sucrose solution. Temperature, 28 °C. Straight line: absorbance as measured; broken line: absorbance corrected for fraction of lysed cells and, for sucrose concentrations above 300 mM, also for the increase of the refractive index of the sucrose solution.

Fig. 6. Double reciprocal plot of absorbance (450 nm) versus sucrose concentration. 100 μl cell suspension was added to 10 ml sucrose solution. Temperature, 12 °C. Straight line, absorbance as measured; broken line, absorbance corrected for fraction of lysed cells and, for sucrose concentrations above 300 mM, also for the increase of the refractive index of the sucrose solution.

As can be seen, at 28 °C the curve is shifted as a result of the correction for lysis, but the correction has no profound influence on the shape of the curve. The corrected curve exhibits a linear relationship from 200 mM down to very low sucrose concentrations. Assuming that $1/A$ is directly proportional to the cell volume [2–5] this indicates that under these conditions the cell is a good osmometer. Above 200 mM there is a deviation from the linear relationship. At 12 °C the result is quite different. The uncorrected graph is linear over a very small concentration range. After correction however, the absorbance comes to a constant value at concentrations below about 150 mM sucrose. At higher concentrations there probably is a deviation from this constant value, even though the experimental error made in this region is high. From Fig. 5 we can conclude that cells with membranes in the liquid-crystalline phase are able to swell and behave as ideal osmometers over an extended concentration range (50–200 mM sucrose). Concerning the non-linear part of the curve, it has been found by Lemcke [12] from electron-microscopy pictures that cells of *A. laidlawii* in hypertonic media have a collapsed shape. Further it has been found [5, 12] that cells grown on elaidic acid have a rod-like shape in their growth-medium (isotonic to 270 mM sucrose [12]), but are spherical in a 200 mM sucrose solution [5]. So, we conclude that the deviation from the linear relationship at 28 °C is due to the fact that cells in sucrose solutions with concentrations above 200 mM are no longer spherical. Summarizing, it can be stated that cells resuspended in strongly hypertonic media (e.g. 1200 mM sucrose) at 28 °C have a folded structure. When the osmolarity is lowered, the cell will unfold, becoming rod-shaped at 300 mM and reaching the spherical form at 200 mM. From here the cell is able to swell as a spherical particle, stretching the membrane (see Fig. 5).

It follows from Fig. 6 that those cells that survive at 12 °C in any sucrose concentration below 150 mM have a constant volume and are unable to swell. The deviation from the constant absorbance value at 12 °C starts at about the same osmotic concentration as the deviation from the linear relationship at 28 °C. We assume that cells of *A. laidlawii* at 12 °C are spherical in osmotic concentrations below 150 mM and shaped otherwise in solutions of higher concentration. No data are known about the actual shape of a cell with the membrane in the gel phase. This leads to the assumption that by lowering the osmotic concentration, the cell will swell until it reaches the spherical form at about 150 mM (see Fig. 6). At lower concentrations the cell is unable to swell any further, resulting in a sharp increase in the amount of lysis at about 150 mM sucrose (see Fig. 4). This is due to the poor elasticity of the cell membrane in the gel phase. These results are in agreement with the observations of M.C. Blok (personal communication), who found that liposomes (made of synthetic phospholipids, dimyristoyllecithin and dipalmitoyllecithin) in the gel-phase are permeable to water. When cells of *A. laidlawii* are resuspended in isotonic solutions of glycerol or erythritol, they start swelling due to the applied gradient. The volumes gone through successively during this swelling are the same as those obtained in the various sucrose solutions. So in isotonic solutions above 200 mM the cell in the liquid-crystalline phase will first swell until the spherical form has been reached, and swell from there as a spherical particle obeying Boyle-van 't Hoff's law. This relationship between volume of the cell and the osmotic concentration of the solution, could also be calculated from phase-contrast microscopic pictures (B. de Kruyff, personal communication). During the first part of the swelling there will not be much resisting

force due to the stretching of the membrane, resulting in a much higher swelling rate compared with the second swelling. Only for this second swelling is the volume-change directly related to the measured change in absorbance at 450 nm. In the gel phase the cell will be able to swell in isotonic solutions of glycerol or erythritol above 150 mM until the spherical form has been reached. Because there is no possibility for any further swelling, all change in absorbance measured after this first swelling will be due to lysis of the cells. In agreement with suggestions made in an earlier contribution [5] it can be concluded that it is impossible to describe the swelling behaviour of cells with membranes in the gel phase by measuring the change in absorbance.

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