Biochimica et Biophysica Acta, 352 (1974) 64–70

BBA 76640

PHASE TRANSITIONS IN THE YEAST CELL MEMBRANE THE INFLUENCE OF TEMPERATURE ON THE RECONSTITUTION OF ACTIVE DRY YEAST

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(Received November 21st, 1973)

SUMMARY

Active dry yeast, with a water content of about 7%, can be reconstituted in water at 38–42 °C. When the dried cells are rehydrated in cold water, the cells lose their viability. The influence of temperature on the reconstitution process shows that a phase transition is involved in this cold-shock effect. The experimental results indicate that, in addition to membrane lipids, vicinal water may be involved in this phase transition.

INTRODUCTION

By careful drying, the water content of yeast cells can be decreased from the normal value of about 70 to about 7%. This dried yeast retains its enzymatic activity [1] and has much better storage properties than fresh yeast [2].

It has been shown previously that dry yeast cells lose their viability when rehydrated with a cold medium. This "cold-shock effect" was ascribed to a physical transition between different states in the cell membrane [3]. No suggestions were made, however, concerning the nature of this transition.

Phase transitions may be visualized by discontinuities in Arrhenius plots of physiological functions [4]. Therefore a detailed study of the influence of temperature on the reconstitution of active dry yeast was made. Further, an attempt was made to elucidate the nature of the phase transition causing the cold-shock effect. The results of these studies are presented in this communication.

METHODS

The active dry yeast used in these experiments was obtained from the Royal Netherlands Fermentation Industries Ltd. Rehydration experiments were carried out by incubating 500 mg dry yeast in a flask, placed in a shaking thermostat bath at a given temperature. After 15 min, 50 ml water of the same temperature was added. The resulting suspension was further incubated at the same temperature.

Slow rehydration was performed by passing humidified, oxygen-free nitrogen over dried yeast in a rotating flask, during 4–48 h at room temperature. The water content of yeast samples was measured by drying at 105 °C to constant weight.

Leakage of K^+ was determined by measuring K^+ in the supernatant with a flame photometer. Fermentation of glucose by yeast, rehydrated at different temperatures, was measured using the standard Warburg technique at 25 °C.

The percentage of non-viable cells was measured by selective staining of non-viable cells as described previously [5] and by the plating method. With the latter procedure the yeast cell suspension was diluted in 1% NaCl and the number of cells was counted with a TOA electronic particle counter. The percentage of viable cells was then determined by plating the diluted suspension on culture plates, containing 1% glucose, 0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 1.5% agar. The plates were incubated for 48 h at 28 °C.

Lipid extraction of yeast cells was carried out as described by Deierkauf and Booij [6]. Differential scanning calorimetry was performed with a Perkin–Elmer DSC-1B, as described by Blazyk and Steim [7].

RESULTS

The influence of the rehydration temperature on viability is shown in Fig. 1. The percentage of viable cells increased slowly with increasing temperature between 2 and 15 °C, and much faster at higher rehydration temperatures. Viability reached a maximum of about 75% at temperatures of 38–42 °C. The plating method and the procedure of counting of damaged cells by selective staining gave identical results.

The percentage of non-viable cells after rehydration at 40 °C varied slightly between different dried yeast batches. It seems likely that the yeast cells, remaining non-viable even under optimal rehydration conditions, have been damaged irre-

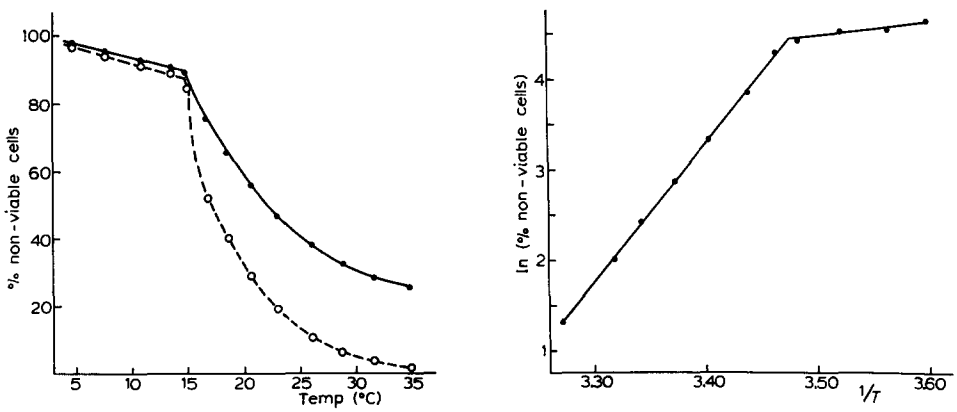


Fig. 1. The influence of rehydration temperature on viability. ●–●, percentage non-viable cells, measured experimentally; ○–○, percentage non-viable cells after correction of the experimental results for the number of cells, damaged irreversibly during drying.

Fig. 2. The relationship between the ln of the percentage of non-viable cells and the reciprocal of the rehydration temperature.

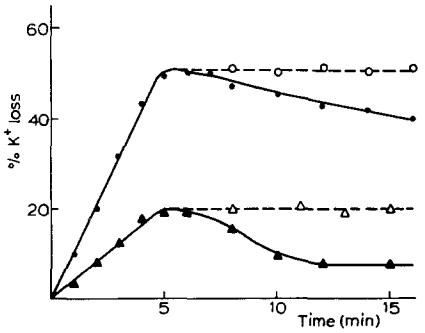
versibly during the drying process [2]. The percentage of non-viable cells was much smaller (about 10%) if the dried yeast was sieved prior to use, discarding all pellets smaller than 0.6 mm. Correction of the results for this damaged fraction yields the dashed curve in Fig. 1. In all further experiments the results were corrected for the fraction of the total population that remains non-viable after rehydration at 40 °C.

In Fig. 2 the natural logarithm of the percentage of non-viable cells is plotted against the reciprocal of the rehydration temperature. The curve has a break at 14.7 °C.

The leakage of K^+ during rehydration is depicted in Fig. 3. Maximal K^+ loss is reached after about 5 min, coinciding with the moment that the yeast is fully suspended. This time interval can be decreased by more vigorous shaking. The secondary decrease of the K^+ concentration in the medium appeared to be caused by K^+ uptake by viable cells and can be abolished by blocking cell metabolism with 1 mM iodoacetate in the rehydration medium (dashed curve in Fig. 3). In control experiments it was verified that 1 mM iodoacetate had no influence on viability. Therefore, further experiments on K^+ loss during rehydration were carried out in a iodoacetate-containing medium, measuring K^+ loss after an interval of 15 min.

The relationship between K^+ loss and rehydration temperature is shown in Fig. 4. Again a residual K^+ loss of about 25% was found at the optimal rehydration temperature of 40 °C, corresponding closely to the experimental data on viability. A plot of the logarithm of fermentation velocity at 25 °C against the reciprocal of the rehydration temperature gave identical results. Both curves showed a break between 14 and 15 °C.

To study the possible role of water structures in the cold shock effect, different rehydration media were compared. Rehydration was carried out in water, in 25% dimethylsulfoxide and in 5% thiourea, all media containing 1 mM iodoacetate. These solutes are known to perturb water structures [8]. In preliminary



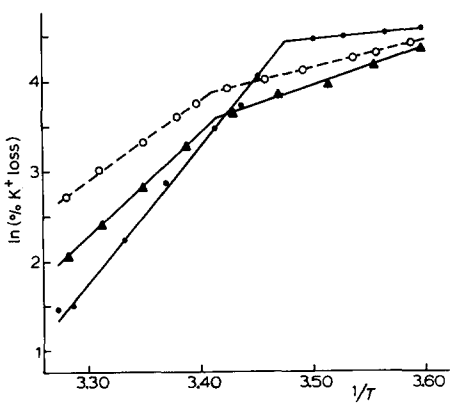


Fig. 3. K^+ leakage during rehydration of dried yeast. ●–●, rehydration in water at 17 °C; ○–○, rehydration in 1 mM iodoacetate, 17 °C; ▲–▲, rehydration in water, 22 °C; △–△, rehydration in 1 mM iodoacetate, 22 °C.

Fig. 4. The relationship between the ln of the percentage of K^+ loss and the reciprocal of the rehydration temperature in various rehydration media. ●–●, water; ▲–▲, 25% dimethylsulfoxide; ○–○, 5% thiourea.

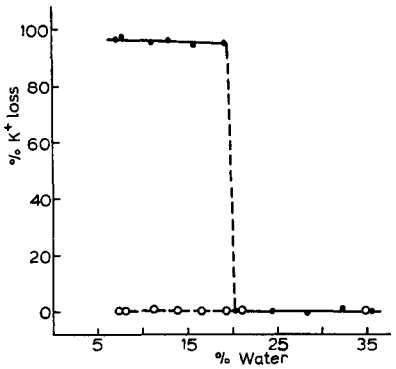


Fig. 5. The relationship between the water content of dried yeast and the K^+ loss, caused by rehydration at 4 °C (●–●) and at 40 °C (○–○).

experiments it was shown that these media did not cause K^+ loss from normal fresh yeast, nor from dried yeast cells reconstituted in distilled water at 40 °C. The results of rehydration of dried yeast in these media at different temperatures are shown in Fig. 4. Dimethylsulfoxide and thiourea obscure the breaking point which is simultaneously shifted to a higher temperature (about 22 °C).

In further experiments dried yeast with a water content of 7% was rehydrated slowly at room temperature in a stream of oxygen-free, humidified nitrogen. Yeast batches with a water content of 7 to 35% were thus obtained. The partially rehydrated yeast was subsequently suspended in water at 4 or at 40 °C. The resulting K^+ loss is shown in Fig. 5. Apparently the cold-shock effect disappears abruptly after slow rehydration over 20% in humidified nitrogen. These results agree with similar observations of Echigo et al. [3].

The possible occurrence of a phase transition in the lipids of the cell membrane was studied by differential scanning calorimetry. Thermograms of the lipid extract from yeast cells and of whole cell suspensions did not reveal a transition in the range from −20 to +40 °C.

DISCUSSION

Probably about 25% of the yeast cells has been damaged irreversibly during drying. This can be deduced from the fact that under optimal rehydration conditions in water (38–42 °C) 25% of the cells are non-viable. The same percentage of dead cells was found after slow rehydration in humidified nitrogen at room temperature. In addition, this percentage was very constant in any particular yeast batch but varied in different yeast batches from 20 to 28%. As shown, the percentage of non-viable cells was much smaller in the bigger yeast pellets, indicating again that the cell damage should be attributed to the drying process. The results were corrected for this percentage of damaged cells. It should be emphasized, however, that omission of this correction did not essentially affect the results. Without correction the same temperature irregularities were found.

In all experiments there was a close correlation between viability, staining of dead cells, K^+ loss and fermentation after rehydration. Apparently the cells respond

in an all-or-none fashion to rehydration, either staying intact, or losing all K^+, with a concomitant loss of viability and fermentation capacity.

The relationship between cell survival and rehydration temperature shows a break at 14.7 °C (Fig. 2). Temperature irregularities as reflected by breaks in Arrhenius plots can be considered as the thermodynamic consequence of a phase transition [4]. In analogy, it seems very likely that a phase transition is involved in the cold-shock effect of dried yeast. The existence of such a phase change has been suggested already by Echigo et al. [3] as an explanation for the cold-shock effect.

According to present knowledge, phase transitions at physiological temperatures can occur in two membrane components: the membrane lipids [9–13] and the vicinal water at the cell interface [8, 14]. Thermal transitions of proteins occur at higher temperatures [15].

In recent literature most attention has been paid to phase transitions in the membrane lipids, and several breaks in Arrhenius plots of physiological functions have been ascribed to the order–disorder transition of the hydrocarbon chains of membrane lipids [16, 17]. It seemed likely, a priori, that a transition in the lipid phase is also involved in the cold-shock effect.

In the present experiments no lipid phase transitions could be found with differential scanning calorimetry. Hinz and Sturtevant [18] demonstrated that the endothermic transitions obtained with phospholipids disappeared after addition of cholesterol. In accordance with this observation, no phase transitions are found in the cholesterol-containing red blood cell membranes and lipids [19]. It seems probable that the high ergosterol concentration in yeast cell membranes has a similar effect as cholesterol, keeping the hydrocarbon chains of the phospholipids in an intermediate fluid condition [19], thus preventing phase transitions.

Several observations indicate that breaks in Arrhenius plots of physiological functions cannot be explained solely by phase changes in membrane lipids. As pointed out by Steim [10], the sharp breaks in Arrhenius plots can hardly be attributed to the transitions in membrane lipids, occurring over a broad range of about 25 °C. Moreover, as shown by Lenaz et al. [20], several enzymes bound to the same membrane exhibit breaks in Arrhenius plots at different temperatures. Esfahani et al. [21] observed disparities between the transition temperature of membrane lipids and the temperature of discontinuities in the Arrhenius plots of some membrane processes in *Escherichia coli*. This led these authors to conclude that the distribution of lipids within the membrane is heterogeneous. Overath et al. [22] presented evidence, however, for randomization of the lipid phase of *E. coli* membranes. Therefore, the only possibility for a heterogeneous distribution would be a specific interaction between membrane proteins and certain phospholipids. As shown by Chapman and Urbina [23], such protein–phospholipid interactions may cause a considerable shift in transition temperatures, thus obscuring direct correlations between transitions in isolated membrane lipids and transitions in membrane functions.

Watson et al. [24] also observed that two enzymes bound to yeast mitochondria showed breaks in Arrhenius plots at different temperatures. For both enzymes the transition temperature changed when the fatty acid composition of the membrane lipids was changed experimentally. Therefore, these authors concluded that, although the lipid composition of the membrane plays a role in the interpretation of discontinuities in Arrhenius plots, other factors should be considered as well.

The present experiments indicate that one of those factors may be the structure of vicinal water. Changes in the structure of vicinal water usually occur around 15, 30 and 45 °C [8]. The break at 14.7 °C found in the present experiments corresponds rather closely to one of these transition temperatures. Further, dimethylsulfoxide and thiourea tend to disrupt water structures [8]. This may explain their influence on the cold-shock effect, obscuring and shifting the temperature break (Fig. 4). Finally, as shown in Fig. 5, the water content of dried yeast is of crucial importance for the occurrence of the cold shock effect. If the water content is increased slowly in humidified nitrogen at room temperature to 20%, the cold shock effect disappears very abruptly. This phenomenon can be understood if the vicinal water structure plays an important role in the cold shock effect. It is also in accordance with observations which indicate that a certain degree of hydration is indispensible to maintain structural integrity of cellular membranes [25].

The described experiments are not conclusive with respect to the exact nature of the phase transition involved in the cold-shock effect. It is quite conceivable that membrane lipids play an important role. As shown, several observations indicate that presumably a change in the structure of vicinal water is also involved in this process. This suggests that phase transitions in biological structures may be very complicated, being a cooperative effect involving both membrane lipids and vicinal water.

ACKNOWLEDGMENT

The authors are much indebted to Miss K. Christianse for her skilful technical assistance and to the Netherlands Organization for the Advancement of Pure Research (Z.W.O.) for financial aid.

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