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TEMPERATURE EFFECTS ON THE PROLINE TRANSPORT SYSTEM OF *SACCHAROMYCES CHEVALIERI*

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SUMMARY

1. The effects of temperature on the operation of a proline transport system in yeast are described.

2. The kinetics of L-proline accumulation are not greatly altered by changes in temperature.

3. The initial velocity of L-proline uptake remains constant up to 120 min when cells are heated at 30°, but decreases sharply after 40 min when cells are heated at 42°. This sudden change was found to occur immediately when cells grown at 20° were heated at 42°.

4. The decrease in the initial rate of L-proline uptake after heating at 42° is only partially reversed when the cells are slowly cooled to 30°. On the other hand, the kinetics of accumulation are little affected. Also, the maximum capacity to accumulate L-proline is practically unaltered after heating at 42°.

5. Cells devoid of their cell wall show a sharp decrease in the initial velocity of uptake when heated even only at 32°. The change may be detected after one minute preincubation at 32°. Thus, the cell wall appears to behave as an insulator.

6. The results are discussed in terms of the hypothesis that a temperature increase primarily causes changes in the liquid-crystalline structure of membrane lipids.

INTRODUCTION

In the last few years it has become clear that some specific proteins play a fundamental role in the process of transport across biological membranes. This information has been reviewed by PARDEE[1]. However, far less is known about the role of lipids in transport phenomena. Although it is generally accepted that the structure of lipid–water systems and lipid–water–protein systems are essential for the maintenance of normal structure and function of biomembranes (see reviews by STEIN[2], KAVANAU[3] and also by GLAUERT AND LUCY[4]) few reports on this matter have appeared. LUZZATI AND HUSSON[5] have proposed that the changes in lipid–crystalline phases found with a phospholipid–water mixture as a function of temperature, may also occur in lipoprotein complexes *in vivo* and produce important alterations of the selective properties of biomembranes.

One possible approach to the problem of the role of the lipids in membrane function, is the study of the effects of temperature on transport. This line has been explored by KABACK[6] in the study of the α-methylglucoside transport system. In fact, he has proposed that the temperature effects displayed by this system may be explained in terms of phase changes.

Here we describe the effects of temperature on the proline transport system of *Saccharomyces chevalieri*, which has been described by us previously[7]. Our results suggest that the temperature changes in proline transport may be explained by phase changes in the membrane lipids.

MATERIALS AND METHODS

Strain of yeast used and growth conditions

Galactose-adapted cells of *S. chevalieri*, strain C_1, were used. The strain originated from culture NRRL-Y of the Northern Regional Laboratories (U.S.A.). Unless otherwise specified, the yeast cells were grown at 30° in standing test tubes in complete medium with galactose as carbon source, as previously described[7].

Chemicals

Ingredients for media were purchased from Difco Laboratories. Galactose was a Pfanstiehl product. Cycloheximide was a gift from the Upjohn Co. Uniformly ^{14}C-labelled L-proline was obtained from Schwarz. Its specific activity of 262 mC/mmole was adjusted with unlabelled L-proline to 0.118 mC/mmole.

All other chemicals were of analytical grade.

Derepression

Exponentially growing cells were derepressed by nitrogen starvation as previously described[8].

Measurement of proline uptake by cells

Derepressed cells were adjusted to $2 \cdot 10^7$ cells/ml in 66 mM KH_2PO_4 (pH 5.5) containing 10 mM D-galactose (phosphate–galactose buffer). 2 ml of cell suspension were preincubated at 30° for 5 min with 2.1 μg of cycloheximide *plus* the additions, where indicated, and phosphate–galactose buffer to a final volume of 2.9 ml. At zero time, 0.1 ml of uniformly ^{14}C-labelled L-proline was added and the cells were incubated in a shaker at 30°. The final proline concentration in the medium was 50 μM. Incubation was terminated after 5 min by filtration through a Millipore filter (pore size, 0.45 μm), the filter was washed 3 times with 5-ml portions of distilled water at room temperature, and dried and counted in a thin window gas-flow counter (Nuclear Chicago). The efficiency of the counting method is 30 %.

Determination of intracellular water

Packed cell volume was determined from the microhematocrit of a dense cell suspension ($3 \cdot 10^9$ cells/ml). Considering that intracellular water accounts for 47 % of the packed cell volume[9], the intracellular water volume corresponding to $1 \cdot 10^7$ cells was found to be 0.35 μl.

Assay for temperature effects with cells

The incubation mixture, phosphate–galactose buffer and 0.7 μg/ml cycloheximide but without cells, was preheated at the desired temperature for 5 min. Derepressed cells adjusted to $2 \cdot 10^8$ cells/ml were then added (2 ml of cell suspension for 27 ml of incubation mixture). Samples of 2.9 ml were taken at intervals, rapidly cooled to 30° by sampling into a precooled test tube (this operation lasted from 5 to 8 sec) and the accumulation of L-[^{14}C]proline assayed at 30° as described.

Preparation of protoplasts

Protoplasts from *S. chevalieri* were prepared from derepressed cells as described by SCHWENCKE *et al.*[10].

Measurements of proline uptake into protoplasts

Protoplasts collected by centrifugation were washed once with 600 mM KCl, 20 mM KH_2PO_4 (pH 5.5), 5 mM $MgCl_2$ *plus* 10 mM D-galactose (KCl-buffer), resuspended in KCl-buffer and adjusted to a final concentration of $1.5 \cdot 10^7$ protoplasts/ml. A 0.9-ml aliquot of protoplast suspension was preincubated at 30° for 5 min in the presence of 0.7 μg of cycloheximide. At zero time, 0.1 ml of uniformly ^{14}C-labelled L-proline (spec. act. 0.118 mC/mmole) was added and the mixture incubated in a shaker at 30° and 60 rev./min. The final L-proline concentration in the medium was 0.15 mM. No protoplast rupture occurred during this period. Incubation was terminated by centrifugation for 4 min at 3500 × *g*. The pellet was washed once with 3 ml KCl buffer, recentrifuged and the protoplasts resuspended in 0.5 ml of water and allowed to lyse for 2 min. The lysate was boiled for 5 min, then cooled and the entire lysate plated onto planchets and counted.

Assay for temperature effects with protoplasts

Protoplasts washed as described were adjusted to $2 \cdot 10^8$ protoplasts/ml in KCl buffer. The incubation mixture of KCl buffer and 0.7 μg/ml cycloheximide was preheated at the desired temperature for 5 min. A small volume of the adjusted protoplasts was then added (0.5 ml for every 6 ml of preheated medium), the mixture was incubated in a shaker and 0.9-ml samples were taken at appropriate intervals, rapidly cooled to 30° and the accumulation of L-proline assayed as described in the previous paragraph.

RESULTS

Kinetics of L-proline accumulation

The internally accumulated L-proline reaches a maximum at times which depend on the temperature of the assay (Fig. 1). At 22°, the maximum is obtained after 45 min while at 30° it occurs after 30 min. At 42° the maximum is reached after 22 min. However, the maximum capacity for L-proline accumulation is the same in all cases. No other significant difference in the shape of the curves is found.

The interesting kinetics of L-proline uptake clearly differ from those previously reported by us for DL-proline[7]. The decay of internal radioactivity may be explained in terms of an increase of exit, a decrease of uptake or a combination of both. It may also be due to a release of L-proline metabolites. These alternatives are under active study

and will be reported separately. However, preliminary results at least indicate that the phenomenon is characteristic for L-proline and not for the transport system. Thus, although sarcosine uses the same transport system, its kinetics of uptake are not oscillatory.

The uptake of L-proline is active as indicated by the fact that 20 mM azide and 1 mM dinitrophenol inhibit the initial velocity of uptake by 97 % and 98 %, respectively. Furthermore, the L-proline concentration amounts to a maximum of 43 μmoles/ml of intracellular water, which is 860 times higher than the extracellular L-proline concentration.

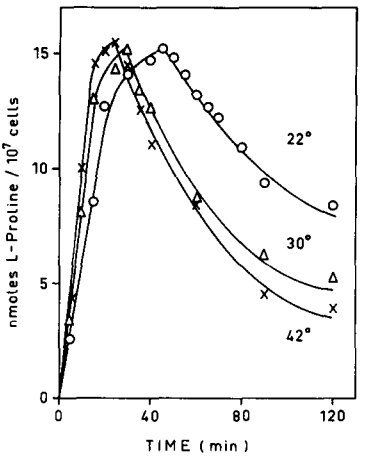
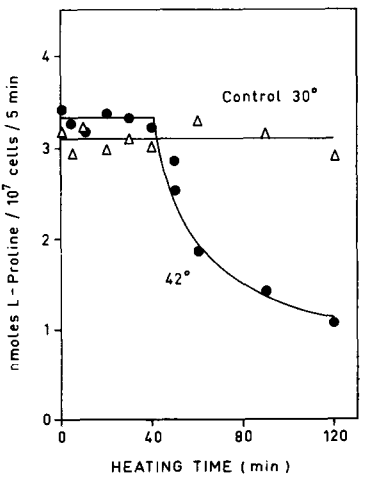
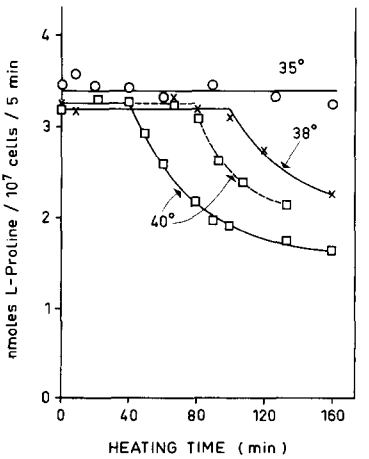


Fig. 1. Effect of temperature on kinetics of L-proline uptake. Cells resuspended in phosphate–galactose buffer were incubated at the indicated temperatures in the presence of L-[^{14}C]proline. Samples were taken at intervals and their internal radioactivity measured as described in MATERIALS AND METHODS.





Figs. 2 and 3. Effect of temperature on initial velocity of uptake. The phosphate–galactose buffer was equilibrated at the indicated temperatures for 5 min, then a small volume of a concentrated cell suspension was added and incubated. Samples were taken at intervals, rapidly cooled to 30° and the accumulation of L-proline measured as indicated in MATERIALS AND METHODS.

Because the internal pool of radioactive proline increases and then decreases as a function of time (Fig. 1), L-proline uptake was measured for only 5 min in all cases to facilitate the interpretation of the results. We have previously described that the internal radioactivity measured after proline accumulation comprises 80 % free proline[7]. Thus, the observed effects on accumulation are due to variations in the free proline concentration accumulated by the cells.

The initial velocity of uptake after preheating at various temperatures

As shown in Fig. 2, the initial rate of uptake for cells preheated at 30° remains constant up to 120 min. However, for cells preheated at 42°, the initial rate remains unaltered up to 40 min and then abruptly decreases in an exponential manner.

In Fig. 3 it can be seen that the initial rate of uptake for cells preheated at 35° remains unaltered up to 120 min, but when cells are preheated at 38°, it decreases sharply at 100 min. When cells are preheated at 40°, the phenomenon is also observed, but the decrease in initial rate may occur either at 40 min or at 80 min.

Table I shows that when cells are heated at 42°, viability is practically unaltered up to 180 min of heating.

TABLE I

EFFECT OF TEMPERATURE ON VIABILITY

Cells were heated at the temperatures indicated. Samples were taken at intervals and rapidly cooled to 30°. After dilution, appropriate aliquots were plated in triplicate onto agar plates containing complete medium (see MATERIALS AND METHODS) *plus* eosin and Methylene Blue. Colony counting was performed after 3 days of growth at 30°. The percentage was calculated by considering the number of colonies found at zero time of heating to be 100 %. Values are means of two experiments.

Heating time (min)	*Temperature*					
	30°		*35°*		*42°*	
	Colonies	%	*Colonies*	%	*Colonies*	%
0	310	100	298	100	344	100
30	284	92	322	108	328	95
60	302	97	279	94	357	104
90	330	106	319	107	305	89
120	289	93	271	91	324	94
180	297	96	288	97	337	98

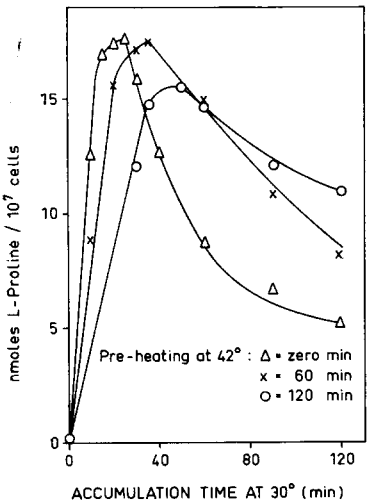
Kinetics of accumulation after preheating at 42°

In order to study the recovery of transport properties after preheating, the kinetics of accumulation were analyzed. As seen in Fig. 4, the kinetics of accumulation are apparently not greatly disturbed although the initial rate of uptake is diminished. Also maxima for proline uptake are reached after longer incubation times. The maximum internal capacity of accumulation is little effected by preheating at 42° either for 60 or 120 min.

Recovery of initial velocity of uptake after preheating at 42°

Fig. 5 shows that the decrease in the initial rate of uptake due to preheating at 42° is only partially reversed when the cells are cooled to 30° and incubated at this

temperature. Maximum recovery, of up to 20 %, occurs after 12 min. To assure reproducibility, preheated cells were pipetted into polypropylene test tubes and allowed to cool to 30° for a period of 4 min. When cooling was performed rapidly, recovery was very poor. Further attempts to enhance recovery were unsuccessful.



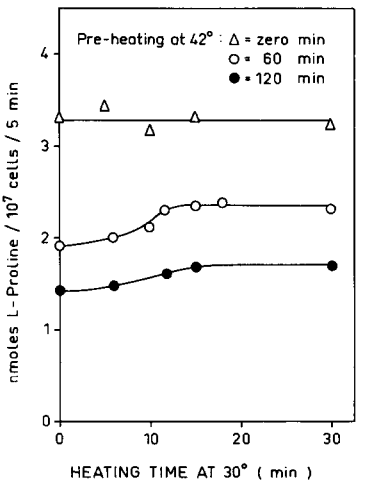


Fig. 4. Effect of preheating at 42° on the kinetics of accumulation. Cells were preheated in phosphate–galactose buffer for the indicated times. Appropriate aliquots were taken, rapidly cooled to 30° and radioactive L-proline added. The kinetics of accumulation were measured as described for Fig. 1.

Fig. 5. Recovery of initial velocity of uptake after preheating at 42°. Cells were preheated at 42°. At the indicated times, aliquots were taken, pipetted into polypropylene test tubes and allowed to cool to 30° at room temperature. This process was completed after 4 min. The cell suspension was then incubated at 30° and 2.9-ml samples were taken at the intervals indicated on the abscissa. 0.1 ml L-[^{14}C]proline was added and accumulation measured as indicated in MATERIALS AND METHODS.

Effect of preheating on the initial rate of uptake in cells grown at different temperatures

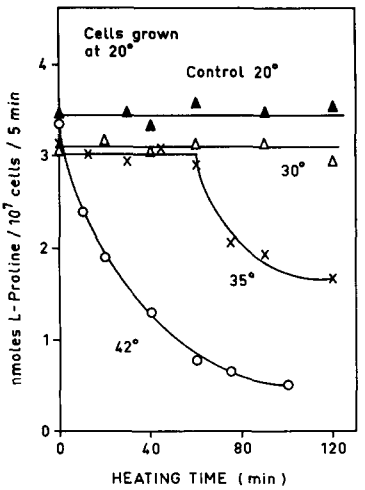
Fig. 6 shows that when cells are grown and derepressed at 20°, the initial velocity of uptake remains unaltered when they are preheated at 20° or 30°. However, preheating at 42° produces an immediate very rapid decay of the initial velocity of uptake. Cells preheated at 35° maintain their initial rate of uptake up to 60 min and then a rapid decay is observed. Thus, cells grown at 20° are more sensitive to preheating at 42°.

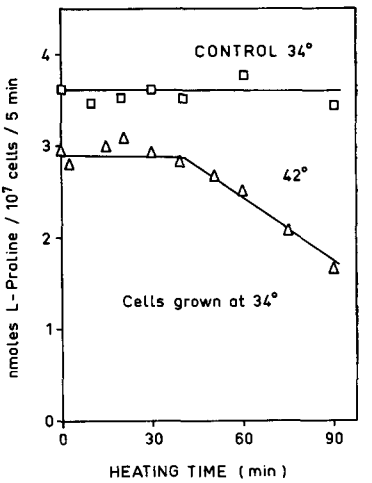
On the other hand, cells grown at 34° also show changes in the initial rate of uptake. Curiously, the decay also occurs after 40 min of heating at 42°, but it is linear rather than exponential (Fig. 7).

Effect of preheating on the initial rate of uptake in protoplasts

From Fig. 8a it can be seen that protoplasts are far more sensitive to preheating than cells. A sharp decrease in the initial velocity of uptake is observed when protoplasts are preheated at 38°, 36° or 34°. The initial velocity was reduced to 50 % of its original value after 7.5 min of heating in all these cases. Furthermore, the change was

also noted when protoplasts were preheated at 32° even after only 1 min of preheating (Fig. 8b).





Figs. 6 and 7. Effect of temperature on the initial rate of uptake in cells grown and derepressed at temperatures other than 30°. The experiment was performed as described for Fig. 2, except that the cells used were grown and derepressed at 20° for Fig. 6 and at 34° for Fig. 7. Proline uptake was measured as described but at 20° for Fig. 6 and at 34° for Fig. 7.

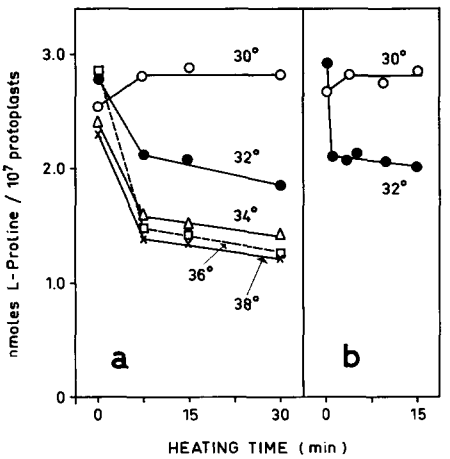


Fig. 8. Effect of preheating at various temperatures on the initial rate of uptake in protoplasts. A small volume of protoplast suspension was added to KCl buffer heated at the temperatures indicated. Samples were taken at intervals, rapidly cooled to 30° and the capacity of the protoplast suspension to accumulate L-proline assayed at 30° as described in MATERIALS AND METHODS.

DISCUSSION

The effect of temperature on the velocity of uptake may be due to a number of causes comparable to those described for enzymes[11]. Because transport systems are located in membranes one may attempt primarily to distinguish between effects due to changes in the protein or changes preferentially related to lipids.

The fact that the decrease in initial velocity of uptake depends on the growth temperature suggests, but does not prove, that changes occur in lipids rather than in proteins, since the composition of membrane fatty acids of microbial cells depends on the temperature of growth[12]. In addition, it has been reported that the physicochemical properties of phospholipids critically depend on the structure of fatty acid components[13–15].

On the other hand, the delicate protein–lipid interactions of biomembranes[16] are probably not easily rebuilt after alterations by thermal transition since recovery of initial velocity of uptake is very small.

Whole cell results are complicated by the rather high temperature and long incubation period necessary to obtain the sudden decrease in initial velocity of uptake. A number of biochemical changes affecting the enzymatic machinery coupled to active transport may occur in the meantime. Nevertheless, none of these problems influences our results with protoplasts since the change is detected even one minute after heating at 32°. Furthermore, in these cases the changes are clearly from one state to another and do not decay exponentially. The fact that the changes reported here are very abrupt indicate a very rapid phenomenon, as may be expected for membrane lipids or lipoproteins undergoing structural changes[5].

The finding of STEIM *et al.*[13] that transitions in whole membranes caused by temperature changes occur at the same temperature as the transitions in the isolated membrane lipids, and the report that changes in membrane lipid composition alter the temperature characteristics of thiomethylgalactoside accumulation[17] are in line with the results reported here.

In spite of some uncertainties, our results are compatible with the proposition that the changes observed may be explained in terms of changes in the lipid–crystalline structure of the yeast membrane.

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