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TEMPERATURE DEPENDENCE OF THE APPARENT AFFINITY AND THE MAXIMUM VELOCITY OF THE MEMBRANE-BOUND MONOSACCHARIDE TRANSPORT SYSTEM IN THE YEAST *RHODOTORULA GRACILIS*

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Summary

Analysis of the temperature dependence of the monosaccharide transport system in the yeast *Rhodotorula gracilis* (ATCC 26194, CBS 6681), as tested with D-xylose, revealed that the apparent affinity of the transport system, measured as the reciprocal of the half-saturation constant K_T, increased when transport velocity was stimulated by temperature (15—30°C) and decreased when the rate of uptake was reduced at temperatures above 30°C. Breaks in Arrhenius plots were accompanied by corresponding breaks in van't Hoff plots. Whereas untreated cells exhibited in the van't Hoff plot a discontinuity at 28—30°C this was not observed in heat-treated cells (at either 37 or 45°C). In heat-treated cells the maximum transport velocity was always lower and the apparent affinity higher than in untreated cells at the same temperature; the optimum temperature for both transport velocity and apparent affinity was shifted to higher values.

The data are interpreted in terms of a reversible phase transition of membrane lipids effecting an irreversible alteration of membrane structure. The temperature-induced reversible alkalinization of unbuffered yeast suspensions supports this interpretation.

Introduction

Transport-mediating molecules (carriers) are believed to be proteins spanning the membrane. The mobility and the conformation of these membrane-embedded proteins may depend on interaction with their lipid environment. Wisnieski et al. [1] showed that the biological activity of membrane proteins

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associated with lipids is related to the physical state of these lipids. De Kruijff et al. [2] concluded from their results obtained with *Acholeplasma laidlawii* that transport is performed only in membrane domains with lipids in the liquid-crystalline state. Lipids in liquid state may disturb membrane-bound processes due to the altered environment of the proteins.

The obligatory aerobic yeast *Rhodotorula gracilis* possesses a membrane-bound active uptake system for monosaccharides [3,4], the efficiency of which obviously depends on the phase state of the membrane as demonstrated by the effect of temperature on the transport [5]: Below 15°C there is virtually no transport, above 30°C the transport becomes inactivated by increasing temperature and only between the two temperatures the transport system can function in an optimal way. Temperatures above 30°C lead to irreversible alterations in the membrane architecture as indicated, e.g., by a different sensitivity toward the polyene antibiotic nystatin [5,6]. The reduction of the transport velocity above 30°C has been postulated to be due to a phase change of the phospholipids as described for *Escherichia coli* [7] and for mitochondrial membranes [8].

The present communication demonstrates that the postulated phase change of the membrane phospholipids is accompanied by drastic and irreversible alterations in the apparent affinity of the transport system for monosaccharides.

Materials and Methods

The strain used, *Rhodotorula gracilis* (taxonomically *Rhodosporidium toruloides* Banno mating type a, ATCC 26 194, CBS 6681) was cultivated, washed and aerated as described earlier [9].

D-Xylose was used as a representative substrate of the monosaccharide uptake system for it is transported rapidly and not catabolized during the first 30 min of incubation [10]. The transport kinetics were analyzed by measuring the time-dependent changes of the intracellular pentose concentration at the temperature indicated, as already described [5]. The values of the half-saturation constant, K_T, as a measure of the apparent affinity of the transport system for D-xylose, and the maximum initial rate of uptake, V_0, at each temperature, were extrapolated from double-reciprocal plots of the initial velocity of uptake against D-xylose concentrations.

Heat treatment of an aerated yeast suspension was performed by warming the suspension to either 37 or 45°C for 10 min and rapidly cooling to the temperature indicated. The reaction was started at once by adding the substrate. The pH value of the yeast suspension was measured in a temperature-controlled cuvette; for details see ref. 11. All chemicals used were of analytical grade and purchased from commercial sources.

Results

The temperature dependence of the transport velocity of D-xylose uptake through the plasmalemma of *R. gracilis* and the temperature-dependent alteration of the carrier's apparent affinity for its substrate were measured between 15 and 45°C (cf. ref. 5).

The results shown in Fig. 1 demonstrate that the acceleration of sugar uptake was accompanied by an increase of the apparent affinity of the transport system up to 28°C. At about 28—30°C both the initial velocity and the apparent carrier affinity were highest. Above this critical temperature the rate of uptake and the apparent affinity decreased. The slope in the van't Hoff plot exhibits a discontinuity at the critical temperature to a much higher affinity, which decreased again continuously. Around 45°C it reached approximately the same value as at 30°C, however, the transport velocity at this temperature was reduced to about 15% of the maximum value.

If the same type of experiment was performed with heat-treated cells the following alterations in the temperature dependence of the kinetic parameters of the transport system were observed (Fig. 2).

(i) The transport velocity was considerably lower at all temperatures; the higher the temperature of the heat treatment the lower the transport rate.

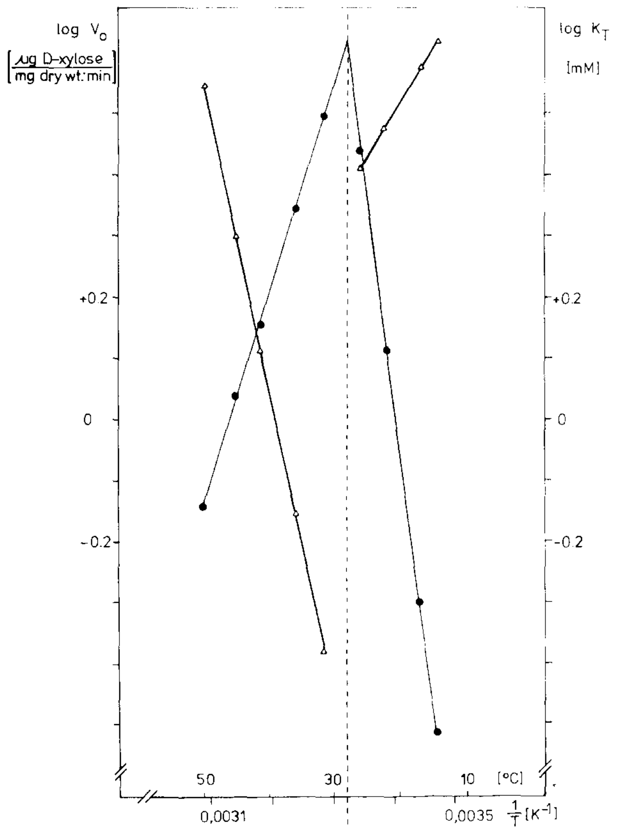

Fig. 1. Arrhenius plot of the maximum initial transport velocity, V_0, and the van't Hoff plot of the apparent affinity of the monosaccharide transport system, expressed as the half-saturation constant, K_T. The values of V_0 and K_T plotted were extrapolated from Lineweaver-Burk plots of kinetic data obtained with D-xylose concentrations between 3.3 and 26.6 mM at each temperature indicated. ●, log V_0; △, log K_T. Experimental conditions: portions of the aerobic cell suspension of *R. gracilis* (about 5 mg dry weight/ml) in 120 mM potassium phosphate buffer (pH 4.5) were incubated at the given temperature without any pretreatment. The reaction was started by the addition of D-xylose.

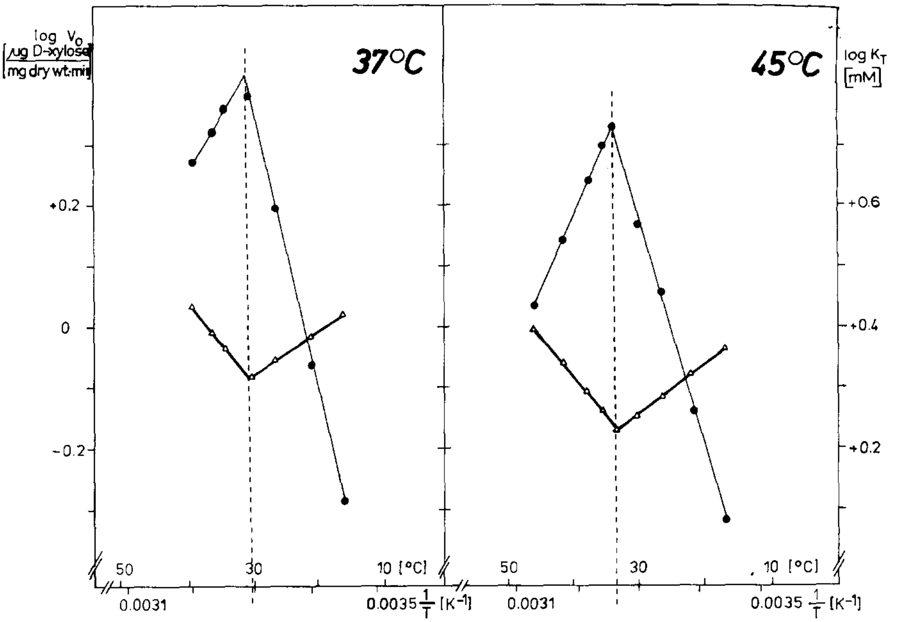

Fig. 2. Arrhenius plot of the maximum initial transport velocity, V_0, and van't Hoff plot of the apparent affinity of the monosaccharide transport system, K_T, of heat-treated suspensions of *R. gracilis*. ●, log V_0; △, log K_T. Estimation of the kinetic parameters and experimental conditions as in Fig. 1, except for the cell suspensions were heat-treated either at 37 or at 45°C prior to the addition of D-xylose (see Materials and Methods).

(ii) The optimum temperature for the transport function was shifted to higher values, depending on the temperature of the heat treatment.

(iii) The breaks in the van't Hoff plot corresponded to those in the Arrhenius plot.

(iv) The activation energies of stimulation and deactivation were decreased by increasing the temperature of the heat treatment.

(v) The apparent affinity below the optimal temperature for transport (between 15 and 28°C) was higher in heat-treated cells than in untreated cells; however, the corresponding velocity of sugar uptake was lower.

Both the heat-treated and the untreated cells exhibited an increasing apparent affinity when the velocity of uptake increased, a decreasing velocity was accompanied by a reduction of the apparent affinity. In heat-treated cells the discontinuous break in the apparent affinity, which in untreated cells coincided with the optimum temperature for transport (28—30°C), is no longer detectable.

The effect of raising the temperature on the pH value of unbuffered cell suspensions of *R. gracilis*, pH_e, is depicted in Fig. 3. The data demonstrate that the higher the temperature above the optimum region of 28—30°C the lower the resulting pH gradient maintained across the cell membrane. Independent measurements of the intracellular pH value, pH_i, by means of weakly acid indicators (5,5-dimethyl-[2-^{14}C]oxazolidine-2,4-dione and bromophenol blue) lead to a value of 6.2—6.5 for unbuffered cell suspension at 28°C [12]. The

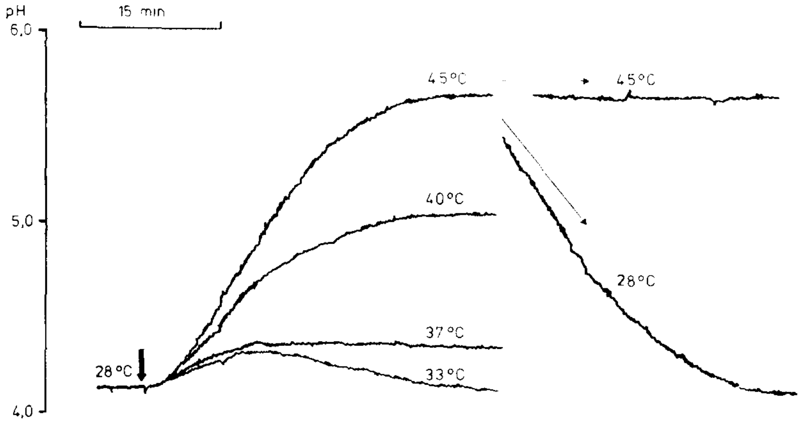

Fig. 3. Temperature-dependent changes of the external pH of unbuffered *R. gracilis* suspensions. Experimental conditions: unbuffered cell suspensions (about 5 mg dry weight/ml) in water containing 1 mM $CaCl_2$ (for more details see ref. 11) temperature as indicated. Temperature changes started at the arrow; an aliquot of the 45°C-treated suspension was cooled to 28°C, again leading to a restoration of the pH gradient.

effect of temperature on the pH_e was reversible. A simple cooling of the cell suspension resulted in restoration of the original pH gradient.

Discussion

The postulated phase change of the lipids in the plasmalemma of the yeast *R. gracilis* [5] has not yet been demonstrated by physical methods. However, such a phase change is likely for there is evidence in the literature in favour of a correlation of breaks in the Arrhenius plot and phase transitions in the lipids (cf. refs. 1 and 7).

A lipid phase transition may also effect a protein clustering in that lipid region with the preferred physical environment, as was observed in *Tetrahymena pyriformis* [13]. In *E. coli* it was found that different proteins of a multicomponent membrane system were separated as a result of lateral phase separation of membrane lipids [14]. The importance of the physical state of the lipids for membrane-embedded transport processes has been shown very impressively by Overath and co-workers [15].

Applying these results to the data obtained with *R. gracilis* we postulate a phase transition at 28°C which allows the membrane-bound transport protein (monosaccharide uptake system) to reorientate itself in the membrane, thus resulting in a better accessibility of the carrier to its substrate (measured as a decrease of the half saturation constant); concomittantly the proton gradient across the membrane was reduced.

Since the cell respiration was not reduced at temperatures above 30°C [5] the temperature-dependent increase of the extracellular pH_e can be brought about only by an increased proton permeability of the plasma membrane. At 33°C the permeability increase can still be compensated by a more active operation of the proton pumping assembly; cf. ref. 11. At higher temperatures the enhanced leakage of protons through the cell membrane cannot be compensated any more and new lower steady-state pH gradients are reached (Fig. 3). The proton gradient across the membrane may be involved in the energization of the monosaccharide transport [12] so that the reduced gradient and the altered membrane structure effect a decreased transport velocity.

If the alkalinization of the medium is due to enhancement of the passive diffusion of protons through the more fluid membrane, cooling down the cell suspension should restore the gradient by resealing the membrane (a lipid phase transition should be completely reversible), as was the case in Fig. 3. The optimum surroundings of the transport protein(s) cannot be restored by cooling.

The fact that the break in the Arrhenius plot is accompanied by a corresponding discontinuous break in the van't Hoff plot supports our earlier postulate that at 28—30° C the membrane lipids undergo a phase change leading to a sudden reorganization of membrane constituents due to altered protein-lipid interactions in the membrane, cf. also ref. 8. Consequently, the transport efficiency becomes reduced in spite of an increased apparent affinity of the transport system for its substrate.

The discontinuity in the van't Hoff plot may be equally well explained by an irreversible conformational change of the carrier molecule itself independent of the phase transition of the membrane lipids. The experimental data do not favour one explanation or the other. However, since both the apparent affinity and the proton permeability changed in the same temperature region (28—30° C) we assume that both processes occur on the same basis, namely the phase transition of the membrane lipids.

Acknowledgement

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