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EVIDENCE FOR AN INDUCIBLE GLUCOSE TRANSPORT SYSTEM IN *KLUYVEROMYCES LACTIS*

PAULETTE W. ROYT and ANTHONY M. MACQUILLAN

Department of Microbiology, University of Maryland, College Park, Md. 20742 (U.S.A.)

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SUMMARY

To find the cause of delayed glucose oxidation in succinate-grown *Kluyveromyces lactis*, glucose transport was studied in glucose- and in succinate-grown cells. The initial rate of 2-deoxyglucose (2-dGlc) accumulation, as well as the appearance of 2-deoxyglucose 6-phosphate, was higher in the glucose-grown cells. In both cell types, 2-dGlc was apparently transported in the free form to be phosphorylated intracellularly. In glucose-grown cells the level of free 2-dGlc in the pool was always less than the external concentration. Exchange transport in starved, poisoned cells loaded with unlabeled 2-dGlc was 140-fold greater in glucose- than in succinate-grown cells, probably because of the presence of an inducible transport component. The development of the increased rate of transport in a succinate-grown uracil-requiring auxotroph after transfer to glucose depends on the presence of uracil.

INTRODUCTION

When succinate-grown *Kluyveromyces lactis* cells are transferred to a medium with glucose as the carbon source, they exhibit a lag in growth during which their rate of glucose oxidation, initially very low, progressively increases for about 4 h [1]. A number of possibilities might explain this apparent adaptive utilization of glucose. For example, diminished levels of one or more glycolytic enzymes in succinate-grown cells might account for the lag. However, no significant differences in levels of glycolytic enzymes in cells grown on glucose and on succinate (Royt, P. W. and MacQuillan, A. M., unpublished data) were found. High levels of activities of major gluconeogenic enzymes in succinate-grown cells may also retard glucose utilization, but we would expect reduction of such levels via metabolic controls to result in more rapid changes in glucose oxidation rates than we observed.

It is also possible that the adaptive lag on transfer to glucose reflects a deficient glucose transport capacity of the succinate-grown cells. Accordingly, we have studied glucose transport in *K. lactis* cells grown on glucose and on succinate, and conclude that these cells possess different levels of inducible glucose transport components.

Abbreviations: 2-dGlc, 2-deoxyglucose; 2-dGlc-6-*P*, 2-deoxyglucose 6-phosphate.

The glucose transport system in *Saccharomyces cerevisiae* has repeatedly been reported to be constitutive [2–5]. An inducible transport system for glucose in *K. lactis* might constitute an important regulatory difference between these yeasts, which also differ in their ability to utilize tricarboxylic acid cycle intermediates for growth [6].

MATERIALS AND METHODS

Organisms and growth conditions

Kluyveromyces lactis Y123 was used in the majority of experiments. A uracil-requiring derivative of *K. lactis* Y123 was obtained after treatment of cells with ultraviolet irradiation.

Cells were grown in synthetic medium [7] at pH 5.4 containing 0.2 % of the appropriate carbon source and harvested as previously described [1]. For washed cells in suspension an absorbance of 1.0, recorded at 600 nm, is equivalent to 0.125 and 0.129 mg dry weight of succinate- and glucose-grown cells, respectively.

Oxidation studies

The evolution of $^{14}CO_2$ from [U-^{14}C]glucose (International Chemical and Nuclear Corp., Irvine, Calif.) was measured. Each flask contained: 0.067 M phosphate buffer (pH 5.4), 9.5 ml; cell suspension (absorbance = 10), 2.0 ml; water, 2.5 ml; and 20 % KOH in the sidearm, 5 ml. After equilibration for 10 min at 30 °C, labeled glucose (250 μmol/ml, specific activity $6.4 \cdot 10^{-4}$ Ci/mol),1 ml, was added. Samples (0.1 ml) of KOH were withdrawn and added to 0.9 ml water in a scintillation vial. 9 ml of scintillation fluid were added, and the samples were counted as previously described [8]. Counting efficiency, determined by the channels ratio method, was 70 %. The presence of KOH in these samples caused no significant quenching.

Transport studies

The transport, at 20 °C, of 2-deoxy-D-[U-^{14}C]glucose (2-dGlc) (International Chemical and Nuclear Corp.; specific activity $6.4 \cdot 10^{-3}$ Ci/mol) was studied using published methods [9–11]. Samples were washed in ice-cold phosphate buffer, filtered, washed and counted by liquid scintillation. Corrections for adsorption of 2-dGlc to cells and to filters were made by subtracting counts obtained from cell suspensions incubated on ice. Results are expressed as μmol/g dry weight of cells per min.

(*a*) *Accumulation.* To determine the total accumulation of label in cells, the above procedure was performed on washed, exponential-phase cells. Inhibitors sodium azide, $3.2 \cdot 10^{-4}$ M, and iodoacetate, $5 \cdot 10^{-3}$ M, were added 10 min prior to substrate.

(*b*) *Uptake.* Washed cells were starved for 3 h (glucose cells) and 4 h (succinate cells) by shaking at 30 °C in buffer. Cells were then poisoned with both $3.2 \cdot 10^{-4}$ M azide and $5 \cdot 10^{-3}$ M iodoacetate for 15 min. Uptake studies were performed on these cells utilizing the transport procedure.

(*c*) *Exchange.* Starved, poisoned cells were loaded with unlabeled 2-dGlc (Calbiochem Co., Los Angeles, Calif.) by shaking at 30 °C for 1 h (glucose cells) and 2 h (succinate cells). These cells do not accumulate 2-deoxyglucose 6-phosphate

(2-dGlc-6-*P*) (see Results). Cells were loaded to various levels such that in each experiment the initial internal to external concentration ratio of total 2-dGlc was constant at 5 to 1. Following loading, samples were centrifuged and the cell pellets cooled on ice. The pellets were washed once in ice-cold buffer, centrifuged and supernatant fluids decanted immediately. Buffer, preincubated at 20 °C, containing labeled substrate was added to the pellets, and the usual transport procedure followed.

(*d*) *Efflux*. Starved, poisoned cells were loaded as described in (c) with 100 mM labeled 2-dGlc and washed. Exchange was carried out against 6 mM unlabeled 2-dGlc, using the transport procedure and measuring the decrease of label in cells. Zero time internal 2-dGlc concentration was determined in loaded cells not exposed to unlabeled 2-dGlc.

Competition

Competition uptake studies were done adding 2-d[^{14}C]Glc and 10 mM glucose simultaneously to starved, poisoned glucose-grown cells.

Chromatography of cell-free extracts

The intracellular distribution of label was determined in cells previously starved for 1 h at 30 °C, then loaded with 100 mM unlabeled 2-dGlc for 15 or 30 min (glucose and succinate cells, respectively). The standard transport procedure was performed using 6 mM 2-d[^{14}C]Glc. Filters containing washed cells were immediately transferred to centrifuge tubes containing boiling water, and the samples boiled in a water bath for 10 min. After a $1000 \times g$ centrifugation, 500 μl of supernatant fluid were spotted on Whatman No. 1 filter paper. Separation of labeled products was developed by descending chromatography in ethyl acetate/*n*-propanol/water (20 : 60 : 20 by vol.) [11]. Non-labeled 2-deoxysugars were detected by the method of Weidemann and Fischer [12]. Labeled 2-deoxysugars were quantitatively determined by eluting from chromatographic strips for 10 min with distilled water then adding scintillation fluid and counting.

Labeled products in aqueous extracts of boiled cells were also chromatographed [13] using ethyl acetate/pyridine/water (80 : 20 : 10 by vol.). Additionally, an ethanol extract of 1 h-starved cells was chromatographed [14] in *n*-butanol/acetic acid/water (40 : 10 : 50 by vol.).

Determination of intracellular volume

A modification of the volume of distribution technique of Conway and Downey [15] was used to determine the intracellular volume of this yeast grown in glucose-containing medium. Since glucose-grown cells were impermeable to succinate [1, 8], this compound was used to determine cell wall volume.

Hexokinase assay

Cell-free extracts were prepared by shaking a heavy suspension of cells in 0.05 M phosphate buffer (pH 7.4) with an equal volume of 0.45 mm glass beads (VWR Scientific) on a Braun MSK Cell Homogenizer for 20 s. The extract was decanted and centrifuged in the cold at $6000 \times g$ for 10 min to remove beads, cell debris and unbroken cells. The supernatant fluid was then centrifuged for 1 h at $100\,000 \times g$ on a Beckman Model L ultracentrifuge to sediment mitochondria and

mitochondrial fragments. The pellet was discarded and the supernatant fluid retained for analysis. Hexokinase was assayed spectrophotometrically [16].

The ability of the yeast hexokinase to phosphorylate 2-dGlc was determined by the method of Brown and Romano [9].

Protein in the extracts was determined according to Lowry et al. [17] with crystalline bovine serum albumin (Sigma Co.) as standard.

Development of the glucose transport system

A uracil-requiring mutant of *K. lactis* Y123 was isolated after irradiation with ultraviolet light to 25 % survival. The mutant was grown in succinate synthetic medium supplemented with 25 μg uracil/ml. Exponential-phase cells were washed 3 times aseptically in 0.067 M phosphate buffer (pH 5.4) and starved for 1 h by shaking at 30 °C. Cells were then centrifuged, resuspended in buffer and inoculated into the media described in Results. One aliquot of cells served as a zero time accumulation control. The cultures were shaken at 30 °C for 4 h, centrifuged and washed. Accumulation of labeled 2-dGlc, 6 mM, was determined by the standard transport procedure.

RESULTS

The low rate of glucose utilization by resting cells of succinate-grown *K. lactis*, originally shown by Warburg oxidation studies [1], was further indicated by measurement of $^{14}CO_2$ evolution from labeled glucose (Fig. 1). In contrast to the high constant rate of $^{14}CO_2$ production by cells grown on glucose, succinate-grown cells produce $^{14}CO_2$ slowly after an initial 30–40 min lag.

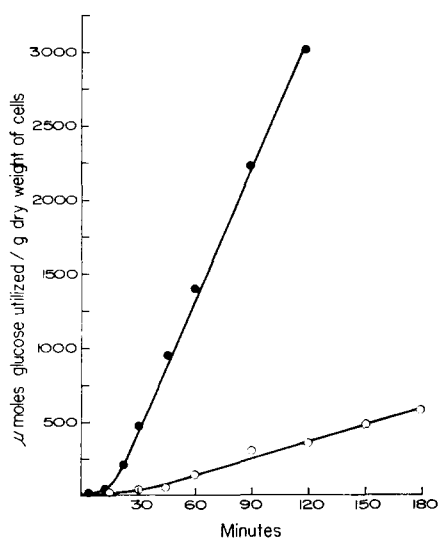

Fig. 1. Glucose utilization by (●) glucose-grown and (○) succinate grown cells. Measured by $^{14}CO_2$ evolution from [U-^{14}C]glucose.

2-dGlc metabolism

A similar experiment was performed using 2-d[^{14}C]Glc. No $^{14}CO_2$ was evolved by cells grown on glucose or on succinate, suggesting lack of glycolytic consumption of 2-dGlc by these cells.

A comparison of two procedures to measure radioactivity taken into cells, one in which whole cells were counted and one in which materials extracted from cells by boiling water were counted, revealed comparable total counts accumulated. These results indicated that, during the time course of an experiment, no significant amount of 2-dGlc was incorporated into the cell wall as has been reported [18] to occur in *S. cerevisiae* grown in the presence of 2-dGlc.

It has been reported that 2-dGlc is phosphorylated in *S. cerevisiae* [11] and in *Aspergillus* [9]. Hot water extracts of *K. lactis* given radioactive 2-dGlc were chromatographed using the three solvent systems described. Approximately 95 % of the label was recovered in two major spots: one corresponding to a known sample of 2-dGlc, and the other to a known standard of 2-dGlc-6-*P*. An assay [9] with cell-free extract showed that the hexokinase of *K. lactis* is, indeed, capable of phosphorylating 2-dGlc. These results indicated that in *K. lactis* phosphorylation of 2-dGlc to form 2-dGlc-6-*P* does occur.

The recovery of at least 95 % of the label as either 2-dGlc or 2-dGlc-6-*P* indicated that further metabolism, if any, of 2-dGlc in this yeast during the course of an experiment is probably not significant.

Competition

The ability of glucose to inhibit uptake of labeled 2-dGlc was studied in starved, poisoned, glucose-grown cells. We observed, as has been reported for other yeasts [19–23] and for *Aspergillus* [9], that glucose competitively inhibits 2-dGlc uptake. These results indicate [24] a common permeability system for the two sugars and, therefore, to eliminate complicating metabolic reactions, we have used 2-dGlc to study the glucose transport system in *K. lactis*.

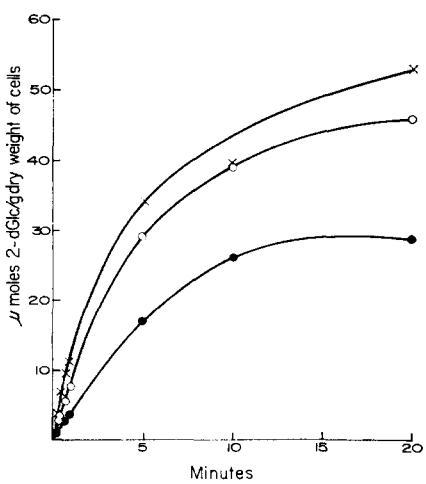

Fig. 2. Accumulation of 2-d [^{14}C]Glc by glucose-grown *K. lactis*. Initial concentration of 2-dGlc: (●), 0.75 mM; (○), 3 mM; (×), 12 mM.

Inducible nature of 2-dGlc accumulation

The accumulation of radioactivity during transport of 2-dGlc by glucose-grown *K. lactis* cells is illustrated in Fig. 2. Both rate and pool size increased with increasing external substrate concentrations. Cells grown in succinate medium accumulated labeled 2-dGlc more slowly (Fig. 3). After 20 min, accumulation by the succinate-grown organisms was still linear, and its extent was less than half that of the cells grown in glucose.

We examined the possibility that this difference in rates of 2-dGlc accumulation was due to an inducible rather than a regulatory element. The Y123 strain of *K. lactis* is cycloheximide-resistant. A uracil-requiring auxotrophic derivative was therefore employed. Washed succinate-grown cells were transferred to glucose media with and without uracil. After incubating the cells in these media for 4 h, their rates of 2-dGlc accumulation were examined. Fig. 4 shows that cells incubated in the presence of uracil have increased their rate of accumulation markedly over that shown by cells deprived of uracil. It would, therefore, appear from the uracil dependence that RNA synthesis and probably protein synthesis are necessary for this development. Succinate enhanced the rate of appearance of the 2-dGlc accumulation system, probably by providing a more readily usable energy substrate for the early stages of the induction process.

Double reciprocal plots [25] of the initial rates of accumulation in Figs. 2 and 3 were linear (correlation coefficient 0.99) and yielded values for V of 12.5 and 1.1 μmol 2-dGlc/g dry weight of cells per min, and for $K_{m_{app}}$ of 1.7 and 3.4 mM for glucose- and succinate-grown cells, respectively. Since *K. lactis* cells are capable of phosphorylating 2-dGlc to yield 2-dGlc-6-*P*, the apparent compliance of the accumulation process with Michaelis-Menten kinetics might, in principal, be due to any one of the following possibilities: simple diffusion, facilitated diffusion or active transport of free 2-dGlc, each followed by hexokinase activity, or group translocation to yield internal 2-dGlc-6-*P* followed by dephosphorylation. The different rates of accumula-

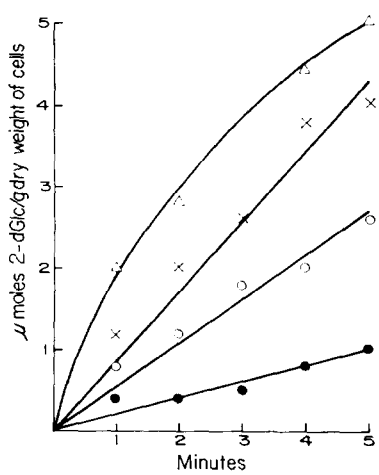

Fig. 3. Accumulation of 2-d[^{14}C]Glc by succinate-grown *K. lactis*. Initial concentration of 2-dGlc: (●), 0.75 mM; (○), 3 mM; (×), 6 mM; (△), 12 mM.

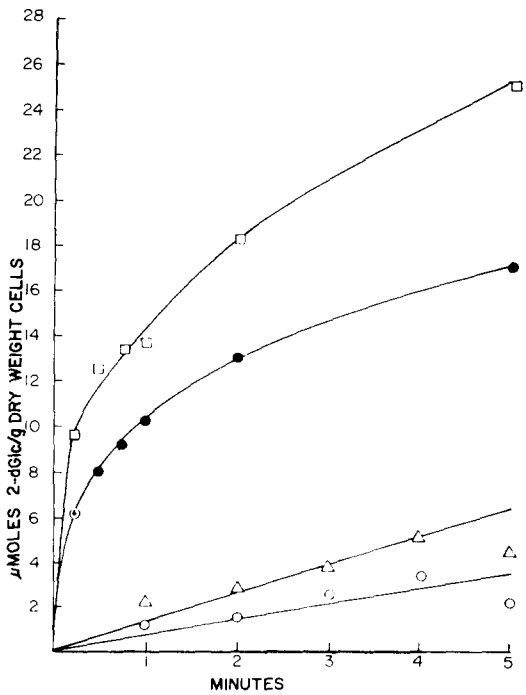

Fig. 4. Accumulation of 2-dGlc by *K. lactis* Y123 ura-1. Cells were grown on succinate medium containing 0.025 % uracil, washed and transferred to glucose media containing the following additions: 0.025 % uracil (●), 0.025 % uracil and 0.2 % succinate (□), none (△). After 4 h, cells were harvested and washed, and accumulation of labeled 2-dGlc was determined. Initial extracellular 2-dGlc concentration was 6 mM. Accumulation was also measured at zero time (○) in the succinate-grown cells.

tion of total labeled compounds from external 2-dGlc found for *K. lactis* cells grown in glucose and succinate might, therefore, be attributable to different levels of either hexokinase activity or transport components.

An examination of hexokinase content in crude cell-free extracts of glucose- and succinate-grown cells revealed specific activities of 0.102 and 0.120 units/mg protein, respectively. It appears that cells grown on either carbon source have similar capacities to phosphorylate 2-dGlc once it enters the cell. Although regulatory properties, such as those exhibited by hexokinase from *S. cerevisiae* [26], may serve to control hexose accumulation by yeast cells, we do not know if hexokinase activity from *K. lactis* is subject to similar modifications. Moreover, these properties would not answer the evident need for an inducible component in the overall accumulation process. We, therefore, examined the glucose transport system of *K. lactis* in search of an inducible component which might account for the adaptive lag on transfer to glucose.

Transport of 2-dGlc by K. lactis

The question of whether transport of free hexose or its group translocation occurs in *K. lactis* was examined by methods similar to those of Kuo and Cirillo [4].

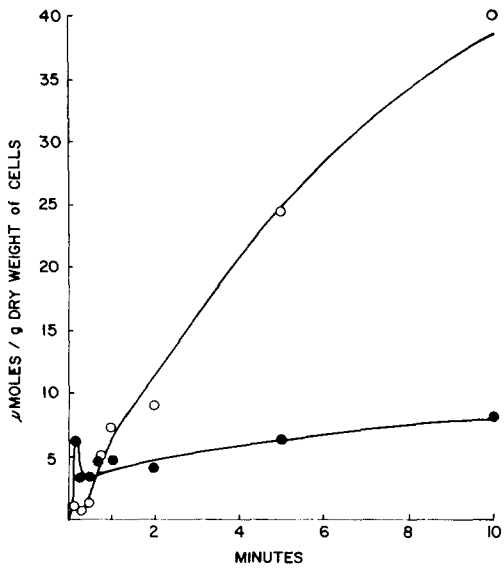

Fig. 5. Distribution of 2-dGlc (●) and 2-dGlc-6-*P* (○) in glucose-grown *K. lactis*. Cells were starved for 1 h and loaded with 100 mM unlabeled 2-dGlc before exposure to 6 mM 2-d[^{14}C]Glc. Corrections for adsorption of 2-d[^{14}C]Glc to cells and to filters were made by subtracting counts obtained from cell suspensions incubated on ice.

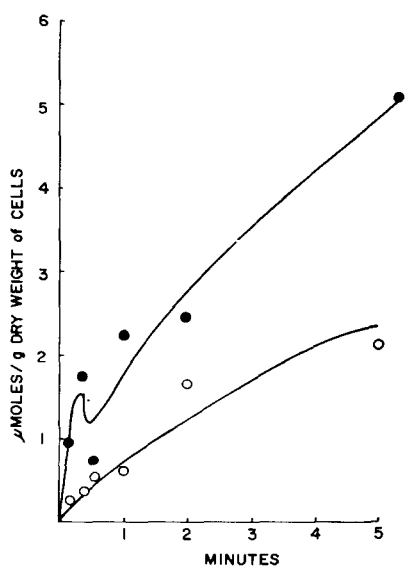

Fig. 6. Intracellular concentration of 2-dGlc (●) and 2-dGlc-6-*P* (○) in succinate-grown *K. lactis*. Experimental conditions were similar to those for Fig. 5.

We measured the rates of appearance, during the course of labeled 2-dGlc transport, of labeled 2-dGlc and 2-dGlc-6-*P* in the pools of partially starved, loaded cells, previously grown on glucose (Fig. 5) and on succinate (Fig. 6). By loading cells with unlabeled 2-dGlc, the pools should accumulate free 2-dGlc and 2-dGlc-6-*P*, thereby minimizing the interconversion of transported labeled compounds by virtue of phosphatase or kinase activities.

The results with both glucose- and succinate-grown cells show slightly higher amounts of free labeled 2-dGlc than of 2-dGlc-6-*P* during the initial phase of transport Taken by itself this finding might indicate that transport of free hexose occurs, which is then followed by the conversion to hexose phosphate. More striking, however, is the observation that in glucose-grown cells (Fig. 5) the concentration of 2-dGlc-6-*P* soon exceeds the free 2-dGlc and rises to high levels, while in succinate-grown cells (Fig. 6) the hexose phosphate appears in the extractable pool much more slowly. The small spike shown in the plot for free 2-dGlc in Fig. 6 has been observed repeatedly. The slower accumulation of 2-dGlc-6-*P* in the cells grown on succinate may be the result of reduced rates of free 2-dGlc transport. An alternative explanation, however, might be that succinate-grown cells are deficient in an inducible glucose group translocation system.

The equivocal nature of these results led us to consider transport of free 2-dGlc by other mechanisms, which might encompass inducible components. The property which best indicates the capability of cells to catalyze the active transport of a substrate is the accumulation of that substrate against a concentration gradient. The rapid phosphorylation of 2-dGlc by *K. lactis* precludes an indefinite incubation to equilibrate the intracellular and extracellular levels of free 2-dGlc. However, it appears from Fig. 5 that the level of 2-dGlc in the pool of glucose-grown cells reaches an equilibrium or steady state after about 1 min. Determinations of the concentrations of 2-dGlc in the pool after 1 min might indicate if this steady-state level is maintained by active transport.

The intracellular concentration of the unphosphorylated compound was determined in unpoisoned, glucose-grown cells. The external concentrations of substrate used were 0.75, 6 and 100 mM. The results (Table I) show lower intracellular than extracellular concentrations of 2-dGlc. These findings do not eliminate the possibility that active transport can occur, but do indicate that the steady-state level of free 2-dGlc is not maintained by an active process. The increasing internal levels with increasing external amounts of 2-dGlc suggest that the pool concentration equilibrates with the external concentration. The unequal intracellular and extracellular concentrations of 2-dGlc may be due to the inaccessibility of all cellular space to the free hexose.

TABLE I

STEADY STATE CONCENTRATIONS OF 2-DEOXYGLUCOSE IN GLUCOSE-GROWN *K. LACTIS* 1 MIN AFTER EXPOSURE TO RADIOACTIVE HEXOSE

Extracellular 2-dGlc concentration (mM)	Intracellular 2-dGlc concentration (mM)
0.75	0.25
6.0	2.3
100.0	30.0

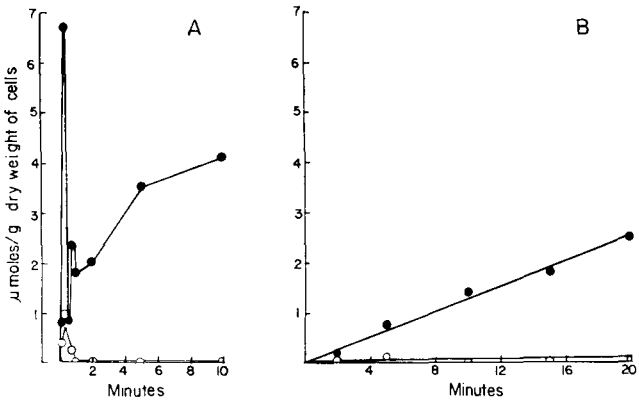

Fig. 7. Distribution of (●) 2-dGlc and (○) 2-dGlc-6-*P* in starved, poisoned (A) glucose-grown and (B) succinate-grown *K. lactis*. Cells were shaken in buffer for 3 or 4 h, respectively, and inhibited with $5 \cdot 10^{-3}$ M iodoacetate and $3.2 \cdot 10^{-4}$ M azide. Initial substrate (2-dGlc) concentration was 6 mM.

There are many reports [4, 10, 14, 27–29] that hexose transport in *S. cerevisiae* can occur via non-energy-requiring routes. We, therefore, compared the rates of transport of 2-dGlc by similar routes in *K. lactis* cells grown on glucose and on succinate. To study transport rather than accumulation, which process appeared to involve the activities of hexokinase or possibly a group translocation system, transport had to be uncoupled from energy-utilizing steps. A search for a hexokinaseless mutant failed. We chose, therefore, to use inhibitors of energy-generating metabolism.

To eliminate effectively the phosphorylation of 2-dGlc, cells were starved until their endogenous respiration was minimal, then poisoned with both azide and iodoacetate. Fig. 7 shows the amounts of labeled pool components extracted from such cells. Negligible amounts of 2-dGlc-6-*P* were detected, assuring us that phosphorylating activities had been minimized. These results indicate that 2-dGlc can enter the cells in a non-phosphorylated form and that, therefore, neither group translocation nor, probably, active transport is obligatory for 2-dGlc transport in *K. lactis*. The initial very rapid influx and efflux of 2-dGlc, forming a spike in the plot for the starved, poisoned, glucose-grown cells has been found consistently. Van Steveninck [11] has reported a similar phenomenon during 2-dGlc transport by iodoacetate-treated cells of *S. cerevisiae*, but the peak contained greater amounts of 2-dGlc-6-*P* than of 2-dGlc. Our results in Fig. 7 show labeled 2-dGlc almost exclusively in the cellular pools of *K. lactis*, and indicate that these experimental conditions should permit us to compare the rates of non-energy-requiring 2-dGlc transport in cells grown on glucose and on succinate.

Comparisons of rates of uptake, exchange and efflux of 2-dGlc by succinate- and glucose-grown cells

The rates of 2-dGlc uptake by starved, poisoned, succinate- and glucose-grown cells were observed to increase with increasing external concentrations of 2-dGlc. Double reciprocal plots of the data yielded values for V of 0.4 and 1.4 μmol/g dry weight of cells per min for cells grown on succinate and on glucose, respectively. These saturation kinetics, illustrated only for glucose-grown cells in Fig. 8A, indicate

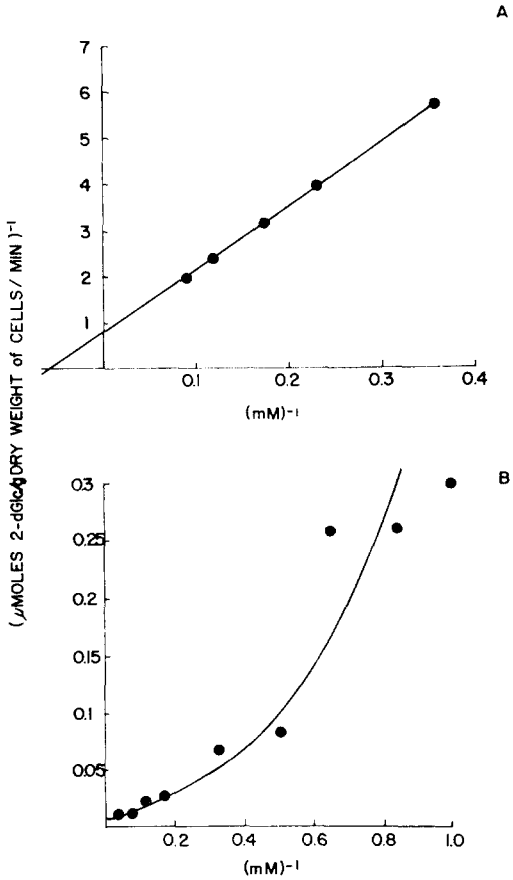

Fig. 8. Double-reciprocal plots of entry of 2-dGlc into glucose-grown *K. lactis*. (A) Uptake of 2-dGlc into the starved, poisoned cells. (B) Exchange of 2-dGlc into the starved, poisoned, loaded cells.

that 2-dGlc uptake by cells grown on either carbon source is carrier-mediated. However, the V for uptake by glucose-grown cells is much lower than V for accumulation, calculated from Fig. 2. If no active process were involved in 2-dGlc transport, the maximum rates should be the same in poisoned and unpoisoned cells. Efflux of substrate from the cells may lower the net initial uptake rates and account for this disparity. The difference in 2-dGlc transport capabilities of succinate- and glucose-grown cells may also be modified by efflux. Therefore, rates of exchange of external, labeled 2-dGlc with internal, unlabeled 2-dGlc were determined. The data for succinate-grown cells yielded values for V of 0.5 μmol/g dry weight of cells per min, and for $K_{m_{app}}$ of 3.8 mM. These values are not significantly different from those calculated for uptake in succinate-grown cells. The exchange data for glucose-grown cells are more complex. A double-reciprocal plot of the data (Fig. 8B) is not linear but appears to be parabolic. The V of exchange from these data is 71 μmol/g dry weight of cells per min, and the $K_{m_{app}}$ is 5.8 mM. The rates of 2-dGlc transport by succinate- and glucose-grown *K. lactis* determined from the exchange methodology appear to be distinctly different.

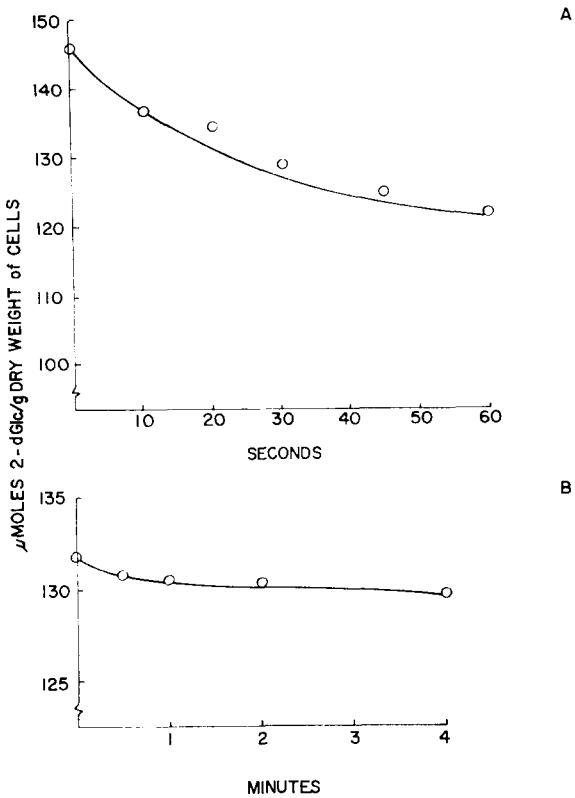

Fig. 9. Efflux of labeled 2-dGlc from (A) glucose-grown and (B) succinate-grown *K. lactis*. Cells were starved, poisoned and loaded with 100 mM 2-d[^{14}C]Glc. Efflux was carried out against 6 mM unlabeled 2-dGlc.

A facilitated diffusion system catalyzing exchange of free hexose across the cell membrane should exhibit a similar difference in rates of efflux from cells grown on succinate and on glucose. When starved, poisoned cells were loaded with radioactive 2-dGlc and analyzed for rates of efflux against external, unlabeled 2-dGlc (Fig. 9), glucose-grown cells, as expected, showed much higher exit rates.

DISCUSSION

Our conclusion that the formation of an inducible component of the glucose transport system accounts, at least in part, for the adaptive utilization of glucose by *K. lactis* is based on several observations. First, the uracil-dependent development of increased rates of 2-dGlc accumulation by the uracil auxotroph. A similar finding that histidine stimulated development of 2-dGlc accumulation in a histidine auxotroph reinforced the conclusion that protein synthesis is required for an increased capacity to accumulate hexose. Second, the similar levels of hexokinase activity in glucose- and succinate-grown cells suggest that transport steps, rather than accumulation events subsequent to transport, are catalyzed by the inducible entity. Third, the markedly

different V values for exchange transport with cells grown on succinate and on glucose indicate significantly different levels of a hexose carrier system in these cells. We suggest that the carrier may be an inducible transport component.

We have shown that rapid efflux of 2-dGlc occurs from poisoned, glucose-grown cells. Although ice-cold buffer was used to minimize it, undoubtedly some efflux will have occurred during sample washing, and presumably to a greater degree in the glucose-grown cells. It is possible, therefore, that the V values for 2-dGlc transport estimated by the exchange method may be somewhat low, especially for the glucose-grown cells. Hence, the difference in transport capacities between these cells and those grown on succinate may be greater than we have determined.

If succinate-grown *K. lactis* cells are also deficient in a glucose group translocation system, as might be interpreted from Figs. 5 and 6, then a second inducible glucose transport component may exist. It is possible, of course, that the inducible carrier might function in both energy-requiring and non-energy-requiring transport modes.

The inducibility of a glucose transport system in *K. lactis* is interesting in that most yeasts investigated are reported to be constitutive in this respect. In *K. lactis* the low levels of an inducible glucose transport system during glucogenesis may serve the purpose of conserving hexose units synthesized by the cells, effort which would be wasted, possibly, if the cells retained high levels of an efficient efflux system.

If non-energy-requiring glucose transport catalyzed by an inducible carrier is important in *K. lactis*, then the rates of transport of free 2-dGlc into succinate- and glucose-grown cells illustrated in Figs. 5 and 6 might be expected to show a greater difference. However, transport here occurs under conditions where hexose-phosphate can be formed. Kotyk and Kleinzeller [30], and more recently Serrano and DelaFuente [5], have suggested that an intermediate of glucose metabolism may regulate the activity of the glucose carrier in yeast. Conceivably, regulation of this kind might diminish the magnitude of the difference in rates of free 2-dGlc transport by *K. lactis* grown in succinate and in glucose.

Our investigations do not exclude the possibility that energy-requiring transport of glucose occurs in *K. lactis*: the data are equivocal. The mode of transport of hexoses in *S. cerevisiae* has been the subject of intensive study which has led to two discrepant views. Cirillo [4, 10, 27, 28] and Kotyk [14, 29] and their co-workers have presented evidence favoring the view that hexoses are transported by a carrier-mediated, non-energy-requiring mechanism, while results from Van Steveninck's laboratory [31–34] support a group translocation mode of transport. Kotyk and Michaljanicova [14] have recently reported that the conversion of transported galactose to trehalose precursors may obscure the precursor relationships between this sugar and its phosphorylated derivative. They suggested that this conversion might be responsible for the different results found by Van Steveninck [32] and by themselves, which have led them to favor opposing mechanisms of transport. Kotyk and Michaljanicova [14] also state that in *S. cerevisiae* metabolism of 2-dGlc results in the appearance of several products. Our data with *K. lactis* suggest that dideoxytrehalose is not formed as readily as in *S. cerevisiae*, for pools from *K. lactis* extracted and chromatographed by three methods, one identical to that described by Kotyk and Michaljanicova [14], revealed only 2-dGlc and 2-dGlc-6-*P*.

The curvilinear appearance of the exchange data with glucose-grown cells may

reflect the influence of efflux of unlabeled substrate from the internal cell pool. By diluting the extracellular labeled substrate, efflux from the cells would alter the true rate of exchange, with a greater effect on exchange at low external substrate concentrations. By keeping the ratio of internal to external concentrations of substrate constant, any outward diffusion effect would be standardized. Therefore, the parabolic double-reciprocal plot of the data may not be entirely due to diffusion (efflux) effects. Parabolic-shaped, double-reciprocal plots of enzyme velocity-substrate relationships can indicate allosteric properties of the enzyme. Sugar transport is the first step in a series of catabolic reactions, many of which are metabolically regulated. That transport, too, in yeast might be so controlled seems reasonable, and has been previously suggested [5, 30, 35] by other investigators.

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REFERENCES

1 MacQuillan, A. M. (1969) J. Bacteriol. 100, 288–294
2 Azam, F. and Kotyk, A. (1967) Folia Microbiol. 12, 115–120
3 Heredia, C. F., Sols, A. and DelaFuente, G. (1968) Eur. J. Biochem. 5, 321–329
4 Kuo, S. C. and Cirillo, V. P. (1970) J. Bacteriol. 103, 679–685
5 Serrano, R. and DelaFuente, G. (1974) Mol. Cell. Biochem. 5, 161–171
6 Barnett, J. A. and Kornberg, H. L. (1960) J. Gen. Microbiol. 23, 65–82
7 Spiegelman, S. and Halvorson, H. O. (1954) J. Bacteriol. 68, 265–273
8 Zmijewski, Jr., M. J. and MacQuillan, A. M. (1975) Can. J. Microbiol. 21, 473–480
9 Brown, C. E. and Romano, A. H. (1969) J. Bacteriol. 100, 1198–1203
10 Kuo, S. C., Christensen, M. S. and Cirillo, V. P. (1970) J. Bacteriol. 103, 671–678
11 Van Steveninck, J. (1968) Biochim. Biophys. Acta 163, 386–394
12 Weidemann, G. and Fischer, W. (1964) Z. Physiol. Chem. 336, 192–194
13 Farkas, V., Bauer, S. and Zemek, J. (1969) Biochim. Biophys. Acta 184, 77–82
14 Kotyk, A. and Michalianicova, D. (1974) Biochim. Biophys. Acta 332, 104–113
15 Conway, E. J. and Downey, M. (1950) Biochem. J. 47, 347–355
16 Maitra, P. K. and Lobo, Z. (1971) J. Biol. Chem. 246, 175–188
17 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
18 Biely, P., Kratky, Z. and Bauer, S. (1972) Biochim. Biophys. Acta 255, 631–639
19 Blakley, E. R., Myoda, T. and Spencer, J. F. T. (1966) Can. J. Biochem. 4, 927–935
20 Cirillo, V. P. (1968) J. Bacteriol. 95, 603–611
21 Heredia, C. F., DelaFuente, G. and Sols, A. (1964) Biochim. Biophys. Acta 86, 216–223
22 Kotyk, A. (1967) Folia Microbiol. 12, 121–131
23 Spoerl, E., Williams, J. P. and Benedict, S. H. (1973) Biochim. Biophys. Acta 298, 956–966
24 Cirillo, V. P. (1961) Annu. Rev. Microbiol. 15, 197–218
25 Lineweaver, H. and Burk, D. (1934) J. Am. Chem. Soc. 56, 658–666
26 Purich, D. L., Fromm, H. J. and Rudolph, F. B. (1973) Adv. Enzymol. 39, 249–326
27 Cirillo, V. P. (1961) Trans. N.Y. Acad. Sci. Ser. II 23, 725–734
28 Cirillo, V. P. (1968) J. Bacteriol. 95, 1727–1731
29 Kotyk, A. and Haskovee, C. (1968) Folia Microbiol. 13, 12–19
30 Kotyk, A. and Kleinzeller, A. (1967) Biochim. Biophys. Acta 135, 106–111

31 Rothstein, A. and Van Steveninck, J. (1966) Ann. N.Y. Acad. Sci. 137, 606–623
32 Van Steveninck, J. (1972) Biochim. Biophys. Acta 274, 575–583
33 Van Steveninck, J. and Dawson, E. C. (1968) Biochim. Biophys. Acta 150, 47–55
34 Van Steveninck, J. and Rothstein, A. (1965) J. Gen. Physiol. 49, 235–246
35 Kleinzeller, A. and Kotyk, A. (1968) in Aspects of Yeast Metabolism (Mills, A. K., ed.), pp. 33-45, F. A. Davis Co., Philadelphia