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Sugar and Amino Acid Transport in Yeast.

I. Glucose Transport during the Sporulation Stage with Reference to the Vegetative Stage.

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효모세포의 당과 아미노산의 운반에 관한 연구

——I. 생장시기와 포자형성기의 포도당 운반——

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ABSTRACT

During the sporulation stage in *Saccharomyces cerevisiae* J170, the incorporation of D^{14} C-glucose into starved cells of sporulation stage as well as the vegetative one is appeared higher at pH 6.0. Glucose transport system, in both the vegetative and sporulation stage, is associated with "energy dependent" as the result of repression by such a respiratory inhibitor as 2,4-dinitrophenol. The Km value of glucose uptake in vegetative stage and sporulation stage was 2.1 mM and 2.5 mM respectively, indicating that the glucose is considerably required for vegetative growth. Competition and countertransport of glucose by fructose and galactose are more distinct in vegetative stage, comparing with sporulation stage. The main sugar components of yeast cells consists of ribose, mannose, and α, β-glucose. Amounts of mannose is lower in the sporulation stage than that in the vegetative stage.

INTRODUCTION

Sporulation of the yeast *Saccharomyces cerevisiae* can be induced in nearly all of the members of diploid yeast population under appropriate conditions. Diploid cells of yeast undergo meiosis and the subsequent four spores form within an ascus.

The studies of yeast sporulation has been examined from a various biochemical

and genetical aspects(Esposito *et al.*,1969; Powell. 1969). Acetate utilization and macromolecular synthesis during sporulation(meiosis) of homothallic strains of *Saccharomyces cerevisiae* were studied. When diploid cells are transferred from glucose nutrient medium to acetat sporulation medium at early stationary phase, the ascospore development was essentially complete(Esposito *et al.*, 1969). Sporulation of several strains of the yeast grown in a variety of carbon sources was more synchronous than the sporulation of cells grown in medium containing dextrose. (Fast, 1973)

By using the YEP pregrowth procedure, information has been obtained concerning the effects of this mutant on macromolecular synthesis, genetic recombination, and fine structural development during sporulation.

In early papers(van Steveninck *et al*, 1964, 1968, 1969, 1975; Rothstein and Van Steveninck, 1966) evidences have been presented for the existence of two sugar transport systems in yeast. A passive, carrier mediated facilitated diffusion and an active, metabolically linked transport mechanism. The active mechanism is operative in glucose transport. Experimental results indicated a permease carrier transport system in which a phosphorylating reaction takes place, with polyphosphate as phosphate donor.

van Steveninck, (1968) indicated that, based on the model of transmembrane sugar transport, the kinetic of mutual inhibition between pairs of sugars were reported. Two modes of transport of faciliated diffusion and active transport, both utilizing the same carrier.

In this experiment, to examine glucose uptake into vegetative cells and sporulated cells in synchronous sporulation system, an attempt was made to investigate the effects of pH, kinetic parameters, the presence of active transport system, inhibition of galactose or fructose upon glucose uptake, and sugar composition in two different stages of yeast.

METHODS AND MATERIALS

1. Materials

1) ^{14}C-glucose: Radioactive D-glucose was a gift from Dr. Nisizawa Kazutosi, Nihon University, Tokyo, Japan.

2) Yeast strain: The mutant of yeast strain used throughout these studies was a gift from Dr. H. O. Halvorson, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Massachusetts. Homothalic diploid strain of *Saccharomyces cerevisiae* was used in this experiment: J170 (AP-1). The genotype of J170 is a/α, aden 1/+, aden 2/aden 2, gal 1/+, tyr 1/+, his 7/+, ura 1/+, +/leu 1, +/cyh 2, try/try, +/can. The symbols are as follows: a and α, mating type alleles; aden, adenine auxotroph; can, canavinine resistance; cyh, cyclohexamide resistance; gal, galactose fermentation; his, histidine auxotroph; leu, leucine auxotroph; try, tryptophan auxotroph; tyr, tyrosin auxotroph; and ura, uracil auxotroph.

2. Methods

1) Media: Yeast extract peptone(YEP) medium(Esposito *et al.*, 1969) was used for the growth medium of *Saccharomyces cerevisiae*, J170, YEP medium consisted of 20g of dextrose, 20g of Bacto-peptone, and 10g of yeast extract. Sporulation medium is composed of 20g of potassium

acetate and 1g of yeast extract.

2) Preparation of vegetative and sporulated cells: The cells for this initial inoculum were obtained from 2 days colonies grown on solid YEP medium. Diploid cells to be sporulated were inoculated into liquid YEP medium at an initial cell dinsity of 10^5 cells/ml and in cubated with rotary shakcr at room tem perature. The cells were harvested by filtration after 15–20hr of growth in Y EP medium at logarithm icphase, washed 4times with sterile distilled water, and the yeast suspension was used for vegetative cells. This vegetative cells were resuspended in sporulation medium at an initial cell density of 5×10^7 cells/ml, incubated for 20 hr in sporulation medium, and the sporulatcd ycast suspenension was washed with distilled water several times on centrifugation. This yeast suspenension was used fors porulated cells. A 20mg of amount of tetracycline per liter was routinely added to sporulating cultures to prevent bacterial contamination.

3) Procedure of cell counting: Yeast cell number was determined by hemocytometer counts of 300 to 500 cells. Cell number include buds and cells irrespective of size.

4) Standard uptake assay: The standard assay system contained the appropriate radioactive glucose, and cells at a concentration of 2.32×10^6/ml. Incubation were carried out at 30°C in a final volume of 1.0ml. After the appropriate time period, one mililiter of reaction mixture was filtered on a Millipore filter(pore size is 0.45μm), washed with cold distilled water several times, and dried in air. The dried filters were them placed in liquid scintillation counting vials and counted in a toluene based liquid scintillation fluid containing 2,5-diphenyloxazole(PPO) (4g) and 1,4-bis[2-(4-methyl-5-phenyloxazolyl)] benzene(POPOP)(0.1 g) per liter of toluene.

5) Determination of sugar composition in vegetative and sporulated cells: Five milliliters of yeasts solution of starved cells of vegetative or sporulation stage contained 3.01×10^8/ml was added with 2.5ml of 2N HCl, respectively. Acid hydrolysis was carried out for 6 hours in reflux system at 100°C and the hydrolysates were concentrated to remove HCl. This hydrolysates were applied to gaschromatography(Holligan, 1971).

RESULTS

The yeast cells grown in YEP medium are optimally adapted to sporulation in early stationary phase. This stage of growth corresponds to the time of transition from fermentation to respiration as the dextrose in this medium is exhausted, and suggests that actively respiring cells are required for sporulation(Cross 1967; Esposito *et al* 1969; Halbach-Keup and Ehrenberg 1971; Fast, 1973).

The yeast cells grown in a semidefined medium containing acetate as a carbon source sporulate optimally(Fast, 1973; Roth and Halvorson, 1969; Simchen *et al.*, 1972). Since acetate is not fermentable, the yeast cells are respiring during logarithmic phase growth.

This routine puocess shows in a higher sporulation synchrony as well as a high percentage of asci.

Growth in YEP medium.

To harvest the vegetative cells during vegetative growth, diploids were inoculated into liquid YEP medium at an intial cell density of 10^5 cells/ml and incubated with rotary shaker at room temperature. The growth curve of yeast cell, J170. in YEP medium is shown in Fig. 1.

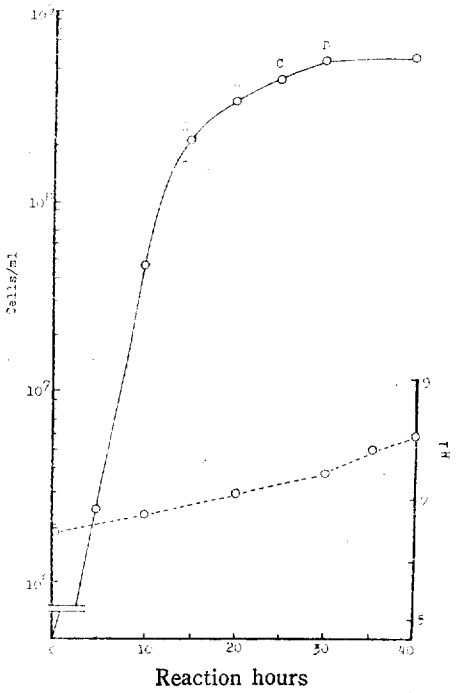

Fig. 1. Growth of diploid J170 in YEP medium. Logarithmically growing cultures were inoculated into the growth YEP medium from an initial inoculum of 10^5 cells/ml. At the time indicated by the arrows, cells were transferred to sporulation medium.

Exponential growth in YEP medium was shown until 15 hours incubation period at room temperature. pH change during the growth period increased from the initial pH 6.5 to pH 8.0 of stationary phase for 40 hours incucation in growth medium.

Sporulation

Samples (A—D) were taken in logarithmic phase and early stationary phase from each of the growth media as indicated by the arrows in Fig. 1 and inoculated into sporulation medium. The time course of the appearance of asci are shown in Fig. 2.

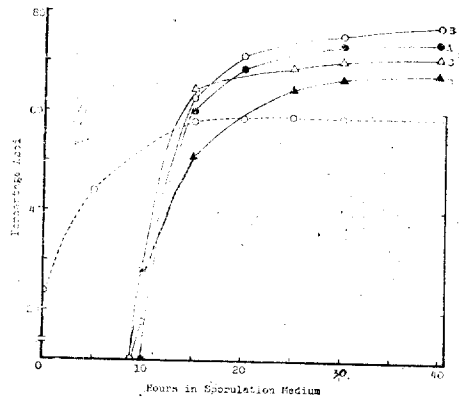

Fig. 2. Ascus formation of J170 in sporulation medium. Cultures of J170 grown in YEP media, were obtained during the growth as indicated in Fig. 1 with various time intervals and were inoculated in sporulation medium. Samples were removed at time intervals and examined for the presence of asci.

The results indicate that, when the cell number is no longer increasing exponentially, synchronous sporulative ability decrease.

The higher pecentage of asci was formed in the cells, which was incubated for 20hr in sporulation medium after 20 hours incubation in YEP growth medium. As a result, an early stationary phase is approached, the rapidly formation of asci and final percentage of asci reached maximal levels (*ca.* 80%).

From the result described above, the authors have taken a B sample of 20hr incubation in YEP medium and incubated for 20 hours in sporulation medium. This B sample was used for sporulated cells of the yeast.

The general fact is that the most

amount of glucose is consumed for respiration in active vegetative stage. However, macromolecular synthesis by acetate were studied during sporulation of yeast. When diploid cells are transferred from glucose nutrient medium to acetate sporulation medium at early stationary phase, 16% of acetate is incorporated into macromolecular cellular components. In relation to physiological properties the authors investigated the glucose uptake during sporulation.

Effect of pH on glucose uptake

To examine the optimal pH on glucose uptake into vegetative and sporulative stage cells, 0.45ml of yeast suspension containing 2.32×10^6/ml, 0.05ml of ^{14}C-glucose containing 0.02μCi, that is, 0.0816 mM of glucose, and 0.5ml of various phosphate buffer solution were mixed and incubations were carried out for 10 min at 30°C. The pH-activity curve of glucose uptake in vegetative cells and sporulated cells is shown in Fig. 3.

Incorporation of ^{14}C-glucose into vegetative cells and sporulated cells can be seen at pH 6.0, where maximum incorporation was observed. Glucose uptake into vegetative cells was effective than that in sporulated cells. This result seems that vegetative cells have more active metabolic process than that in during sporulating period.

The velocity of glucose transport in vegetative cells and sporulated cells appears to be pH-dependent. Only at extreme, pH value has the rate of transport decrease slightly.

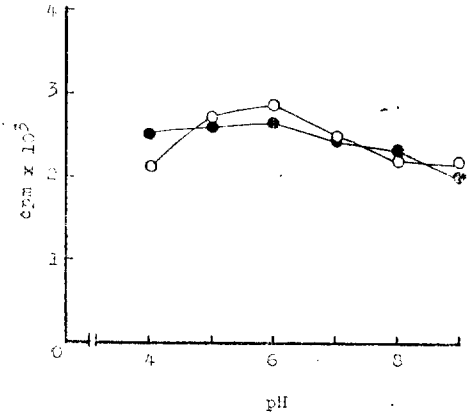

Fig. 3. Effect of pH on glucose uptake into vegetative cells(○—○) and sporulated cells(●—●) of yeast.

Active transport of glucose

To acertain the active transport of glucose uptake into vegetative cells and sporulated cells, the reaction mixture consisted of 0.20ml of glucose containing 0.02μCi and 2.80ml of yeast suspension containing 2.32×10^6/ml and pH adjusted at 6.0. The reaction mixture is incubated at 30°C and 1 ml of sample was removed to determine the glucose transport for 10

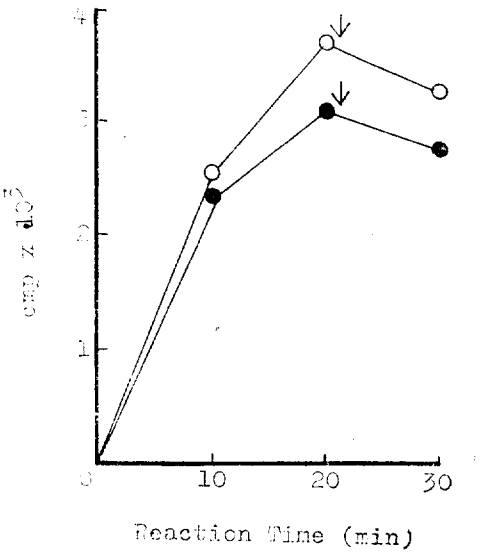

Fig. 4. Time-course of glucose transport and the effect of 2,4-dinitrophenol. Vegetative cells(○—○) are grown on YEP medium for 20hr incubation. Sporulated cells(●—●) are sporulated for 20hr in sporulation medium which the vegetative cells are inoculated. Arrow lines indicate the addition of 2,4-dinitrophenol.

min and 20 min, respectively. After the addition of 1 ml of 0.02M 2,4-dinitrophenol to the remain suspension(1 ml), the total 2 ml of reaction mixture was incubated for 10 min to estimate the glucose uptake.

As shown in Fig. 4, glucose uptake increase for 20min. However, after the addition of 2,4-dinitrophenol to the reaction mixture, glucose uptake decrease for 10 min during the vegetative cell growth and sporulation period. Therfore, the glucose transport system is inducible, and is repressed by a kind of respiratory inhibitor such as 2,4-dinitrophenol. This result means that glucose transport system is associated with energy dependent in both of vegetative growth phase and sporulation period.

Kinetics of glucose uptake

The yeast suspension(0.45ml) adjusted at pH 6.0 was added to 0.65ml of various concentrations of ^{14}C-glucose and cold glucose. Incubation condition was for 10 min at 30°C.

Table 1. Kinetics of glucose uptake into vegetative and sporulated cells.

Stages of yeast J170	Km (mM)	Vmax (μ moles/ml/min)
Vegetative stage	2.1	15.38
Sporulation stage	2.5	14.26

The glucose concentration is expressed in mM and the rate of glucose uptake μmoles/ml of yeast suspension per min.

The constants for vegetative yeast cells is 2.1mM for Km(Michaelis constant) and 15.38μ moles/ml for Vmax. In sporulating cells, Km value is 2.5mM, and the Vmax almost same value 14.26/ml of yeast suspension per min. This result shows that glucose uptake into vegetative cells is higher than that into sporulated cells.

Competition and countertransport studies

To investigate the effects of galactose and fructose on glucose uptake into vegetative and sporulated cells, 0.05ml of ^{14}C-glucose 0.45ml yeast suspension adjusted at pH 6.0 and 0.5ml of two kinds of concentration of galactose or fructose solution was mixed, respectively, and incubated for 10min at 30°C. The results are shown in Table 2.

Table 2. Inhibition by galactose and fructose on glucose transport*.

Inhibitor	Concentration of inhibitor(%)	Inhibition percentage of glucose uptake	
		Vegetative cells	Sporulated cells
Control	—	100.0	100.0
Galactose	0.5	12.0	4.1
	5.0	13.7	6.5
Fructose	0.5	21.4	12.2
	5.0	23.7	17.1

* Glucose concentration as a substrate was 0.0816mM.

As shown in Table 2, in vegetative cells glucose uptake is inhibited by fructose and galactose as well as sporulated cells. Fructose inhibit glucose transport remarkably more than galactose and also the rate of inhibition by sugars on glucose uptake in vegetative cells is higher than in sporulated cells. This results show the evidence that metabolism of glucose uptake was inhibited in vegetative stage with the competition of other sugars.

Sugar composition of yeast cells

Comparing with vegetative diploid and sporulating haploid stage in biochemical aspect, the cells of two stage were hydrolyzed with HCl. The sugar composition of diploid and haploid yeast cell by gas chromatography are shown in Fig. 5.

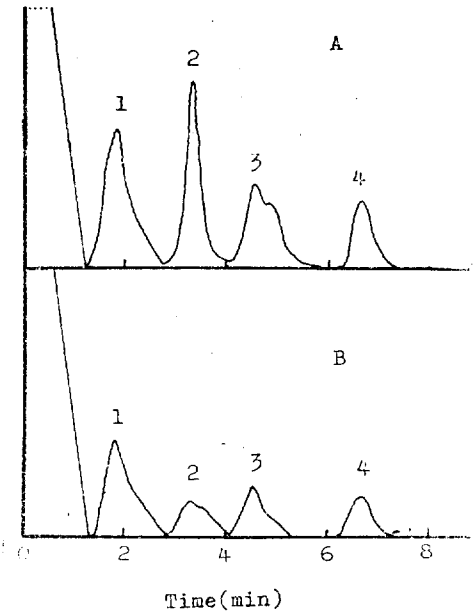

Fig. 5. Gas chromatogram of TMS derivatives of sugars in yeast cell components for vegetative(A) and sporulating stage(B). The analysis was carried out on a 4mm×1.5m stainless-steel column containing 3%60—80 mesh chromosorb W, programmed from 170—200°C with 45ml/min(nitrogen). Peaks correspond to thefollowing sugars: ribose(1); mannose(2); β-glucose(3); and α-glucose(4).

The sugar components of vegetative and sporulating cells are consisted of ribose, mannose, and α, β-glucose. Generally the amounts of all sugars in vegetative cells are richer than sporulating cells, coinciding with other papers (Chung and Nickerson, 1954; Rainbow and Rose, 1963). It is interesting that only mannose is lower constituent in sporulating stage with reference to that of vegetative stage, suggesting that mannose is less required for spore formation.

DISCUSSION

Studies on meiosis and sporulation in yeast reported(Fast, 1973; Wilkins, 1967) and suggested that sporulation is dependent upon acetate utilization and on the physiological age of the cells when transferred to sporulation medium. Glucose transport of yeast cells was compared with diploid vegetative cells and haploid sporulating cells during respiration and macromolecular synthesis.

The velocity of glucose transport in vegetative cells and haploid sporulating cells appears to be pH-dependent. The location of the peaks is somewhat different for different substrates, depends on the yeast strain and physiological conditions of yeast. For instance, van Steveninck *et al.*, (1968) found two maxima for glucose fermentation at pH 4.5 and 6.5. From kinetic studies it has been concluded that hexose uptake in yeast is a carrier-mediated process. Evidence has been presented indicating the existence of two different sugar

transport mechanisms in yeast: a metabolically linked, active transport and passive, carriermediated, facilitated diffusion(van Steveninck and Dawson, 1968).

In this experiment, glucose transport during haploid spore formation is also active process and pH-dependent.

The apparent Michaelis constants for glucose transport in vegetative diploid cells is 2.0mM. This Km value is similar to that of bakers yeast(Wilkins, 1967), which the maximal rate of glucose transport could be estimated. In sporulating haploid cells, the Km value is 2.5 mM. This suggests that spore formation has less requirement of glucose for macromolecular synthesis.

Competition of sugars for the hexose transport system in yeast cells has been kinetically presented by van Steveninck (1968). Countertransport studies and demonstration of mutual inhibition of transport with pairs of sugars indicate that a number of sugars share a common carrier. In our experiment, inhibition of glucose transport in vegetative and haploid cells is presented by the addition of fructose and galactose.

This result is in agreement with the common carrier theory and mutual inhibition of sugar transport. However, it seems that the rate of inhibition on glucose transport system is dependent upon cell conditions since it has variable value.

적 요

Saccharomyces cerevisiae J170, mutant를 사용하여 YEP 배지에서 20시간 진탕 배양하여 생장이 왕성한 영양시기의 효모세포를 얻었다. 이 세포를 sporulation medium에 접종하여 30°C에서 20시간 진탕 배양하면 약 80%의 ascospore를 형성함을 볼 수 있었다. 포자형성시기와 생장시기를 택하여 D-^{14}C-glucose 운반에 관하여 비교 검토한 결과는 다음과 같다. haploid인 포자형성시나 diploid인 생장시기의 포도당 흡수는 pH 6.0에서 최대이었다. 이 두 시기의 포도당 운반은 2, 4-dinitrophenol에 의하여 저해되었으므로 포자형성기의 포도당운반도 역시 energy를 요구하는 능동수송과 연관이 있음을 알게 되었다. 생장시기의 포도당 흡수정도는 포자형성기 보다 높았으며 이때에 fructose나 galactose와 포도당흡수의 관계는 서로 경쟁적으로 작용함을 알 수 있었다. 효모세포의 당 성분은 ribose, mannose, α, β-glucose로 되어 있으며 특히 포자형성기의 mannose 함량은 영양세포 때보다 현저히 적었다.

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