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Sugar and Amino Acid Transport in Yeast.

Ⅱ. Leucine Transport during the Sporulation and Vegetative Stage.

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효모세포의 당과 아미노산의 운반에 관한 연구

—Ⅱ. 포자형성기와 생장시기의 로이신 운반—

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ABSTRACT

Saccharomyces cerevisiae J170, a mutant, was used for DL-^{14}C-leucine uptake during the sporulation and vegetative stage. ^{14}C-Leucine uptake into yeast cells appeared the highest at pH 6.0, indicating the same result of glucose transport. ^{14}C-Leucine uptake in sporulation period was higher than in growth phase, showing the evidence that leucine is more required for protein synthesis. This tendency has been also supported from the result of Km values of leucine uptake in two stages of yeast. Leucine uptake was inhibited by 2, 4-dinitrophenol in two stages of yeast. This means that leucine transport system is associated with energy dependent in both stages. The contents of all amino acid in growth phase cells were higher than those of sporulation stage cells, and those of methionine and tyrosine were showed in trace during the sporulation stage. In contrast, the content of glutamic acid in sporulation stage was higher compared with those of other amino acids.

INTRODUCTION

The system for the accumulation of amino acid in *Saccharomyces cerevisiae* acts as an enzymatic active transport system or amino acid permease. All the amino acids were concentrated by the amino acid permease, but with widely varying affinities. The D-forms were also concentrated, but with affinities much lower than those for L-forms (Surdin *et al.*, 1965)

The accumulation of both lysine and glutamic acid occurs only during the metabolism of glucose (Davies *et al.*, 1953). The accumulation of lysine by yeast is accompanied by a loss of sodium and potassium from the cells.

M. Grenson *et al.* (1966) reported the multiplicity of the amino acid permeases in yeast the evidence for a specific arginine and lysine-transporting system. According to a Lineweaver-Burk plot, the initial velocity of entrance of L-^{14}C-

lysine into yeast cells appears to be dependent on two functions. Evidence is presented showing that lysine enters the cell by two distinct system. One of these systems is the arginine permease and the other lysine-uptake system has a higher affinity for lysine.

Recently, D. Mills (1972) was initiated to examine more closely the effect of alanine and leucine uptake into protein synthesis during meiosis and sporulation. Evidence presented that sporulating yeast becomes impermeable to two amino acids. This impermeability can cause a distortion in measurements of macromolecular synthesis during the stage of sporulation. It was shown that in yeast, as with other fungi, a pH optimum exists for uptake of certain precursors of RNA, DNA and protein.

In previous paper (Min et al., 1978), we discussed the glucose transport in vegetative and sporulated cells. In the present experiments, it is investigated that the effect of pH, active transport system, Michaelis constants, inhibition of sugars on leucine into vegetative diploid cells and sporulated haploid cells.

MATERIALS AND METHODS

1. Materials.

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

2). Yeast strain: The mutant of yeast strain used for this experiment 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, ade 1/+, ade 2/ade 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; ade, 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) Preparation of vegetative and sporulated cells: The cells for the 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 density of 10^5cells/ml and inoculated with rotary shaker at room temperature. The cells were harvested by filtration after 15—20 hr of growth in YEP medium at logarithmic phase, washed 4 times with sterile distilled water, and the yeast suspension was used for vegetative cells. This vegetative cells were resuspended in sporulation medium at an initial cells density of 5×10^7 cells/ml, incubated for 20 hr in sporulating medium, and the sporulated yeast suspension was washed with distilled water 4 times on centrifugation. This yeast suspension was used for sporulated cells.

2). Standard uptake assay: The standard assay system contained the appropriate radioactive leucine, and cells at a concentration of 2.32×10^6/ml. Incubations were carried out at 30°C in a final volume of 1.0ml. After the appropriate time period, one milliliter 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—(4methyl- 5-phenyloxazolyl)] benzene (POPOP) (0.1g) per liter of toluene.

3). Amino acid composition in yeast: Yeast suspension was washed with distilled water several times and 5ml of this yeast supensions in vegetative cells or sporulated cells contained 3.01×10^8 per ml, respectively, was added with 5ml of 11N hydrochloric acid. Acid hydrolysis with sealing was performed for 70hrs in reflux system at 100°C. The hydrolysate was concentrated to remove hydrochoric acid and applied to automatic amino acid analyzer (Schmidt, 1966).

RESULTS

During the stages of sporulation in *Saccharomyces serevisiae*, the uptake of leucine into protein was compared with vegetative diploid cells.

pH optimal of leucine accumulation

To examine the optimum pH on leucine uptake, 0.45ml of yeast suspension containing 2.32×10^6 cells/ml was mixed with 0.5ml of various pH of phosphate buffer solution and 0.05ml of leucine solution containing 0.02μCi,0.0593mM. The reaction mixture was incubated for 10min at 30°C and determined the amount of leucine uptake by the method of standard uptake assay. The result is shown in Fig. 1.

From the results, the highest incorporation of ^{14}C-leucine into vegetative cells and sporulated cells can be seen at pH

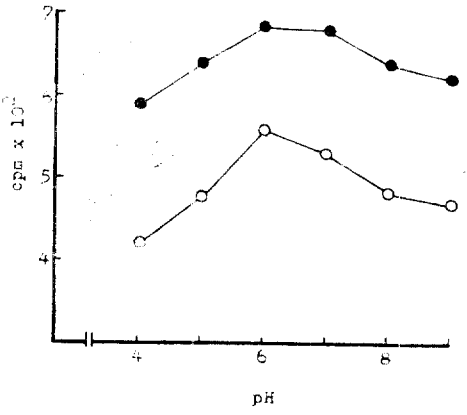

Fig. 1. Optimum pH for leucine uptake into vegetative cells (○—○) and sporulatedc ells (●—●)

6.0. It is interesting fact that the amount of leucine uptake into sporulated cells is higher than that into vegetative cells. This result suggests that the leucine transport into sporulated cells is required remarkably during the spore formation.

Active transport of leucine

In vegetative cells, enzymatic active transport system for the accumulation of amino acids in yeast was reported by Y. Surdin *et al* (1965). However, no evidence is the active transport system for leucine uptake into haploid sporulated cells. Yeast suspension, 2.8ml, containing 2.32×10^6 cells/ml and adjusted at pH 6.0 was mixed with 0.2ml of leucine containing 0.02μCi. The reaction mixture was incubated at 30°C and one milliliter of sample was removed to determine the leucine transport for 10 min or 20 min, respectively. After the addition of 1ml of 0.02M 2,4-dinitrophenol to the remain suspension (1ml), the total 2 ml of reaction mixture was incubated for 10 min to estimate the leucine uptake.

As shown in Fig. 2, leucine uptake increase for 20 min. However, after addition of 2,4-dinitrophenol to the

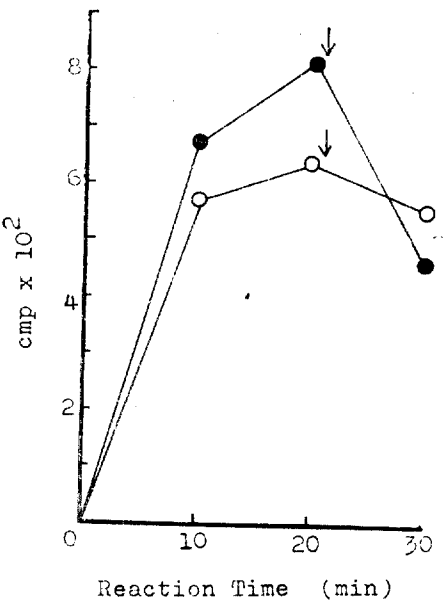

Fig. 2. Time-course of leucine transport and the effect of 2, 4-dinitrophenol. Vegetative cells (○—○) were grown on YEP medium for 20 hr incubation. Sporulated cells (●—●) were sporulated for 20 hr in sporulation medium, which the vegetative cells were inoculated. Arrow lines indicate the addition of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

reaction mixture, leucine uptake decrease for 10min during vegetative stage and sporulating period. The effect of 2, 4-dinitrophenol on leucine uptake during sporulation is more sensitive than vegetative stage. These results show that the active transport system for leucine uptake is present in vegetative cells as well as sporulating cells.

Kinetic paramenters of leucine uptake

Each of yeast suspension, 0.45ml, of vegetative cells or sporulating cells was mixed with 0.55ml of various concentration of ^{14}C-leucine and cold leucine. Incubation condition was for 10min at 30°C.

Table 1. Kinetics of leucine into vegetative and sporulated cells.

Stages of yeast J170	Km (M)	Vmax (moles/ml/min)
Vegetative	5.50×10^{-5}	0.054
Sporulation stage	4.69×10^{-5}	0.086

The Michaelis constant, Km value, for vegetative cells is $5.55\times^{-5}$M and Vmax value 0.054 moles/ml/min. In sporulating cells, Km value is higher, 4.69×10^{-5}M, and Vmax 0.086 moles/ml/min. This result shows that leucine uptake of sporulating cells is also higher than that of vegetative cells

Inhibition of galactose and fructose on leucine uptake

In previous paper, (Min *et al.*, 1978) we investigated the effect of galactose and fructose on glucose uptake.

Yeast suspension, 0.45ml, was mixed with 0.05ml C^{14} leucine solution and 0.5ml of two kinds of galactose or

Table 2. Inhibition of galactose and fructose on^{14}C-leucine uptake.

Inhibitor	Concentration of inhibitor (%)	Inhibition percentage of leucine transport	
		Vegetative cells	Sporulated cells
Control	———	100.0	100.0
Galactose	0.5	0.5	1.5
	5.0	4.7	4.9
Fructose	0.5	2.5	2.8
	5.0	4.2	7.5

fructose solution, respectively. Incubation was carried out for 10min at 30°C. This results are represented in Table 2.

The degree of inhibition of leucine transport by sugar in sporulating cells is more effective cells. This inclination is not in agreement with glucose transport system. And inhibition percentage of leucine uptake by fructose is higher than that by galactose. This result seems that leucine transport is associated with a kind of sugars and their concentration.

Table 3. Amino acid contents of the starved cells before leucine uptake.

Amino acids	Vegetative cells (mg/ml)*	Sporulated cells (mg/ml)*
Asp	1.729	0.737
Thr	0.880	0.345
Ser	0.765	0.287
Glu	1.613	1.182
Pro	0.517	0.245
Gly	0.778	0.289
Ala	0.840	0.325
Val	0.733	0.358
Cys	—	—
Met	0.124	trace
Ileu	0.648	0.263
Leu	0.923	0.355
Tyr	0.339	trace
Phe	0.557	0.223
NH_3	2.177	0.821
Orn	0.105	0.052
Lys	1.394	0.478
His	0.386	0.125
Arg	0.826	0.267

*One milliliter of yeast suspension was contained 3.01×10^8 cells/ml.

Amino acid contents of vegetative and sporulated cells

In Table 3, the contents of all amino acids in vegetative cells is higher than in the cells of sporulation stage. It is noticed that the contents of methionine or tyrosine in vegetative cells is 0.124 mg/ml or 0.923 mg/ml, respectively, but the contents of two amino acids are appeared trace in sporulation stage. In contrast, the content of glutamic acid in sporulation phase is higher compared with those of other amino acids.

Therefore, it is concluded that methionine and tyrosine are not required for spore formation but glutamic acid in relation to other amino acids.

The leucine content of the cells in sporulation stage is not so much as one third of vegetative stage. However, according to the previous results(Fig. 1, 2, and Table 1), leucine is also repuired for spore formation compared with vegetative stge.

DISCUSSION

Meiosis and sporulation in yeast, like sporulation in bacteria (Bernlohr, 1964; Hardwick and Foster, 1952; Monro, 1961: Spizizen, 1965) is associated with extensive protein turnover. Protein synthesis is continuous throughout sporulation and shows two maxima, one stage before asci appears and a second stage during ascospore development. The enchanced uptake and incorporation at low pH is not restricted to RNA synthesis was shown by ^{14}C-alanine and ^{14}C-leucine uptake and incorporation into protein. Maximum uptake of these amino acids again occurred at pH 5.6 or 6.0. Optimal pH of accumulation of arginine, methionine, threonine, glutamic acid, and aspartic acid was demonstrated around pH 5.0—6.0. In our experiment, ^{14}C-Leucine uptake into yeast cells appears highest level at pH 6.0, indicating the same result of glucose transport. This result means that both transport of sugar and amino acid into yeast cells is optimally at pH 6.0.

The effect of 2.4-dinitrophenol on accumulation of ^{14}C-methionine was observed for the asertainment of the active transport system, indicating that leucine transport system is associated with energy dependent in both stages of yeast. Same tendency for leucine uptake in vegetative and sporulated stages is also present in this experiment.

The multiplicity of the amino acid permeases in *Saccharomyces cerevisiae* was reported (Grenson, 1966) that the existence of a lysine-uptake system distinct from the arginine permease became apparant in the course of the study of arginine uptake. And also the other lysine-uptake system has a higher affinity for lysine. However, leucine accumulation of yeast cell in this experiment is higher during the sporulation period than in vegetative stage. This inclination suggests that leucine is more required for protein synthesis during spore formation.

It is interesting fact that the contents of methionine and tyrosine were presented in trace during the sporulaton stage, but glutamic acid was more required for protein synthesis.

적 요

Saccharomyces cerevisiae J170를 사용하여 포자형성기와 생장기를 택하여 DL-^{14}C-Leucine 운반에 관하여 비교 검토하였다. 효모세포의 leucine 흡수는 약 pH 6.0 부근에서 최대를 나타낸 것은 glucose 흡수와 동일한 결과이었다. 이것은 당과 아미노산의 운반은 어느 시기에서나 pH 6.0 근처가 최적임을 의미 한다. 또한 leucine 흡수는 포자형성기가 생장기보다 높은 것은 leucine이 포자형성에 필요함을 암시하고 있다. 2,4-dinitrophenol에 의하여 두시기 모두 leucine 흡수가 억제되는 현상을 볼수 있는데 이 leucine 흡수도 glucose 운반과 같이 energy를 요구하는 운반계와 연관되어 있음을 알았다. Km 값을 비교하여 보아도 포자형성기에 leucine을 보다 많이 필요로 함을 알수있었다. 아미노산의 분석결과 포자형성기에 단백질 합성에 주로 필요한 아미노산은 glutamic acid이었으며 반면에 포자형성기에 비교적 적은 양의 아미노산은 methionine과 tyrosine이었다.

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