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Physiological and Biochemical Studies on Germinating Fungal Spores. IV.[1] Accumulation of Glutamine and Its Origin in Germinating Conidia of *Cochliobolus miyabeanus*

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The compositional changes of free amino acids in germinating conidia of *Cochliobolus miyabeanus* and the origin of the predominant acid in the conidia were studied. Through all stages of germination, the predominant component in free amino acid pools of the conidia was glutamine. The experimental results on the metabolism of protein and carbohydrate in the conidia suggested that proteolytic products of alkali-soluble protein served as the nitrogen source for glutamine generation, while trehalose, which was consumed rapidly from the commencement of conidial germination, did not provide the carbon skeleton of the accumulated glutamine, but was catabolized to CO_2 through the hexose monophosphate pathway and the tricarboxylic acid cycle.

Keywords——glutamine; trehalose; fungal spores; conidia; germination; *Cochliobolus miyabeanus*; glycolytic pathway; tricarboxylic acid pathway; hexose monophosphate pathway

In general, the conidia are regarded as the reproductive organs of fungi, but although many reports have been presented on conidial germination,[3] the detailed mechanisms of this phenomenon remain to be elucidated. In the previous papers, the metabolism of conidial germination of *Cochliobolus miyabeanus* in distilled water was reported.[1] The data indicated that the trehalose stored in conidia was consumed as soon as germination was initiated (incubation time: 0—15 min), while RNA, fatty acids and free amino acids in the conidia were accumulated till the stage of germ-tube formation (incubation time: 0—90 min). Since these experiments were carried out in distilled water, free amino acids accumulated were not supplied from the medium, but were generated in the conidial cells. In this report, therefore, in order to clarify the metabolic pathways at the early stage of conidial germination, the chemical composition and biosynthesis of this accumulated amino acid fraction were studied.

Materials and Methods

Organism and Germinating Conditions——The harvesting procedure for conidia of *Cochliobolus miyabeanus* and the germinating conditions were as described previously.[1]

Incorporation of Glucose-U-^{14}C or Leucine-U-^{14}C into Germinating Conidia——To collect the $^{14}CO_2$ produced from germinating conidia, unless otherwise specified, a vessel fitted with a center well containing 0.3 ml of 2 N NaOH was employed. A mixture of 1 mM glucose (2.5 ml), 2% Tween 80 (1.0 ml), distilled water (10 ml) and conidia (50 mg) was placed in the main compartment of the vessel, which was closed with a rubber stopper and preincubated for 30 min in a 30° bath with shaking. Glucose-U-^{14}C (25 μCi) was then added and the incubation was continued for 15 min. In the case of leucine incorporation, leucine-U-^{14}C (20 μCi) was added without preincubation and incubated for the prescribed time. The germinating conidia were collected on a glass filter, washed with water till no radioactivity was found in the washings, lyophilized, fractionated and measured for radioactivity using a liquid scintillation spectrometer.

Generation of $^{14}CO_2$ by Conidia——The measurement of $^{14}CO_2$ generation from conidia incubated with glucose-1-^{14}C or glucose-6-^{14}C was performed in Warburg flasks. A mixture of distilled water (2.0 ml), 2%

1) Part III: H. Kuroda and M. Matsubara (née Tokuhiro), *Yakugaku Zasshi*, **88**, 308 (1968).
2) Location: *Motoyama-Kita-machi, Higashinada-ku, Kobe.*
3) R. Brambl, H. Wenzler, and M. Josephson, *J. Bacteriol.*, **135**, 311 (1978); M. Orlowski, and P. Sypherd, *ibid.*, 134, 76 (1978); J.C. Schmit, R.C. Fahey, and S. Brody, *Spore*, VI, 112 (1975); P.E. Mirkers, *J. Bacteriol.*, **117**, 196 (1974).

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Tween 80 (0.2 ml) and the conidia (10 mg) was placed in the main compartment, 1 N NaOH (0.15 ml) was placed in the center well, and 60% $HClO_4$ (0.2 ml) and glucose-1-^{14}C or glucose-6-^{14}C (3 μCi) were put separately in the two side arms. The entire reaction vessel was immersed in a water bath at 30° then the labeled glucose was poured into the conidial mixture, and incubation was continued for 15 min with shaking. The reaction was stopped by addition of 60% $HClO_4$ from the side arm, and after further incubation for 30 min, 0.1 ml of the alkaline solution contained in the center well was withdrawn for radioactivity measurement using the liquid scintillation spectrometer.

Fractionation——The lyophilized conidia were fractionated by the procedure shown in Chart 1.

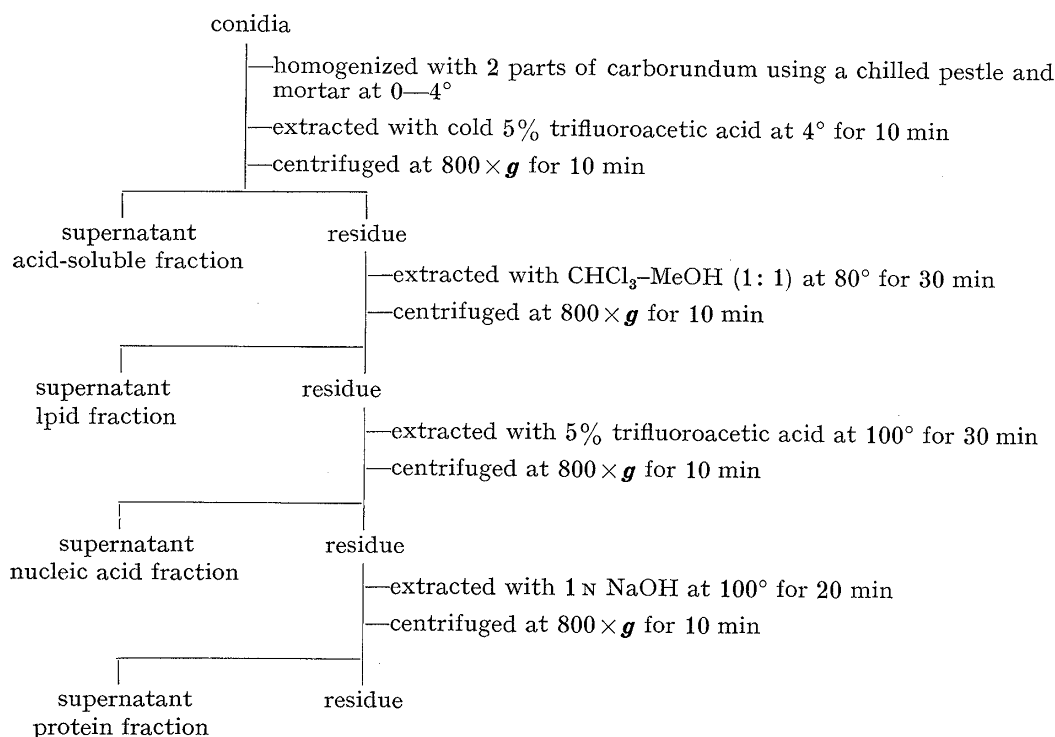

Chart 1. Fractionation of Conidial Components

The conidia were extracted successively with cold 5% trifluoroacetic acid (at 4° for 10 min), a mixture of $CHCl_3$–MeOH (1: 1, at 80° for 30 min), hot 5% trifluoroacetic acid (at 100° for 30 min) and 1 N NaOH (at 100° for 20 min). These extracts were designated as acid-soluble, lipid, nucleic acid and protein fractions, respectively.

Effects of Enzyme Inhibitors on the Accumulation of Glutamic Acid and Glutamine in Germinating Conidia——The conidia (300 mg) were incubated with distilled water (150 ml) in the presence or absence of NaF (120 μmol) or CH_2FCOOH (120 μmol) at 30° for 60 min with shaking. The germinating conidia were collected on a glass filter, washed throughly with distilled water and lyophilized. The acid-scluble fractions of these lyophilized conidia were subjected to quantitative analyses for glutamic acid and glutamine, using an amino acid autoanalyzer.

Thin Layer Chromatography (TLC)——Organic acids contained in the acid-soluble fraction were detected by TLC. An aliquot of the concentrated acid-soluble fraction was developed on a Kieselgel G plate (0.25 mm thick) using EtOH–H_2O–$CHCl_3$–28% NH_4OH (80: 12: 2: 6) or Et_2O–85% HCOOH–Me_2O–H_2O (12: 2: 1: 1) as a solvent, and the acids were detected with I_2 vapor or a radiochromatoscanner.

Analytical Methods——i) Amino Acids: Quantitative analysis of each amino acid contained in the acid-soluble fraction and the hydrolysate of the protein fraction was carried out with a Hitachi automatic recording amino acid analyzer, model KLA-3B, by the standard method. Since it was difficult to isolate glutamine clearly from other amino acids, the content of glutamine in the acid-soluble fraction was calculated from the difference of glutamic acid contents in the samples before and after hydrolysis with 2 N HCl for 30 min at 100°.

ii) Total Nitrogen and Protein: Total nitrogen was determined by the semimicro-Kjeldahl method and protein by the method of Lowry *et al.*[4]

4) O.H. Lowry, N.J. Rosenbrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

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iii) Measurement of Radioactivity: Radioactivity in an aqueous solution (0.1 ml) was determined in 10 ml of counting fluid of following composition: 2,5-diphenyloxazole (PPO, 1.6 g), 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (dimethyl POPOP, 1.6 g), naphthalene (150 g), methyl cellosolve (400 ml), toluene (up to 1000 ml) using a scintillation spectrometer (Fujitsu EA-118 scintillation counter). The radioactivity on paper or thin layer chromatograms was counted with a radiochromatoscanner (Aloka Co., JTC-202B).

Enzyme Assays——The conidia were homogenized with 2 parts of carborundum in an appropriate buffered solution using a chilled pestle and mortar, and the homogenate was centrifuged at $7000\times g$ for 20 min at 0—4°. The following enzyme activities of the supernatant were assayed by the established methods: protease,[5] aspartate aminotransferase (EC 2.6.1.1),[6] alanine aminotransferase (EC 2.6.1.2),[6] glutamate dehydrogenase (EC 1.4.1.4),[7] glutamine synthetase (EC 6.3.1.2),[8] citrate synthetase (EC 4.1.3.7),[9] isocitrate dehydrogenase (EC 1.1.1.41),[10] succinate dehydrogenase (EC 1.3.99.1),[11] malate dehydrogenase (EC 1.1.1.37),[12] glucokinase (EC 2.7.1.2),[13] glucosephosphate isomerase (EC 5.3.1.9),[13] phosphoglycerate kinase (EC 2.7.2.3),[12] phosphoglyceromutase (EC 2.7.5.3),[13] phosphoglucomutase (EC 2.7.5.1),[14] phosphofructokinase (EC 2.7.1.11),[15] fructosediphosphate aldolase (EC 4.1.2.7),[16] phosphopyruvate hydrotase (EC 4.2.1.11),[13] 6-phosphogluconate dehydrogenase (EC 1.1.1.44),[15] glucose-6-phosphate dehydrogenase (EC 1.1.1.49),[15] transketolase (EC 2.2.1.1),[15] and trehalase (EC 3.2.1.28).[17]

Results

Free Amino Acid Composition in Germinating Conidia

Fig. 1 shows the changes in the levels of free amino acids in the germinating conidia. Throughout all stages of germination, the glutamine content was highest among the amino acids examined; it rapidly increased during germ-tube formation (incubation time: 30—60 min). Although the pool sizes of other amino acids were fairly small, there were two types of fluctuation. Threonine, alanine and methionine reached the maximum levels at an early stage of germination (incubation time: 0—30 min), while serine, lysine, histidine and aspartic acid did not reach their maximum levels until the germ-tube formation was completed. Since the glutamine content was so extraordinarily high, the origin of the glutamine was investigated.

Nitrogen Source of Glutamine

Throughout all stages of germination, the total nitrogen content of conidia remained almost constant, while that of the alkali-soluble protein fraction of the conidia began to decrease as soon as the germination was initiated (Fig. 2). As shown in Fig. 3, the protein content of the alkali-soluble fraction decreased by an amount corresponding to the loss of nitrogen of the same fraction indicated in Fig. 2. Further, although the proteolytic enzyme activity in the ungerminated conidia was high, with the progress of germination, this activity became

5) S. Akabori, "Koso Kenkyuho," Vol. 1, Asakurashoten, Tokyo, 1956, p. 237.
6) M.K. Schwartz, "Methods in Enzymology," Vol. XVIIb, ed. by S.P. Colowick and N.O. Kaplan, Academic Press, New York and London, 1971, p. 866.
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17) N. O. Suza and A. D. Panek, *Archives of Biochemistry and Biophysics*, **125**, 22 (1968).

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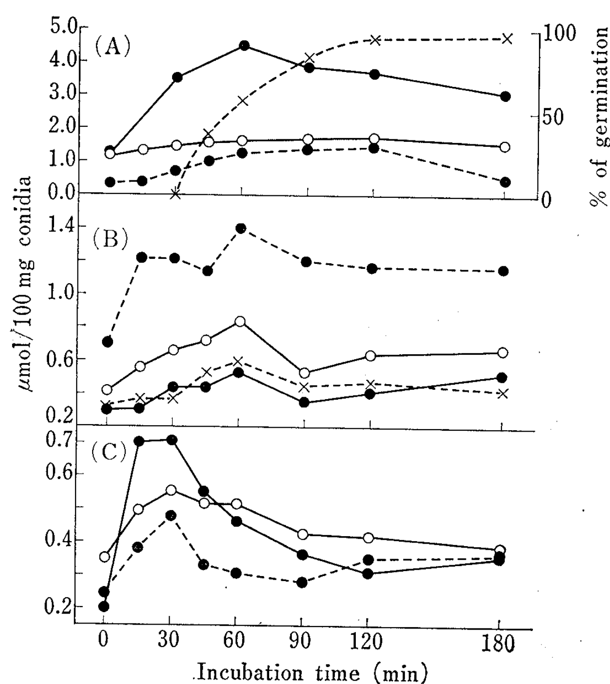

Fig. 1. Amino Acid Contents in Conidia at Different Stages of Germination

The free amino acids were extracted from conidia at different stages of germination with cold trifluoroacetic acid and analyzed with an amino acid analyzer. (A), —●—, glutamine; —○—, arginine; ---●---, glutamate; --×--, germination %, (B), ---●---, aspartate; —○—, serine; --×--, lysine; —●—, histidine; (C), —●—, alanine; —○—, methionine; ---●---, threonine. Contents of glycine, valine, leucine, isoleucine, tyrosine and phenylalanine were less than 0.2 μmol per 100 mg of conidia.

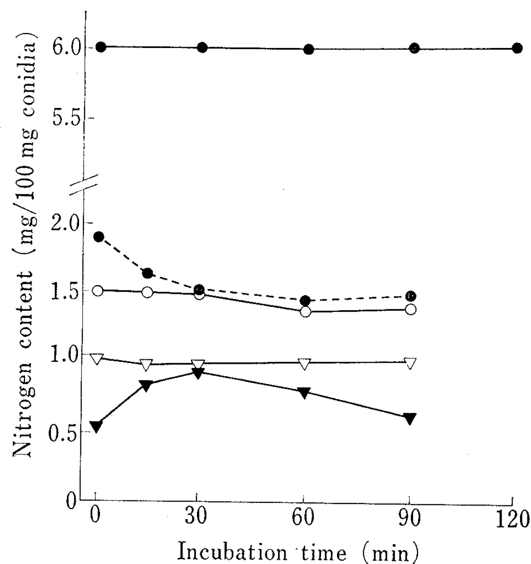

Fig. 2. Nitrogen Contents in Various Fractions of Germinating Conidia

Conidia at different stages of germination were fractionated as described in the text and the nitrogen content of each fraction was determined by the semimicro-Kjeldahl method.

—●—, total nitrogen; ---●---, alkali-soluble protein fraction; —○—, cell wall fraction;[a] —▽—, nucleic acid fraction; —▼—, acid-soluble fraction;

a) Disrupted conidia were washed with 0.9% NaCl and distilled water repeatedly, and insoluble material was harvested as the cell wall fraction by centrifugation.

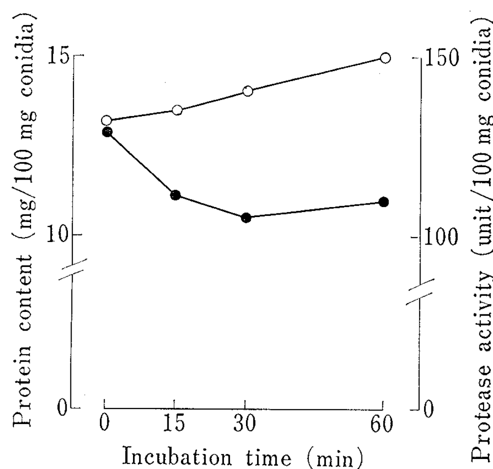

Fig. 3. Alkali-Soluble Protein Contents and Protease Activities in Conidia at Different Stages of Germination

Conidia at different stages of germination were divided into two portions. A portion was extracted with 1 N NaOH at 100° for 20 min and assayed for protein by Lowry's method. The rest was Subjected to assay for proteolytic enzyme activities as described in the text. —○—, protein; —●—, protease.

TABLE I. Amino Acid Compositions of Alkali-Soluble Protein in Conidia at Different Stages of Germination

Amino acid	Incubation time (min) 0	15
Aspartate	6.48[a]	4.68[a]
Glutamate	6.64	4.97
Glycine	6.20	4.70
Alanine	5.41	4.12
Leucine	4.60	3.40
Lysine	3.70	2.71
Valine	3.48	2.60
Arginine	1.24	0.84
Threonine	1.54	1.00
Serine	2.08	1.36
Proline	2.50	2.12
Tyrosine	0.90	0.80
Isoleucine	2.08	1.54
Phenylalanine	1.84	1.46
Methionine	0.42	0.34

a) μmol/100 mg conidia.

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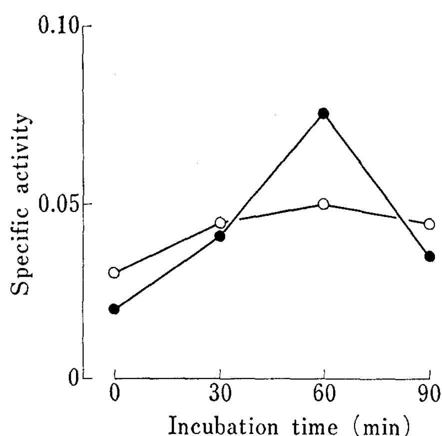

Fig. 4. Activities of Alanine Aminotransferase and Aspartate Aminotransferase in Conidia at Different Stages of Germination

Assay systems are described in the text.
—○—, Aspartate aminotransferase;
—●—, alanine aminotransferase.

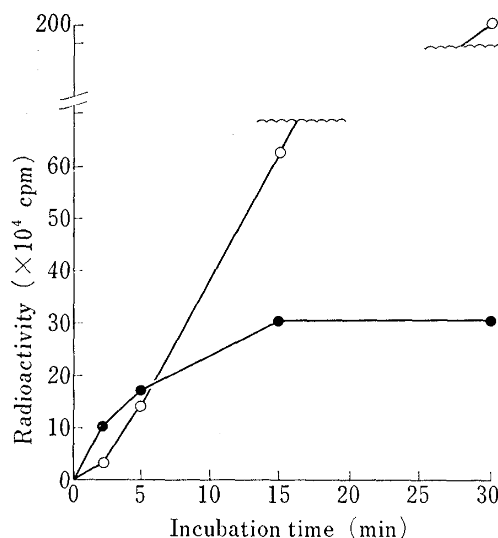

Fig. 5. Incorporation of Leucine-U-^{14}C into Germinating Conidia

Conidia (50 mg) were incubated with leucine-U-^{14}C (20 μCi) at 30° for the indicated times with shaking. After washing with distilled water, the conidia were fractionated and measured for radioactivity with a scintillation spectrometer.
—●—, acid-soluble fraction; —○—, alkali-soluble protein fraction.

even higher. The alkali-soluble protein was hydrolyzed with 6 N HCl at 105° for 20 hr in a sealed tube. As shown in Table I, although this fraction of ungerminated conidia was rich in aspartic acid, glutamic acid, glycine and alanine, the contents of these amino acids decreased remarkably during 15 min after the commencement of germination. The specific activities of aspartate aminotransferase and alanine aminotransferase of conidia are shown in Fig. 4. As the alkali-soluble protein content decreased during the germination, the aminotransferase activities increased.

Incorporation of Leucine-U-^{14}C into Alkali-Soluble Protein of Germinating Conidia

When ungerminated conidia were incubated with leucine-U-^{14}C, some radioactivity was found in the alkali-soluble fraction without any lag time and the amount increased rapidly up to 30 min (Fig. 5). These results (Figs. 2, 3, and 5) suggest that the synthesis and consumption of alkali-soluble protein are carried out simultaneously.

Carbon Skeleton Source of Glutamine

As reported in the previous paper,[1] the trehalose in ungerminated conidia was consumed rapidly (within 15 min after the commencement of germination) and Fig. 6 shows that the trehalase activity also increased at that time. These data suggest that the stored trehalose is hydrolyzed to glucose by trehalase, and that the carbon skeleton of glutamine may be derived from glucose through the tricarboxylic acid cycle. The activities of certain enzymes of the tricarboxylic acid cycle were detected at all stages of germination. On the other hand, a stepwise activation of glutamate dehydrogenase (linked with NADP) was observed, in parallel with the progress of germination, while glutamine synthetase was activated rapidly, within 30 min (Fig. 7). The incubated mixture of glutamic acid-U-^{14}C with the crude enzyme solution prepared from the conidia showed two radioactive spots on a paper chromatogram corresponding to those of authentic glutamic acid and glutamine (Fig. 8). The extract from the radioactive glutamine in Fig. 8a was hydrolyzed to glutamic acid as shown in Fig. 8b. Those results indicate the presence of glutamine synthetase in the conidia.

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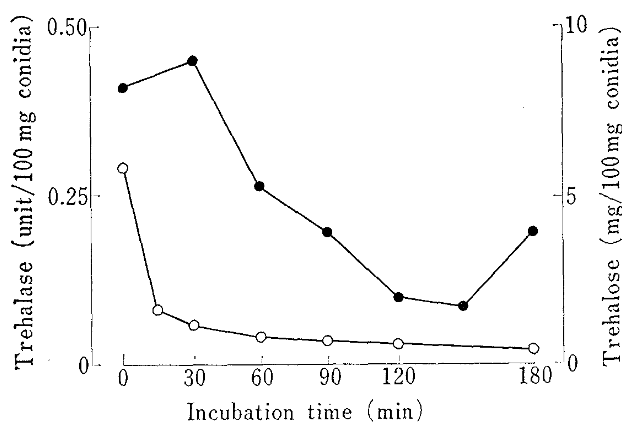

Fig. 6. Trehalase Activities and Trehalose Contents in Conidia at Different Stages of Germination

Conidia at different stages of germination were assayed for trehalase activity as described in the text. Trehalose contents were determined as described in previous paper.[1)]
—●—, trehalase; —○—, trehalose.

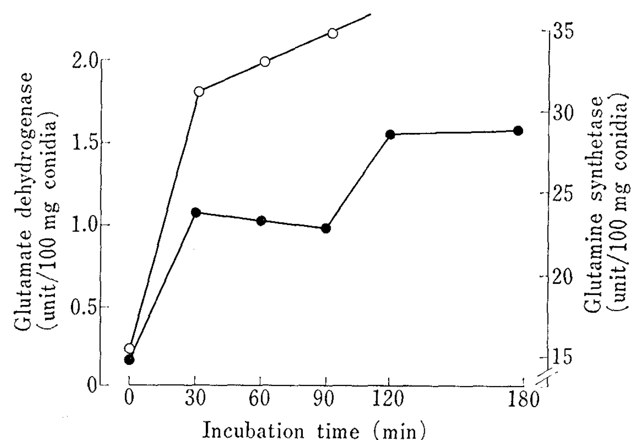

Fig. 7. Activities of Glutamate Dehydrogenase and Glutamine Synthetase in Conidia at Different Stages of Germination

Assay systems and procedures are described in the text.
—●—, glutamate dehydrogenase; —○—, glutamine synthetase.

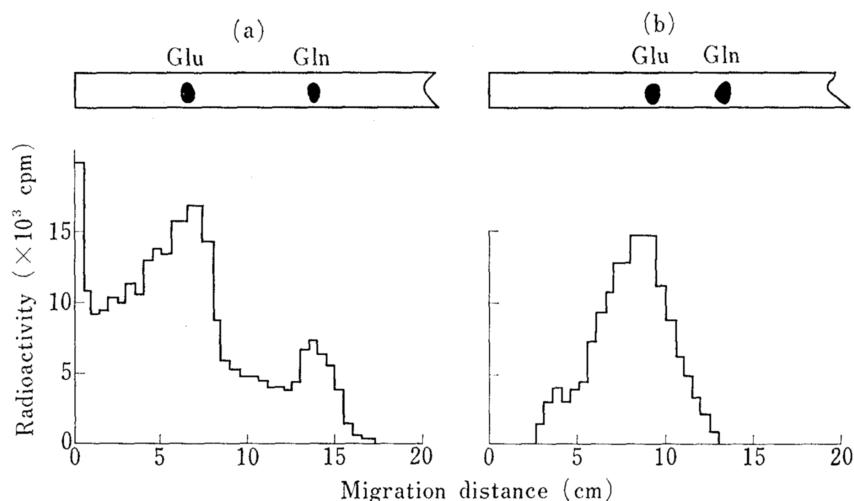

Fig. 8a, b. Paper Chromatography of Glutamine-^{14}C formed from Glutamate-^{14}C by Conidial Enzyme Solution

(a) The incubated mixture of glutamate-^{14}C with the conidial crude enzyme solution was concentrated and developed on Toyo Roshi No. 50 paper using phenol–H_2O (7: 3) mixture. The developed paper was cut into 0.5 cm widths and the radioactivity of each paper piece was determined.
(b) The extract from the radioactive area corresponding to that of glutamine in (a) was hydrolyzed with 2 N HCl at 100° for 2 hr and the hydrolysate was developed on paper under the condition described for (a).
Glu: authentic glutamate; Gln: authentic glutamine.

The fate of glucose-U-^{14}C incorporated into the conidia during 15 min after preincubation for 30 min is shown in Fig. 9. Most of the glucose-^{14}C was catabolized to $^{14}CO_2$. Some of the rest was converted to nucleic acid, cold acid-soluble matter and protein, but none appeared in the lipid fraction. The cold acid-soluble matter in Fig. 9 contained radioactive citrate, malate and fumarate, while radioactive glutamine was not detected (Fig. 10). The effects of additional NaF or CH_2FCOOH on germinating conidia are shown in Fig. 11. The amount of glutamine generated in the cold acid-soluble matter decreased markedly upon the addition of NaF, which is an inhibitor of glutamine synthetase (Fig. 11a). On the other hand, the addition of CH_2FCOOH (an inhibitor of aconitase) had no effect on glutamine generation (Fig. 11b).

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Metabolism of Trehalose

Since the results shown in Fig. 9 suggest that almost of all trehalose stored in conidia was decomposed to CO_2 through the tricarboxylic acid cycle, the activities of enzymes of the glycolytic pathway and the hexose monophosphate pathway were examined (Table II). Although there were different transitional patterns, the enzyme activities participating in both pathways were recognized in the conidia of all germinating stages. Some germinating conidia were withdrawn at intervals and the $^{14}CO_2$ radioactivity released from the conidia on further incubation with glucose-1-^{14}C or glucose-6-^{14}C for 15 min at 30° was assayed (Table III). As shown in Table III, the ratios of released $^{14}CO_2$ derived from ^{14}C-6 or ^{14}C-1 (C-6/C-1) were always less than 0.15 even in the case of conidia incubated for 24 hr. These results indicate that the stored trehalose is metabolized mainly by the hexose monophosphate pathway through all stages of conidial germination.

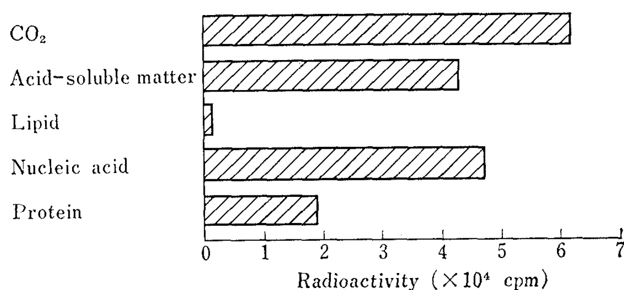

Fig. 9. Distribution of Radioactivity derived from Glucose-^{14}C in Various Fractions of Germinated Conidia

After preincubation for 30 min, the conidia were incubated with glucose-^{14}C for 15 min and fractionated as described in the text. The radioactivity of each fraction was measured with a liquid scintillation spectrometer.

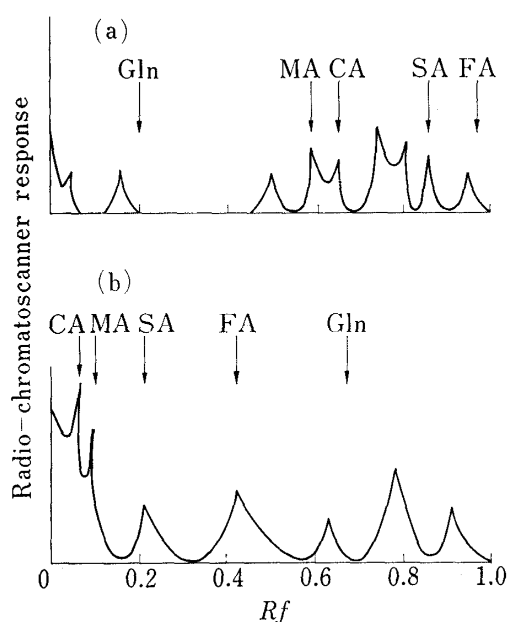

Fig. 10a, b. Radio Thin-Layer Chromatograms of the Acid-Soluble Fraction of Conidia Incubated with Glucose-^{14}C

The acid-soluble matter in Fig. 9 was concentrated and developed on a thin layer plate (Kieselgel G, 0.25 mm thick) using Et_2O-85% HCOOH-Me_2O-H_2O (12: 2: 1: 1, Fig. 10a) or EtOH-H_2O-$CHCl_3$-28% NH_4OH (8: 12: 2: 6, Fig. 10b) mixture as solvents. The radioactivities on developed plates were detected with a radio-chromatoscanner. Some authentic compounds were also developed under the same conditions and located with I_2 vapor.
Gln: glutamine; MA: malate; CA: citrate; SA: succinate; FA: fumarate.

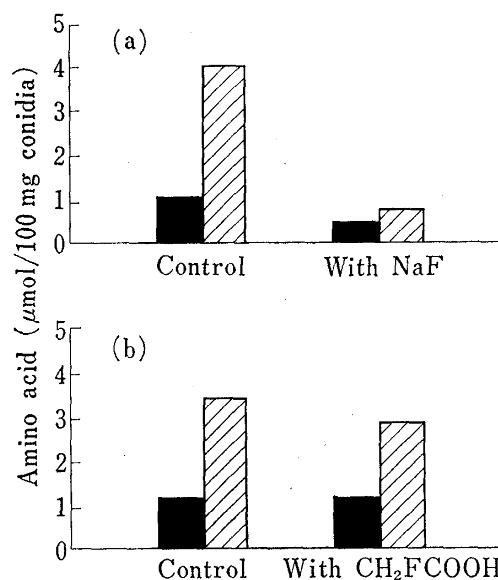

Fig. 11a, b. Effects of Enzyme Inhibitors on the Accumulation of Glutamic Acid and Glutamine in Germinating Conidia

Germinating conidia which had been incubated with 120 μmol NaF (a) or 120 μmol CH_2FCOOH (b) at 30° for 60 min were collected on a glass filter, washed with distilled water and lyophilized. The acid-soluble fractions of these lyophilized conidia were subjected to quantitative analyses for glutamic acid and glutamine using an amino acid autoanalyzer.
■: glutamic acid; ▨: glutamine.

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Table II. Changes of Specific Activities of Enzymes Related to the Glycolytic and Hexose Monophosphate Pathways in Germinating Conidia

Enzyme	Specific activity[a] of crude enzyme solution obtained from conidia incubated for (min)			
	0	30	60	90
Glucokinase	20.16	20.16	19.29	19.29
Glucosephosphate isomerase	292.3	298.2	400.6	367.9
Phosphoglycerate kinase	46.9	64.0	64.0	62.0
Phosphoglyceromutase	257.1	262.1	270.5	247.0
Phosphoglucomutase	0.042	0.036	0.037	0.031
Phosphofructokinase	127.5	131.1	131.6	131.1
Fructosediphosphate aldolase	332.7	322.6	322.6	272.2
Phosphopyruvate hydratase	173.8	209.2	244.5	189.0
Glucose-6-phosphate dehydrogenase	201.6	247.0	223.7	186.5
6-Phosphogluconate dehydrogenase	23.9	36.5	39.0	26.5
Transketolase	25.7	35.8	37.5	37.4

a) Specific activity is expressed as units per mg of protein.

Table III. Release of $^{14}CO_2$ from Glucose-1-^{14}C or Glucose-6-^{14}C by Conidia at Different Stages of Germination

Incubation time (min)	Labeled C of glucose	Radioactivity of $^{14}CO_2$ released by 10 mg of conidia for 15 min (cpm)	Ratio of $^{14}CO_2$ released from C-6 and C-1 (C-6/C-1)
15	C-1	23738	
	C-6	2950	0.124
30	C-1	14107	
	C-6	1772	0.126
60	C-1	11632	
	C-6	1460	0.126
90	C-1	13628	
	C-6	1653	0.121
24[a]	C-1	165487	
	C-6	25553	0.154

a) hr.

Discussion

The main component of the free amino acid fraction in germinating conidia was glutamine, and the variation in the quantity of this fraction was dominated by that in the quantity of glutamine. In contrast to this fungus, in the case of *Neurospora crassa*, Schmit and Brody[18] reported that the main component of the free amino acid pool in conidia was not glutamine but glutamic acid, and they found that this amino acid was exhausted rapidly, within 1 hr after the commencement of germination. As shown in Fig. 1, no free amino acid or amide (including inorganic ammonium ions) decreased in amount sufficiently to counterbalance the glutamine accumulation in conidial germinating processes, and Figs. 2 and 3 show that reduction in the quantity of alkali-soluble protein occurred with germination. Furthermore, high proteolytic enzyme activities were detected in ungerminated conidia (Fig. 3). The results of amino acid analyses on hydrolysates of soluble protein fractions obtained from germinating

18) J.C. Schmit and S. Brody, *J. Bacteriol.*, **124**, 232 (1975).

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conidia showed that the quantities of aspartic acid, glutamic acid or glutamine, glycine and alanine were greatly reduced after germination, while with the exception of glutamine, no amino acid was accumulated in the free amino acid pool of the conidia. These results suggest that the amino acids which are generated from alkali-soluble protein by proteolytic enzymes are metabolized actively and that their nitrogen is utilized for glutamine synthesis. Indeed, the activities of some transaminases in the conidia were high at the time when a rapid decrease of alkali-soluble protein was observed (Fig. 4). A decrease of protein was detected at an early stage of germination by Gottlieb,[19] but on the other hand, Mirkes[20] and Bhagwat[21] reported that radioactive amino acids were incorporated into the protein fraction in conidia of *Neurospora crassa* within several minutes after the commencement of germination. In the case of our fungus, when the conidia were incubated with leucine-U-^{14}C, some radioactivity was found in the alkali-soluble protein fraction of the conidia within 5 min after the commencement of germination, and the level increased with time up to 30 min (Fig. 5). These results suggest that active biosynthesis and hydrolysis of protein occur simultaneously in the conidia at an early stage of germination. On the other hand, as described in the previous paper,[1] the stored trehalose in ungerminated conidia was consumed within 15 min after the commencement of germination and at that time, the activity of trehalase in conidia was raised (Fig. 6). These results and the experiments shown in Figs. 7 and 8 suggest that glucose generated from trehalose participates in the formation of the carbon skeleton of glutamine through the tricarboxylic acid cycle. However, the expected high level of radioactivity was not detected in glutamine which was extracted from conidia incubated with glucose-^{14}C (Fig. 10). Furthermore, as shown in Fig. 11, the inhibition of glutamine formation from glutamic acid by addition of NaF led to little glutamine accumulation in free amino acid pools of conidia, while the inhibition of 2-oxoglutaric acid formation through the tricarboxylic acid cycle by addition of CH_2-FCOOH did not greatly affected the glutamine accumulation. These results indicate that the carbon skeleton of glutamine accumulated in germinating conidia is not directly derived from the catabolite of trehalose. On the other hand, the stored trehalose, which was consumed rapidly from the commencement of conidial germination, is probably catabolized to CO_2 through the tricarboxylic acid cycle, because the germinating conidia generated much $^{14}CO_2$ and some radioactive organic acids related to the tricarboxylic acid cycle on incubation with glucose-^{14}C (Figs. 9 and 10). Tables II and III show that the conidia at all stages of the germination processes (from the early stage of germination to the germ-tube elongation stage) catabolized exogenous glucose consistently by the hexose monophosphate pathway. As regards glucose catabolism by microorganisms, Budd *el al.*[22] indicated that *Neurospora tetrasperma* produced $^{14}CO_2$ by catabolizing glucose-^{14}C, and Patni[13] demonstrated the presence of active enzymes related to the glycolytic pathway in cell-free extracts of *Clostridium themocellum*. Kottel[23] reported that *Microcyclus fiavus* catabolized glucose through the glycolytic pathway. Smith[14] claimed that glucose is catabolized by the hexose monophosphate pathway in young nonsporulating mycelia of *Aspergillus niger*, while in sporulating mycelia metabolism is by the glycolytic pathway. These results suggest that the stored trehalose in conidia is hydrolyzed to glucose rapidly by trehalase on the commencement of germination and then the glucose is metabolized through the hexose monophosphate pathway. The ribose which is generated through this pathway might be utillized for nucleic acid synthesis. The pathway by which the carbon skeleton of glutamine (2-oxoglutaric acid) is produced is under investigation.

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