RELATION BETWEEN UPTAKE OF α-AMINOISOBUTYRIC ACID AND ORGANIC ACIDS METABOLISM BY *PIRICULARIA ORYZAE*

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Introduction

It remains unclear how metabolic energy is coupled to the active transport. Recently, the coupling of an active transport to specific enzyme activity was reported[1,12,16,23,24,26].

It has been shown by ROSEMAN[23] that galactose transport system was mediated by phosphoenolpyruvate phosphotransferase system.

KABACK[1,12] demonstrated that uptake of amino acids and sugars by membrane vesicles of *Escherichia coli* was coupled primarily to D-lactate dehydrogenase. STERN et al[24] proposed that oxalacetate decarboxylase functions as a carrier protein in the uptake of citrate by *Aerobacter aerogenes.*

More recently, KABACK et al[26] reported that a concentrative uptake of amino acids by membrane vesicles of *Staphylococcus aureus* is mediated by L-α-glycerolphosphate dehydrogenase system, and postulated further that "the carrier protein" may be electron transfer intermediates.

Most recently, KONINGS and FREESE[16] have presented the report that active transport of amino acids by membranes prepared from *Bacillus subtilis* is coupled to the oxidation of physiological electron donor and that ascorbate-phenazine methosulfate, an artificial electron donor, is also a very effective electron donor which stimulates amino acids transport. The positive effect of this artificial electron donor for active transport by membrane vesicles was also reported by several researchers[8,17].

NIVEN and HAMILTON[20] demonstrated that the driving force for the active transport of amino acids by *Staphylococcus aureus* is in parallel with the oxidoreduction reaction of electron transport chain and the hydrodehydration reaction of the membrane-bound adenosine triphosphatase. But it has been known[14,16] that added ATP fails to serve as an energy source

for active transport and that ATP production does not couple to active transport.

In the investigations of an energy coupling mechanism for an active transport, it seems to be important to work with intact cells as well as with membrane vesicles. In this respect, it seems to be of great advantage for this purpose to employ *P. oryzae* that is known to possess more endogeneous substrates[19]. Also in this laboratory, it has been known already that the uptake of α-aminoisobutyric acid (α-AIB) and other amino acids by *P. oryzae* is dependent on metabolic energy, in consideration of an increase in the rate of respiration and being poisoned by respiratory inhibitors[11].

Present paper describes studies on the relation between energy-dependent uptake of α-AIB, L-valine, L-aspartate and L-lysine and organic acids metabolism by *P. oryzae*.

From the data obtained, it was concluded that oxidoreduction reaction of organic acids involved in Krebs cycle is coupling to the energy-dependent uptake of amino acids by *P. oryzae*.

Materials and Methods

Preparation of mycelia

Piricularia oryzae No. 1[25] were cultured under shaking for 6 to 7 days at 27°C in a 500 ml Erlenmeyer's flask containing 100 ml of synthetic medium[25]. Mycelia grown were treated with blender for about 40 seconds, and washed with sterilized water. The mycelia obtained were again incubated under shaking for about one day at 27°C in a medium of 100 ml of 0.1 M phosphate buffer (pH 7.0) containing 1.5% sucrose, and washed again with water and further incubated under shaking for another one day with 0.1 M phosphate buffer (pH 7.0). The resting cells were harvested, washed with water once and resuspended in 60 to 80 ml of the same buffer.

Measurements of consumption of oxygen and evolution of carbon dioxide

These were carried out manometrically with Warburg's apparatus.

Measurement of uptake of amino acids

After 5.6 ml of mycelia suspension (30 to 40 mg as dry weight) in 0.1 M phosphate buffer (pH 7.0) in 50 ml Erlenmeyer's flask was kept for 120 min at 25°C under reciprocal shaking, the uptake was initiated by the addition of 0.4 ml of 0.1 M phosphate buffer containing each amino acid (6 μ moles

or 12 μ moles).

At appropriate intervals, an aliquot of the filtrate of the incubation mixture was applied for the determination of residual amino acid.

The determination of α-AIB was made colorimetrically according to the method described previously[11].

L-Valine, L-aspartate and L-lysine were determined colorimetrically according to the ninhydrin method.

Identification of organic acids

The suspension of 2700 g (wet weight) of mycelia in 0.1 N sodium hydroxide solution was frozen with dryice–methanol. After thawing, the filtrate obtained was concentrated, acidified with sulfuric acid solution and absorbed on a dried silica gel. Organic acids fraction was obtained by extraction with water-saturated ether.

The column chromatography for the separation of organic acids was carried out as described by KINNORY[15]. Organic acids were detected by a measurement of electric conductivity.

Identification of organic acids was performed by paper chromatography, thin layer chromatography and infrared absorption spectroscopy.

Paper chromatography was run on Toyo No. 50 filter paper with 95% ethanol–conc. ammonia (100 : 1, v/v) and n-butanol–water–diethylamine (100 : 15 : 1, v/v) for a volatile organic acid.

Thin layer chromatography was carried out on Kiesel gel H with ether (saturated with water)–formic acid (7 : 1, v/v) and propanol–28% ammonia (7 : 3, v/v) for non-volatile organic acids.

Determination of each organic acid

L-Malate was determined with malate dehydrogenase and NAD as described by HOHORST[9]. Fumarate was determined by the modification of the method published by WILLIAMSON[29]. In this case, the assay mixture consists of 0.45 ml of 3 M Tris-HCl–0.4 M hydrazine buffer (pH 9.5) containing 5 mM EDTA, 0.05 ml of 50 mM NAD, 0.5 ml of sample, 0.01 ml of malate dehydrogenase (10 units) and 0.01 ml of fumarase (3.5 units). The determination of citrate was carried out according to the method presented by DAGLEY[4]. The determination of succinate was carried out according to the method described by VEEGER[27]. D-Lactate was determined with D-lactate dehydrogenase by utilizing the method for L-lactate determination as described by HOHORST[10]. L-Lactate was determined with cytochrome b_2 and cytochrome c as reported by BOERI[3]. Acetate was determined according to the method of BERGMEYER[2].

Chemicals

Succinate dehydrogenase was prepared from a bovine heart muscle as described by VEEGER[28].

The method described by HASEGAWA et al[7] was used for the preparation of cytochrome b_2 from Baker's yeast.

Acetokinase was prepared from *Escherichia coli* K-12 by the modification of the methods published by KALMAN[13] and by ROSE[22].

Malate dehydrogenase, fumarase, D- and L-lactate dehydrogenase, citrate lyase, phosphotransacetylase, citrate synthase and cytochrome c were purchased from Boehringer Mannheim.

Results

Uptake of α-AIB and respiratory increase

MORQUIS[18] reported that the more oxygen uptake increased, the more α-AIB was transported in *Bacillus megaterium*. RING et al[21] described that oxygen consumption increased when α-AIB was transported in *Streptomyces hydrogenans*.

As reported already, α-AIB was transported and accumulated in *Piricularia oryzae*, and α-AIB transported was hardly metabolized[11].

As illustrated in Fig. 1, an increase in respiration was observed during the uptake of α-AIB. The rate of respiration during the uptake of α-AIB is higher about three times than that in the absence of α-AIB. An increase in carbon dioxide evolution was in parallel with that in oxygen uptake. After α-AIB was completely transported in *P. oryzae*, the rate of respiration was much the same as in the absence of α-AIB.

It was also observed in present experiments that an amount of α-AIB transported was dependent on the extent of increase in oxygen consumption and an increase in the rate of respiration was independent of the initial amount of α-AIB in the experimental conditions.

As about six hours are required for the induction of α-AIB decomposing enzyme in *P. oryzae*[11], it is reasonable to consider that the increased respiration being accompanied by the uptake of α-AIB directly reflects the requirement of energy for a transport itself.

Furthermore, it is possible to assume that an increased evolution of carbon dioxide might not be due to the decarboxylation of endogeneous amino acids or other compounds but to the oxidation of organic acids in this organism.

From this assumption, it was examined if the contents of organic acids in mycelial cells would change during the uptake of α-AIB.

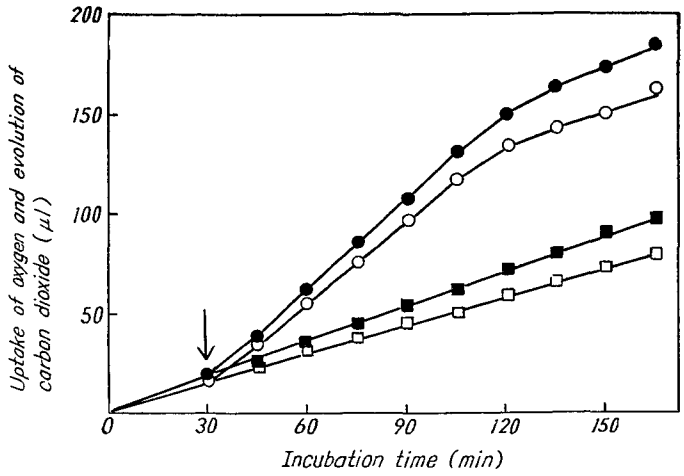

Fig. 1. Effect of α-AIB upon Respiration

After 2 ml of mycelia suspension (13 mg as dry weight) in 0.1 M phosphate buffer (pH 4.5) was preincubated for 120 min at 25°C, the uptake of O_2 and evolution of CO_2 were followed manometrically before and after the addition of α-AIB (3 μ moles) in total volume of 3.2 ml at 25°C. Arrow indicates the addition of α-AIB.

■—■, control of O_2 uptake
□—□, control of evolution of CO_2
●—●, O_2 uptake in the presence of α-AIB
○—○, CO_2 evolution in the presence of α-AIB

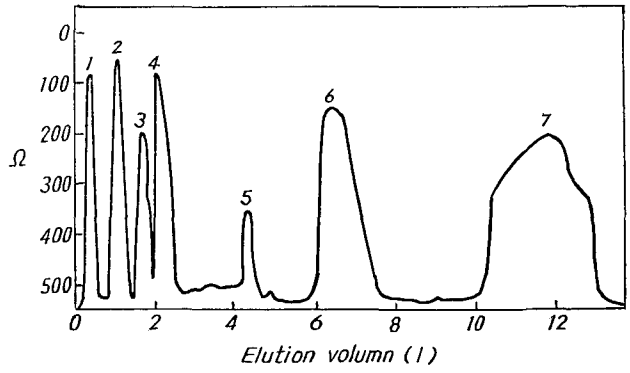

Fig. 2. Elution Pattern of Organic Acids in Column Chromatography

Silica gel (over 100 mesh) suspended in chloroform was packed into a column of 3.0×70 cm. Elution was performed with benzene-water saturated ether. Peak 1 was identified to be acetic acid, peak 2 to be fumaric acid, peak 3 to be succinic acid, peak 4 to be D- and L-lactic acid, peak 6 to be malic acid and peak 7 to be citric acid. Peak 5 could not be identified.

Identification of organic acids in *P. oryzae*

The elution pattern of organic acids in column chromatography is shown in Fig. 2.

Acetic acid, fumaric acid, succinic acid, D- and L-lactic acid, malic acid and citric acid were identified to be each from the results of paper chromatography, thin layer chromatography and infrared absorption spectra. Distinction of D- or L-form of lactic acid was made by the enzymatic method.

Content of each organic acid in *P. oryzae*

Some organic acids are expected to be hardly detectable by the blender and resting treatments.

In order to examine this possibility, the content of each organic acid was measured.

The treated mycelia were prepared by incubation with 0.1 M phosphate buffer (pH 7.0) as shown in "Materials and Methods". And the untreated mycelia were obtained by incubation with the complete medium instead of 0.1 M phosphate buffer (pH 7.0) for two days at 27°C.

As summarized in Table 1, contents of fumarate and malate slightly increased with these treatments. The ratio of malate to fumarate, about four, did not change in spite of these treatments. This value is similar to

TABLE 1. Content of Each Organic Acid in *P. oryzae*

Organic Acids	Content of Organic Acid n moles/mg dry mycelium	
	Intact Mycelia	Treated Mycelia
L-Lactate	1.3	1.5
D-Lactate	15.0	0.5
Acetate	9.2	2.0
Citrate	41.1	19.9
Succinate	6.2	2.8
Fumarate	3.9	5.4
Malate	14.5	18.6

The treated mycelia were prepared by blender treatment and washing with sterilized water and by successive incubation with 0.1 M phosphate buffer (pH 7.0) containing 1.5% sucrose for one day at 27°C, and further, by incubation with 0.1 M phosphate buffer (pH 7.0) for another one day.

Intact mycelia were prepared by incubation with the culture medium[25] for two days at 27°C instead of 0.1 M phosphate buffer (pH 7.0).

the equilibrium constant of fumarase.

On the other hand, the marked decrease of contents of citrate and succinate was observed with these treatments, and the proportion between citrate and succinate remained constant independent of these treatments. But the marked difference was observed in the ratio of the content of succinate to that of fumarate between the rested mycelia and the intact ones.

From these results, it seems that contents of citrate and succinate would relatively fall down and those of fumarate and malate relatively rise up, when mycelia are brought to the state lacking nourishment.

No change was observed in the content of L-lactate inspite of these treatment.

But the content of D-lactate markedly decreased and this acid could hardly be detected in the treated mycelia. This phenomenon is very interesting, but not attainable to be explained at present.

The similar went with acetate.

Effect of uptake of α-AIB on change of content of each organic acid

The procedure for the determination of each organic acid was described in "Materials and Methods".

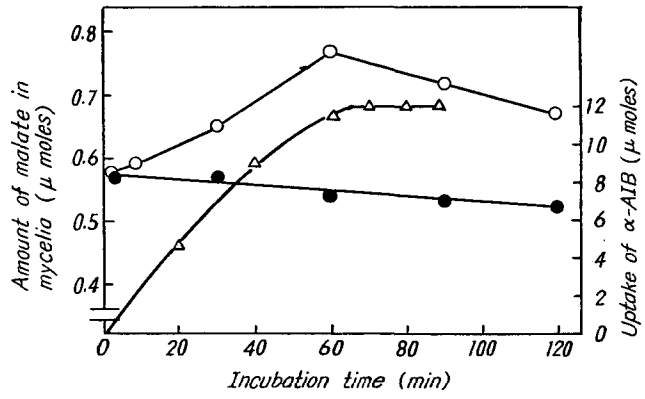

Fig. 3. Change in Content of Malate due to Uptake of α-AIB

After each 50 ml Erlenmeyer's flask containing mycelia suspension (36 mg as dry weight) in 0.1 M phosphate buffer (pH 7.0) was kept for 120 min at 25°C under shaking, α-AIB uptake was initiated by the addition of 12 μ moles α-AIB, and terminated by freezing with dryice-methanol at appropriate intervals.

○—○, the content of malate in the presence of α-AIB
●—●, the content of malate in the absence of α-AIB
△—△, uptake of α-AIB

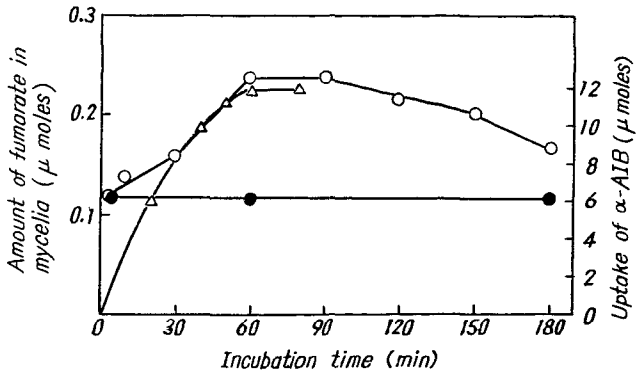

Fig. 4. Change in Content of Fumarate in Mycelia due to Uptake of α-AIB

Incubation conditions were the same as in Fig. 3, except that 34 mg of mycelia (as dry weight) was used.

○—○, the content of fumarate in the presence of α-AIB
●—●, the content of fumarate in the absence of α-AIB
△—△, uptake of α-AIB

The uptake was initiated by the addition of α-AIB to the mycelia suspension in 0.1 M phosphate buffer (pH 7.0), and terminated by freezing with dryice–methanol at appropriate intervals. After the addition of 2 ml of 20% perchloric acid and successive thawing, precipitates were removed by centrifugation, and supernatant was neutralized with conc. potassium hydroxide solution in ice bath. A portion of the neutralized supernatant was used for a determination of each organic acid.

The effect of the uptake of α-AIB on the content of malate in mycelial cells is shown in Fig. 3. The content of malate increased during the uptake of α-AIB and began to fall down when all α-AIB was transported in mycelial cells. The increasing shape of the curve obtained with malate resembles that of the curve of respiration with α-AIB uptake. (Fig. 1)

The similar results were obtained in the case of fumarate. (Fig. 4)

As illustrated in Fig. 5, the content of citrate decreased during the uptake of α-AIB, and began to rise up when the uptake of α-AIB was completed.

Data obtained with succinate (Fig. 6) were very similar to those with citrate.

From the data in Figs. 1, 3, 4, 5 and 6, it was suggested that the oxidation of organic acids existing on Krebs cycle is necessary for the uptake of α-AIB by *P. oryzae*.

On the other hand, no change of the content of L-lactate was observed during the uptake of α-AIB (Table 2), which suggests that L-lactate is unconcerned with the uptake of α-AIB.

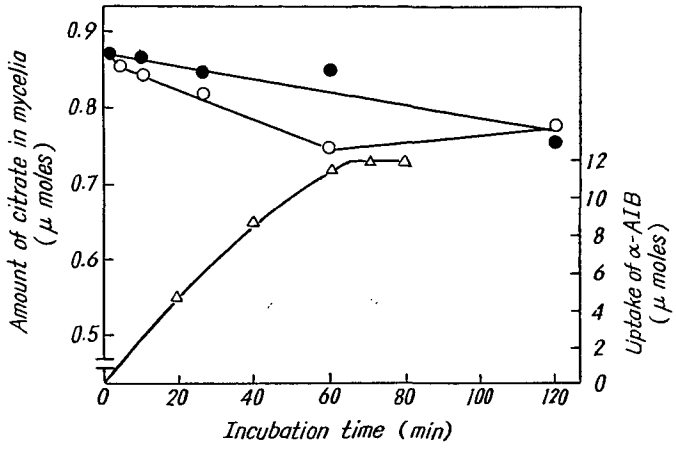

Fig. 5. Change in Content of Citrate in Mycelia due to Uptake of α-AIB

Incubation conditions were the same as in Fig. 3, except that 35 mg of mycelia (as dry weight) was used.

○—○, the content of citrate in the presence of α-AIB
●—●, the content of citrate in the absence of α-AIB
△—△, uptake of α-AIB

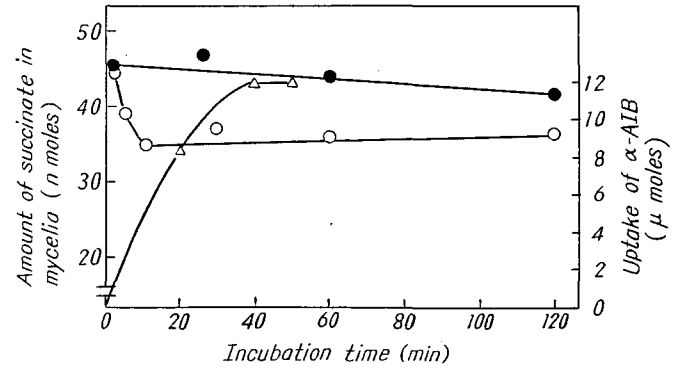

Fig. 6. Change in Content of Succinate in Mycelia due to Uptake of α-AIB

Incubation conditions were the same as in Fig. 3, except that 34 mg of mycelia (as dry weight) was used.

○—○, the content of succinate in the presence of α-AIB
●—●, the content of succinate in the absence of α-AIB
△—△, uptake of α-AIB

TABLE 2. Effect of α-AIB Uptake on Amount of L-Lactate in Mycelia

Incubation Time (min)	Amount of L-Lactate in Mycelia (n moles)		Uptake of α-AIB (μ moles)
	Control	Addition of α-AIB	
0	43.5	43.5	0
30	37.7	38.5	5.3
60	40.2	36.3	9.8
90	—	40.2	12.0
120	38.4	37.7	12.0
180	38.5	38.5	12.0

Incubation conditions were the same as in Fig. 3, except that 30 mg of mycelia (as dry weight) was used.

Effect of addition of D-lactate or acetate on the rate of uptake of α-AIB

As is known from Table 1, D-lactate was found in intact mycelial cells, but its markedly decreased with the blender and resting treatments, and D-lactate could not be detected in mycelia in the uptake experiments.

If the oxidation of D-lactate is coupled with the supply of energy for the uptake of α-AIB, it may be expected that the rate of uptake of α-AIB would be stimulated by the addition of D-lactate. But this was not the case, as is known from Table 3.

Concerning the role of acetate, the same results as in D-lactate were obtained (Table 3).

Effect of uptake of different amount of α-AIB on the extent of change of content of each organic acid

As described already in this paper, the extent of the increase in O_2 uptake and in CO_2 evolution depended on the amount of α-AIB transported. In addition, the extent of the increase in O_2 uptake and in CO_2 evolution was independent of the weight of mycelial cells in the presence of the equal amount of α-AIB.

Accordingly, if an increase of evolution of carbon dioxide by the uptake of α-AIB is assumed to be solely due to the oxidation of organic acids, the extent of change of the content of each organic acid would depend on the amount of α-AIB transported.

As shown in Fig. 7, the extent of an increase in the content of malate

TABLE 3. Effect of D-Lactate or Acetate on Rate of Uptake of α-AIB by Mycelia of *P. oryzae*

(A)

Incubation Time (min)	Uptake of α-AIB (μ moles)	
	None	Addition of D-Lactate
0	0	0
20	5.6	5.8
30	8.3	8.2
40	9.9	10.0
50	12.0	12.0
70	12.0	12.0

(B)

Incubation Time (min)	Uptake of α-AIB (μ moles)	
	None	Addition of Acetate
0	0	0
20	5.3	5.3
30	7.6	7.7
40	9.7	9.5
60	12.0	12.0
80	12.0	12.0

The reaction media containing mycelia suspension in 0.1 M phosphate buffer (pH 7.0) and 10 μ moles of D-lactate (A) or 5 μ moles of acetate (B) were incubated under reciprocal shaking for 60 min at 25°C, and the uptake was started by the addition of 12 μ moles of α-AIB. The uptake of α-AIB was measured at indicated time. Thirty seven mg of mycelia for (A) and 35 mg for (B) were used, respectively.

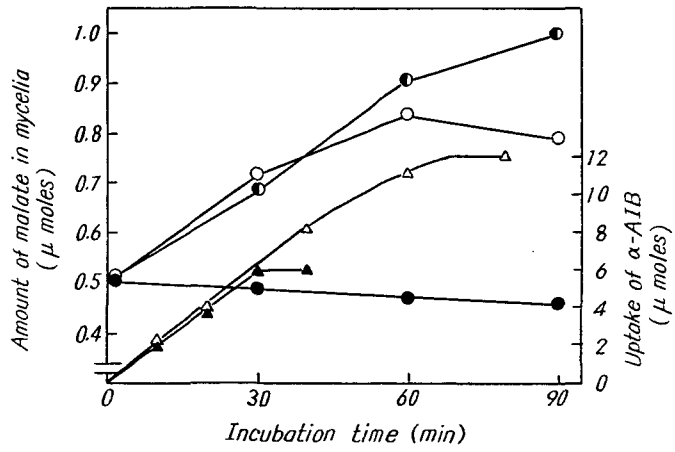

Fig. 7. Change in Content of Malate in Mycelia due to Uptake of Different Amount of α-AIB

Incubation conditions were the same as in Fig. 3, except that 31 mg of mycelia (as dry weight) was used.

◐—◐, the content of malate due to 12 μ moles of α-AIB
○—○, the content of malate due to 6 μ moles of α-AIB
●—●, the content of malate in the absence of α-AIB
△—△, uptake of α-AIB (12 μ moles)
▲—▲, uptake of α-AIB (6 μ moles)

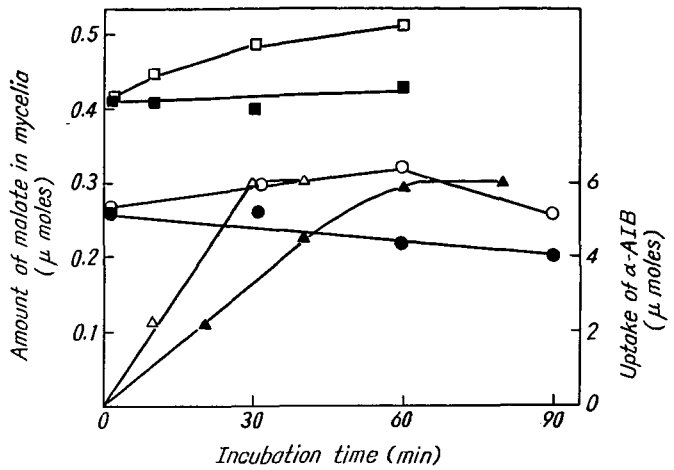

Fig. 8. Change in Contents of Malate due to Uptake of α-AIB by Different Amount of Mycelia

Incubation conditions were the same as in Fig. 3. Thirty two mg or 16 mg of mycelia (as dry weight) and 6 μ moles of α-AIB were used.

□—□, the content of malate in the presence of α-AIB in 32 mg of mycelia
○—○, the content of malate in the presence of α-AIB in 16 mg of mycelia
■—■, the content of malate in the absence of α-AIB in 32 mg of mycelia
●—●, the content of malate in the absence of α-AIB in 16 mg of mycelia
△—△, uptake of α-AIB by 32 mg of mycelia
▲—▲, uptake of α-AIB by 16 mg of mycelia

due to the uptake of 12 μ moles of α-AIB was greater than that due to the uptake of 6 μ moles of α-AIB.

The similar result was obtained in the case of fumarate. (Data are not shown)

Fig. 8 shows the relation between α-AIB uptake by the different amount of mycelial cells and an increase in the content of malate. The reaction mixture contained 6 μ moles of α-AIB, and 16 mg or 32 mg (as dry weight) of mycelial cells. When equal amount of α-AIB was completely transported, the extent of an increase in the content of malate was equal to each other in spite of the different amount of mycelial cells used.

Effect of each organic acid on the rate of uptake of α-AIB

Data in Figs. 3, 4, 5 and 6 suggest the coupling of the oxidation of these organic acids with the uptake of α-AIB. Thus, it is expected that the addition of each organic acid would stimulate the rate of the uptake of α-AIB.

After the reaction mixture containing mycelia suspension in 0.1 M

TABLE 4. Effect of Addition of Each Organic Acid on Rate of Uptake of α-AIB by Mycelia of *P. oryzae*

Incubation Time (min)	(A) Succinic Acid		(B) Citric Acid		(C) Fumaric Acid		(D) Malic Acid	
	Uptake of α-AIB (μ moles)		Uptake of α-AIB (μ moles)		Uptake of α-AIB (μ moles)		Uptake of α-AIB (μ moles)	
	Control	Addition	Control	Addition	Control	Addition	Control	Addition
0	0	0	0	0	0	0	0	0
10	1.7	1.5	1.9	1.7	1.8	1.7	2.4	0.7
20	3.3	3.4	4.2	4.3	3.6	3.3	4.5	3.6
40	6.6	6.7	8.4	8.1	7.3	7.1	8.4	7.3
60	9.1	9.1	10.6	10.8	9.5	9.5	10.8	9.7
80	11.3	11.1	12.0	12.0	10.6	11.1	12.0	11.1

Experimental conditions were the same as in Table 3. Five μ moles of each organic acid were added. The uptake of α-AIB was measured at indicated time. Thirty mg of mycelia for (A), 32 mg for (B), 33 mg for (C) and 36 mg for (D) were used, respectively.

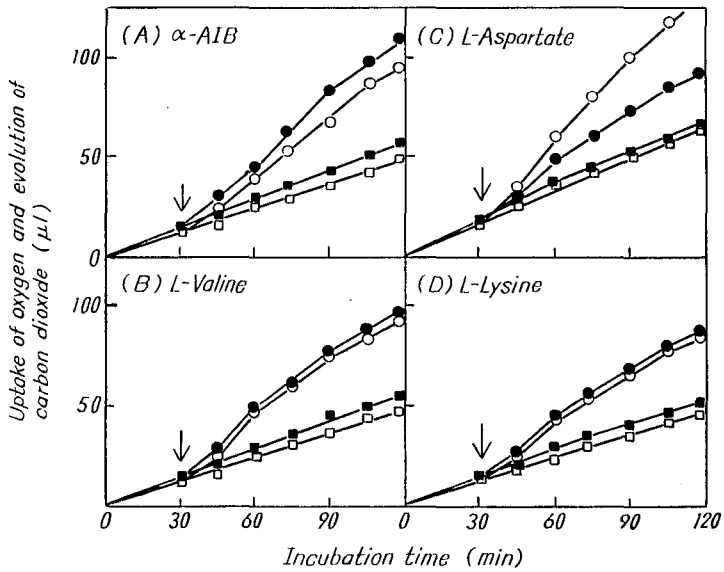

Fig. 9. Increase of Oxygen Uptake and Carbon Dioxide Evolution by *P. oryzae* due to Addition of Amino Acid

Incubation conditions were the same as in Fig. 1. Addition of each amino acid was shown with arrow.

■—■, control of O_2 uptake

□—□, control of CO_2 evolution

●—●, O_2 uptake by the addition of amino acid

○—○, CO_2 evolution by the addition of amino acid

phosphate buffer (pH 7.0) and 5 μ moles of each organic acid was incubated for 60 min at 25°C, 12 μ moles of α-AIB were added.

As Table 4 illustrates, the addition of each organic acid did not stimulate the rate of the uptake of α-AIB, and the addition of malate seems to reduce slightly the rate of the uptake of α-AIB.

In another experiments, it was observed that the intracellular content of these organic acids was not affected by the addition of these organic acids. However, added each organic acid could be permeated into mycelial cells at very smaller rate.

Decrease of content of citrate in mycelia due to the uptake of other amino acids

An increase of respiration due to the uptake of L-valine, L-aspartate, L-lysine or α-AIB is illustrated in Fig. 9. With respect to other amino acids such as L-asparagine, L-glutamine and L-methionine, the pattern of an increase in respiration is similar to that in Fig. 9–(A) or –(B), with acidic amino acids, to that in Fig. 9–(C) and with L-lysine, L-phenylalanine and L-serine, to that in Fig. 9–(D).

Thus, the relation between the intracellular content of citrate and the uptake of L-valine, L-aspartate and L-lysine was examined.

The reaction procedure was the same as in the uptake of α-AIB, except the use of each amino acid in place of α-AIB. Twelve μ moles of valine or 6 μ moles of L-aspartate or 6 μ moles of L-lysine were used, respectively.

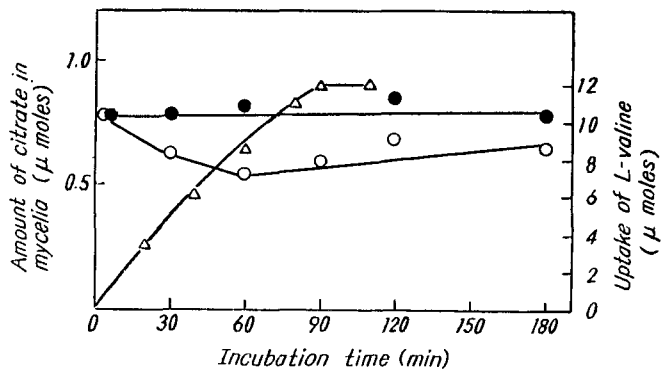

Fig. 10. Change in Content of Citrate in Mycelia due to Uptake of L-Valine

Incubation conditions were the same as in Fig. 3, except that 29 mg of mycelia (as dry weight) and 12 μ moles of L-valine were used.

○—○, the content of citrate in the presence of L-valine

●—●, the content of citrate in the absence of L-valine

▲—▲, uptake of L-valine

The results are shown in Figs. 10, 11 and 12. In a similar way as observed in the uptake of α-AIB (Fig. 5), the content of citrate decreased during the uptake of each amino acid. And just after the complete transport of each amino acid, the content of citrate appeared to begin to increase.

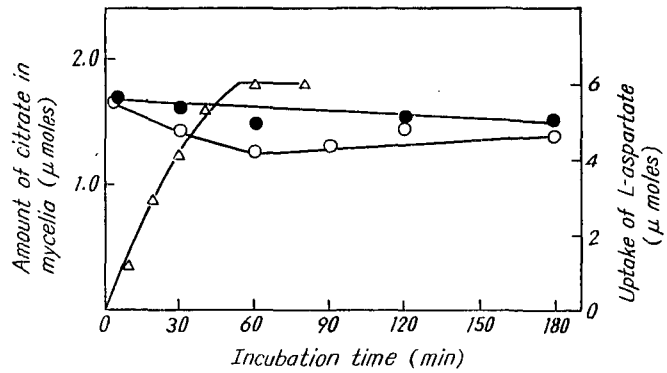

Fig. 11. Change in Content of Citrate in Mycelia due to Uptake of L-Aspartate

Incubation conditions were the same as in Fig. 3, except that 49 mg of mycelia (as dry weight) and 6 μ moles of L-aspartate were used.

○—○, the content of citrate in the presence of L-aspartate
●—●, the content of citrate in the absence of L-aspartate
△—△, uptake of L-aspartate

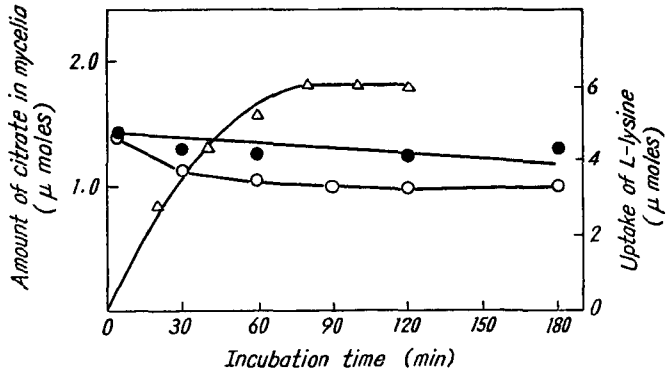

Fig. 12. Change in Content of Citrate in Mycelia due to Uptake of L-Lysine

Incubation conditions were the same as in Fig. 3, except 45 mg of mycelia (as dry weight) and 6 μ moles of L-lysine.

○—○, the content of citrate in the presence of L-lysine
●—●, the content of citrate in the absence of L-lysine
△—△, uptake of L-lysine

Discussion

There have been presented many reports describing the correlation between the uptake of α-AIB and respiration in various organisms[18,21].

Most recently, DECKER and TANNER[5] reported that the addition of 3-O-methylglucose or 6-deoxyglucose, non-metabolized glucose analogues, to the cells of *Chlorella vulgaris* was followed by an increase in the rate of respiration and that 1.18 moles of ATP was required for the transport of one mole of 6-deoxyglucose.

As Fig. 1 shows, when α-AIB, non-metabolized amino acid, was added to mycelial cells of *P. oryzae*, an increase in the rate of respiration was observed. This fact suggests the uptake of α-AIB by *P. oryzae* is energy-dependent. From analogous experiments as in Fig. 1, it was known that this increased respiratory rate remained constant during the uptake of α-AIB and was independent of the initial concentration of α-AIB, and the rate of the uptake of α-AIB was almost constant, being independent of the concentration of this amino acid.

Therefore, it is possible to take into consideration which the respiratory increase is exactly correlated with the uptake of α-AIB itself. In this case, it is assumed that an increase in the evolution of carbon dioxide during the energy-dependent uptake of α-AIB is rather due to the oxidation of organic acids on Krebs cycle than to the decarboxylation of endogeneous amino acids or other compounds. The existence of both Krebs cycle and respiratory chain of heart muscle type in *P. oryzae*[6,19] seems to support this assumption.

It is not clear only from the data (Figs. 3, 4, 5 and 6) why the contents of fumarate and malate increase, and the contents of citrate and succinate decrease, during the energy-dependent uptake of α-AIB. However, it is possible to consider that the very rapid production of energy was required for the quick transport of α-AIB into *P. oryzae*. Thus, these observation might be made only during the energy-requiring uptake of α-AIB by mycelial cells.

Supposing that the energy for the uptake of α-AIB is supplied by the oxidation of organic acids on Krebs cycle, the addition of each organic acid is expected to affect the rate of energy-dependent uptake of this amino acid. However, this was not the case as shown in Table 4. This fact may be caused by the competitive inhibition of the stimulatory effect of addition of organic acids with the permeation of each organic acid or by the participation of added organic acids in various metabolic pathways other than

Krebs cycle. However, these possibilities are unlikely, because the rate of the permeation of each organic acid is very slower compared with the uptake of α-AIB and the contents of organic acid in mycelia does not be affected by the addition of each organic acid.

More recently, KABACK[1] reported that oxidation of D-lactate is coupled with the concentrative uptake of amino acids and β-galactosides by the membrane vesicles isolated from *E. coli*. However, the participation of D-lactate in the energy-dependent uptake of α-AIB by *P. oryzae* was not observed. (Table 3)

The content of L-lactate did not be affected by the uptake of α-AIB. (Table 2)

The similar decrease in the content of citrate was observed during the energy-dependent uptake of other three amino acids. (Figs. 10, 11 and 12) Analogous, but not identical results were obtained with the change in the content of each of succinate, fumarate and malate by the uptake of these three amino acids, compared with the results in Figs. 3, 4 and 6. The effect of catabolism of these amino acids on the contents of succinate, fumarate and malate in mycelial cells was supposed.

Thus, the hypothesis may be possible that the energy-dependent uptake of amino acids by mycelial cells of *P. oryzae* may be primarily coupled to the oxidation of organic acids on Krebs cycle.

The uptake of α-AIB is inhibited by 2, 4-dinitrophenol, azide and cyanide[11]. Therefore, an experiment was attempted to see if nucleotides such as ATP are concerned in the energy-dependent uptake of α-AIB by *P. oryzae*. However, the result obtained indicates no participation of nucleotides in the energy-dependent uptake of α-AIB. But more detailed attempt must be made to know the relation between the uptake of α-AIB and nucleotides metabolism.

Summary

An energy-dependent uptake of α-aminoisobutyric acid (α-AIB) by *Piricularia oryzae* was studied.

It was observed that the uptake of α-AIB caused increase in both oxygen consumption and carbon dioxide evolution which were independent of α-AIB catabolism. In this case, an increase in carbon dioxide evolution was in parallel with that in oxygen consumption.

Based on these results, the relation between organic acids metabolism and uptake of α-AIB was investigated, using *P. oryzae*.

The content of malate and fumarate in mycelial cells increased during

the uptake of α-AIB. When the added α-AIB was completely transported in mycelial cells, the content of these organic acids began to fall down.

On the other hand, the content of citrate and succinate decreased during the uptake of α-AIB. When the added α-AIB was thoroughly transported, the increase in the content of these organic acids was observed.

The content of L-lactate in mycelia did not change during through the uptake of α-AIB. D-lactate and acetate seemed not to be essential for the uptake of α-AIB.

The similar results were obtained in the experiments where α-AIB was replaced with other three amino acids.

It was concluded that the uptake of amino acids by *P. oryzae* is closely related to the metabolism of organic acids involved in Krebs cycle.

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