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# Respiration-driven amino acid uptake system in *Pseudomonads*

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**Abstract** Amino acids uptake in *Pseudomonas putida* was investigated. Respiration-driven glutamate uptake in bacterial cells by means of $H^+$ electrode was examined. The glutamate uptake was insensitive to a proton conductor, carbonyl cyanide m-chlorophenylhydrazone, on the contrary sensitive to a respiration inhibitor, $CN^-$. These results support the idea of the existence of a respiratory glutamate pump in *P. putida* as a primary active transporter.

## INTRODUCTION

In the aerobes, the respiratory chain transfers electrons and extrudes $H^+$ ions establishing proton motive force across the membrane (HAROLD 1972). It is the driving force for ATP synthesis in $H^+$-ATPase complex and for the active transport of many substrates. It has been known that the other cations which are involved in membrane energetics are $Na^+$ (HAROLD 1972) and $Li^+$ (TSUCHIYA *et al* 1984). $Na^+$ is extruded via the $Na^+$-$H^+$ antiporter system and the driving force for the $Na^+$ extrusion is the proton motive force (HAROLD 1972). Therefore, $Na^+$ extrude via the $Na^+$-$H^+$ antiporter (HAROLD 1972). On the other hand, $Li^+$ is recognized by the $Li^+$-proline cotransporter in a secondary transport (CHEN *et al* 1985, and TSUCHIYA *et al* 1984). It was found that the respiration-driven $Na^+$ pump functions as a primary active transporter in *Vibrio alginolyticus* (TOKUDA and UNEMOTO 1982) and *V. parahaemolyticus* (TSUCHIYA and SHINODA 1985). It is insensitive to a proton conductor, carbonyl cyanide m-chlorophenylhydrazone (CCCP), and sensitive to a respiratory inhibitor, $CN^-$, (TSUCHIYA and SHINODA 1985). We studied the active transport of some amino acids in the *Pseudomonas* in order to examine whether the bacterial species also shows some diversification in the active transport system or not.

It is known that *Pseudomonas,* an aerobe possessing catalase, can grow in a wide range of nutritional (from very poor until high concentration of organics) and temperature conditions : 0-40°C (PALLERONI 1984). It can also produce many kinds of oxigenases adapting to the environmental changes. Furthermore, some species, e. g. *P. putida* and *P. fluorescence,* have high proteolytic activity (PALLERONI 1984). By virtue of peculiar characteristics the *Pseudomonas* exhibits strong resistance to many kinds of harmful materials.

This paper describes some evidences for the existence of the respiration-driven and CCCP-insensitive glutamate pump as a new primary active transporter in *P. putida.*

## MATERIALS AND METHODS

Bacteria and growth: *Pseudomonas putida* (ATCC No. 12633) was grown aerobically in peptone water (1% polypeptone, pH 7.0) and harvested at a late-exponential phase of growth. Cells were washed with 20 mM choline chloride (Choline Cl) three times and suspended in the same solution for measurement of pH change and cell concentration was adjusted to 2.0 of absorbance at 660 nm.

Measurement of amino acids transport: Transport of amino acids was evaluated according to the methods reported previously (TSUCHIYA and SHINODA 1985). Washed cells and the reagents required were put together in a 20 ml glass cup and it was sealed with silicon rubber plug. A pH glass electrode was then mounted. Two additional very small holes were present in the plug. One is for introducing $N_2$ gas and solution containing reagents and the other for exaust. For pH measurement an electrode (HITACHI-HORIBA, Type 764-1) was connected to a recorder (TOA ELECTRONICS Ltd., Type FBR-251A) and the pH change showing the $H^+$extrusion or incorporation driven by amino acids uptake was recorded. The cells suspended in 20 mM Choline Cl were incubated in the presence of 20 mM $Na^+$ at 25°C with gentle stirring and addition of an amino acid was made when the pH became constant.

Reagents: Reagents for the examinations were purchased from Wako Pure Chemicals Industry and Dojindo Laboratories, Japan.

## RESULTS

I. *Amino acids uptake driven by the respiration.*

Since *P. putida* is one of the bacteria of high proteolytic activity, aerobic uptake of four kinds of amino acids (glutamate, lysine, proline and leucine which are acid, basic hydrophobic and neutral amino acid, respectively). The pH change showing $H^+$ extrusion or incorporation driven by the addition of 200 $\mu$M amino acid plus 0.01% $H_2O_2$ were observed for all amino acids examined and on the other hand three amino acids uptake resulted in extrusion of $H^+$ only lysine induced $H^+$ incorporation. Although proline and leucine were taken up by two steps at an initial burst, glutamate and lysine uptake were noticed with one step during the initial one to two seconds. Especially glutamate extruded drastically very large amount of $H^+$ (Fig. 1).

II. *Amino acids uptake under an anaerobic condition*

The anaerobic condition was made by the gassing of $N_2$ in the assay medium and pH change was measured by the addition of an anaerobic amino acid (200 $\mu$M) in the presence of 20 mM $Na^+$ (Fig. 2). The $H^+$ extrusion driven by the addition of glutamate occurred in a moment and the amount of the extruded $H^+$ was larger than the observed under the aerobic condition. In the case of lysine, $H^+$ incorporation by the lysine uptake was observed in a moment and the amount of the incorporated $H^+$ was also larger than that observed under aerobic condition. But the amount of the extruded $H^+$ driven by the glutamate uptake was far larger than that of the incorporated $H^+$ driven by the lysine uptake as far as being shown by the pH change. On the other hand, proline and leucine were not taken up under the anaerobic condition, their uptake under anaerobic condition was observed to be sufficient.

III. *The anaerobic uptake of glutamate and lysine is insensitive to $CN^-$ and CCCP.*

From the results described above, it is clear that there is striking contrast between the results of uptake of glutamate and lysine under the anaerobic condition. We had a question whether the uptake of these amino acids is related to the respiration and the difference of the electro-chemical potential of $H^+$ or not. To confirm this, the washed cells suspended in the 20 mM Choline Cl were incubated in the presence of 5 mM KCN, of which concentration inhibited completely, the respiration driven by the addition of $H_2O_2$ or $O_2^-$ saturated pure ethanol at 25°C for 24 h and then the anaerobic uptake of glutamate and lysine after the addition of 20 mM $Na^+$ was examined (Fig. 3). Both glutamate and lysine uptake were found to be inhibited by KCN as the respiration was strongly suggesting that glutamate and lysine uptake depends on the respiration. Next, the effect of CCCP, a proton conductor, on the amino acid uptake was tested. The washed cells were incubated with glutamate and lysine uptake with 20 mM $Na^+$ in the presence or absence of 20 mM CCCP at

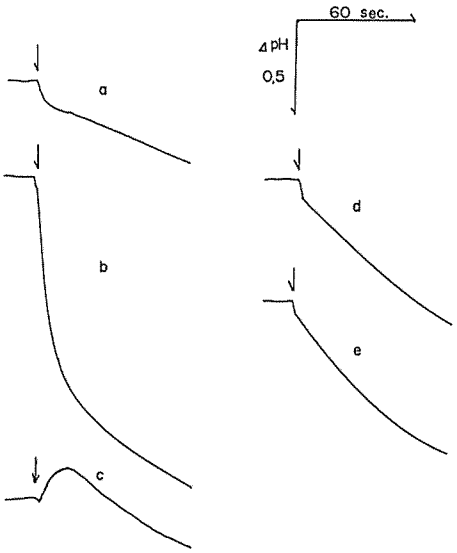


Fig. 1. The pH change driven by the addition of amino acids with 0.01% $H_2O_2$. Cells suspended in 20 mM Choline Cl ($A_{660}$ =2.0) were incubated in the presence of 20 mM $Na^+$ at 25°C with gentle stirring under the aerobic condition (the total reaction volume was 20 ml). At the time point indicated by an arrow, 0.2 ml of $H_2O$ plus 0.1 ml of 3% $H_2O_2$ (a: control), or 0.2 ml of 20 mM glutamate plus 0.1 ml of 3% $H_2O_2$ (b), or 0.2 ml or 20 mM lysine plus 0.1 ml of 3% $H_2O_2$ (c), or 0.2 ml of 20 mM proline plus 0.1 ml of 3% $H_2O_2$ (d), or 0.2 ml of 20 mM leucine plus 0.1 ml of 3% $H_2O_2$ (e) were added. A downward deflection indicates the decrease in pH in the assay medium.

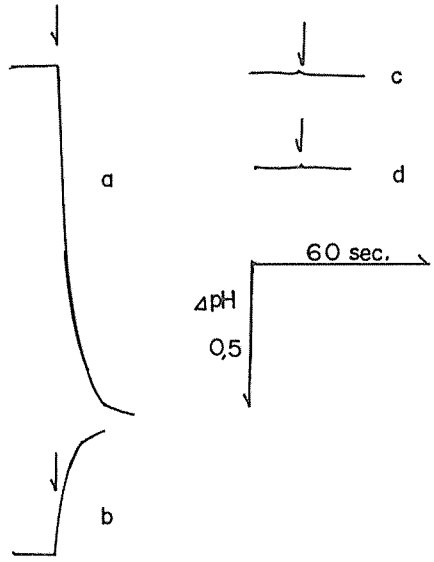


Fig. 2. The pH change driven by the addition of amino acids under the anaerobic condition. Cells suspended in 20 mM Choline Cl ($A_{660}$=2.0) were incubated in the presence of 20 mM $Na^+$ at 25°C with gentle stirring under the aerobic condition (the total volume was 20 ml). At the time point indicated by an arrow, 0.2 ml of 20 mM glutamate (a), or 0.2 ml of 20 mM lysine (b), or 0.2 ml or 20 mM proline (c), or 0.2 ml of 20 mM leucine was added. A downward deflection indicates the decrease in pH in the assay medium.

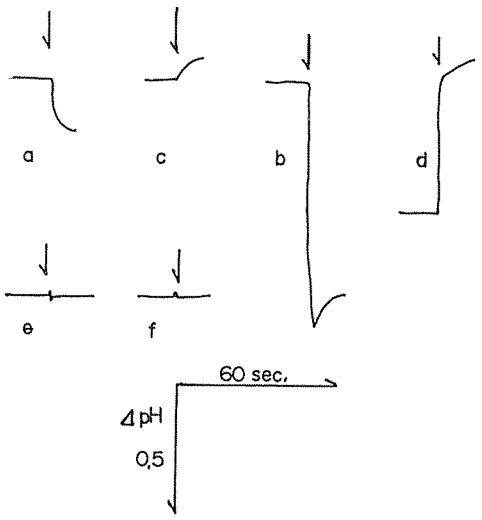


Fig. 3. The resistance of glutamate and lysine uptake to KCN which is shown the pH change anaerobically. Cells suspended in 20 mM Choline Cl ($A_{660}$=2.0) were incubated in the presence of 5 mM KCN at 25°C for 24 h and then, the KCN-treated cells were incubated in the presence of 20 mM $Na^+$ (the total volume of 20 ml) at 25°C with gentle stirring. At the point indicated by an arrow, 0.2 ml of 20 mM glutamate (a), and KCN untreated one (b), 0.2 ml of 20 mM lysine (c), and KCN untreated one (d), and 0.2 ml of 3% $H_2O_2$ (e), and 0.2 ml of $O_2$-saturated pure ethanol (f) was added respectively. A downward deflection indicates the decrease in pH in the assay medium.

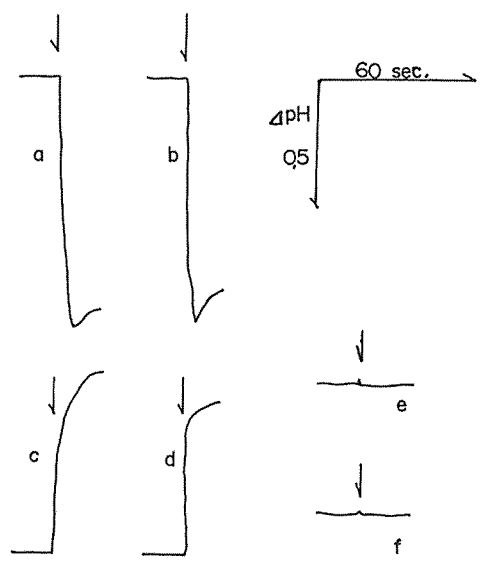


Fig. 4. The resistance of glutamate and lysine uptake to CCCP which is shown the pH change anaerobically. Cells suspended in 20 mM Choline Cl ($A_{660}$=2.0) were incubated in the presence of 20 mM CCCP plus 20 mM $Na^+$ (in the total volume of 20 ml) at 25°C with gentle stirring. At the point indicated by an arrow, 0.2 ml of 20 mM glutamate (a), and CCCP untreated one (b), 0.2 ml of 20 mM lysine (c), and CCCP untreated one (d), and 0.2 ml of 3% $H_2O_2$ (e), and 0.2 ml of $O_2$-saturated pure ethanol (f) was respectively. A downward deflection indicates the decrease in pH in the assay medium.

25°C (Fig. 4). Both extrusion and incorporation indicating glutamate and lysine uptake, respectively, were observed whether the proton conductor was present or not.

## DISCUSSION

Under the aerobic condition, glutamate and lysine were taken up by *Pseudomonas* cells by step within the initial one to two seconds, differing from other amino acids which were taken up by two steps with a small initial burst and the uptake of the former amino acids were far remarkable as compared with that of others (especially glutamate), (Fig. 1).

It was considered that the small initial burst generated by the aerobic uptake of proline and leucine was driven by the respiration upon the addition of 0.01% $H_2O_2$ and then proline and leucine were taken up by antiport to $H^+$ as a secondary active transport but that glutamate and lysine were taken up by the primary active transport because of the extrusion of the large amount of $H^+$ showing these amino acids uptake. We examined glutamate

and lysine uptake under the condition without any of the respiration to confirm its relation to the respiration. For this purpose, the examination was made anaerobically (Fig. 1, 2). The amount of $H^+$ extruded indicating glutamate uptake was far larger than that observed under the aerobic condition within the initial one to two seconds but it was almost the same level of $H^+$ incorporated showing lysine uptake as that observed under the aerobic condition.

To confirm the results, we examined the aerobic uptake of glutamate and lysine in the presence of 5 mM KCN. The use of KCN should enable us to neglect the effect of the respiration completely (Fig. 3). The respiration driven by the addition of 0.03% $H_2O_2$ and $O_2$-saturated pure ethanol was inhibited, and the uptake of glutamate and lysine was almost inhibited. It was, however, found that glutamate and lysine uptake were insensitive to 20 mM CCCP of which concentration was inhibited the $H^+$ extrusion driven by the respiration. The results showed that glutamate and lysine were completely taken up even if the difference of the electro-chemical potential of $H^+$ across the membrane was eliminated by CCCP. The respiration driven by $Na^+$ pump in *V. parahaemolyticus* (Tsuchiya and Shinoda 1985) was known as well as the presence of a CCCP insensitive pump.

Because $Na^+$ is necessary for active transport of many substrates in the secondary transport system, glutamate and lysine uptake in association with $Na^+$ circulation were examined with a $Na^+$ electrode showing the $Na^+$ change driven by the substrates uptake. The $Na^+$ extrusion or incorporation driven by the addition of glutamate or lysine was not detected as the pH change (data not shown). It was considered that glutamate and lysine were transported not to $Na^+$ but to $H^+$. But the effect of $Na^+$ on the transport of these amino acids in *P. putida* should be studied in further detail. Based on the results, we concluded that glutamate and lysine uptake in *P. putida* were driven by the respiration independent of $H^+$ circulation in the secondary active transport (Harold 1972, Tokuda and Kaback 1977, Tsuchiya and Shinoda 1985). Therefore, they were glutamate- or lysine-pump as a primary active transporter. It is well known that $H^+$ and $Na^+$ pump is usually coupled with ATP synthesis (Harold 1972, and Heefner 1980), thus glutamate pump may be related to ATP synthesis but lysine uptake system might not be recognized as a primary active transporter with ATP synthesis because of it's uptake system is not $H^+$-extrusive but $H^+$-incorporative.

In addition, the possibility of the existence of $Na^+$ and $H^+$ transporting ATPase has been reported in some bacteria (Harold 1972, and Heefner 1980). By the analogy with this, the possibility of the existence of the glutamate translocating ATPase would be expected.

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# *Pseudomonads* における呼吸依存性アミノ酸の取り込み

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*Pseudomonas putida* においてアミノ酸の取り込みを［$H^+$］の濃度変化を追跡して調べた。その結果，酸素呼吸によって駆動されるグルタミン酸の取り込みが観察された。このシステムはプロトンコンダクターである Carbonyl cyanide m-chlorophenyl hydrazone によっては阻害されず，$CN^-$ によって阻害された。これらのことから，本細菌には第一次能動輸送として呼吸によって駆動されるグルタミン酸の取り込みシステムがあることが推測された。